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PLENARY LECTURES ON MONDAY, 18 AUGUST 2008

L01 The development of cellulosic biofuels
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The earth receives approximately 4000 times as much energy from the sun each year as total projected human uses in 2050. Thus, because plants can be deployed on a large scale to capture and store solar energy, one way of moving toward the development of carbon neutral energy sources is to use plant biomass for production of fuels. However, there are many inefficiencies in the overall process that must be eliminated in order to make the most efficient use of land and capital. In brief, the efficient production of biofuels by routes other than gasification will require innovation in three main areas: production of feedstocks, conversion of feedstocks to sugars, and conversion of sugars to fuels. At present, the main feedstocks being used for fuel production are corn starch and sugar from sugarcane. However, the demand for fuel vastly exceeds the amount that can be produced from these feedstocks so it is expected that gasoline and diesel replacements will ultimately be derived from cellulosic biomass. The importance of enhancing soil carbon and nutrient retention while minimizing inputs will require an integrated approach to the development of cellulosic energy crops. Parallel technical developments on the biomass-to-fuels processing side also have important implications for how the industry is likely to develop.

L02 Engineered green algae as a future energy source
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Crop plants have traditionally and successfully been used for biofuel production such as bioethanol or biodiesel however, with rather low photon conversion efficiencies. Theoretically, photosynthetic biomass production efficiencies could be increased towards an approximate maximum efficiency of 10% through the use of genetically engineered green algal cells. Of the second generation biofuels, bio-H2 has been identified as one of the most promising sources of clean fuel for the future. The H2 production process depends on the interplay of a wide range of metabolic processes including those where competition between enzymes at multiple stages leads to intricate combinations of different metabolites. We have developed a novel approach for the engineering of complex metabolic pathways in plants based on combinatorial nuclear transformation and the generation of libraries of synthetic mutants. By using co-transformation of multiple genes and direct DNA transfer we were able to recreate complex biosynthetic pathways in their entirety in plants and allowed us to identify and complement rate-limiting steps in specific pathways. Because of the integration mechanism involved, all integrated transgenes end up in one genomic location and they do not segregate in subsequent generations. Combinatorial transformation thus provides an efficient approach for the dissection of complex metabolic pathways, including those where competition between enzymes at multiple stages leads to intricate combinations of different metabolites. We will exemplify applications of the technology in the engineering of a number of multigenic traits in cereal plants.

L03 Getting to the root of cell identity
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Specification and maintenance of cell identity are central processes of development. In an effort to understand the regulatory networks that control cell identity, we have profiled all cell types and developmental stages within a single organ, the Arabidopsis root. To acquire global expression profiles we developed technology that uses sorted marked populations of cells with subsequent hybridization of the labeled RNA to microarrays. We are using computational methods to infer networks functioning within different cell types and developmental stages and have begun to test the hypothesized relationships. Our current efforts are aimed at understanding the responses to environmental stimuli at high spatio-temporal resolution. We are developing new expression analysis platforms and means of analyzing 4-D data sets. We are also analyzing the dynamics of growth of physical root networks using novel non-invasive imaging methods and developing mathematical descriptors of network architecture.

L04 Third generation transgenic crops with enhanced multigenic traits
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We have developed a novel approach for the engineering of complex metabolic pathways in plants based on combinatorial nuclear transformation and the generation of libraries of synthetic mutants. By using co-transformation of multiple genes and direct DNA transfer we were able to recreate complex biosynthetic pathways in their entirety in plants and allowed us to identify and complement rate-limiting steps in specific pathways. Because of the integration mechanism involved, all integrated transgenes end up in one genomic location and they do not segregate in subsequent generations. Combinatorial transformation thus provides an efficient approach for the dissection of complex metabolic pathways, including those where competition between enzymes at multiple stages leads to intricate combinations of different metabolites. We will exemplify applications of the technology in the engineering of a number of multigenic traits in cereal plants.

L05 Natural variation for local adaptation in outcrossing and selfing species
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Natural selection is expected to be efficient in targeting specific traits and loci in large outcrossing highly recombinating populations. Such populations are expected to contain much genetic variation and show low levels of linkage disequilibrium, whereas in selfing populations most variation has been found to be between populations,
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PARALLEL SESSIONS ON MONDAY, 18 AUGUST 2008

PARALLEL SESSION 01: SIGNALLING AND GENE EXPRESSION

S01-01 Growth control in plants by chromatin modifying complexes activating transcription
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Leaf architecture is important for green biomass production and is determined by molecular-genetic network controlling leaf size and shape. Characterization of leaf mutant collection allowed identification of two conserved histone modifying protein complexes, HISTONE MONOUBIQUITINATION1 (HUB1) and ELONGATOR, involved in growth control. Histone modifications result either in transcription activation or repression and act upstream of transcription factors. There is also accumulating evidence that chromatin is reactive to environmental stimuli (Nelissen et al. 2007. Crit Rev Plant Sci). HUB1 is an unconventional ubiquitin E3 ligase that is not involved in protein degradation but mediates histone H2B monoubiquitination. This histone regulation initiates a chain reaction of histone modifications ultimately resulting in transcriptional activation of RNA Polymerase II. hub1-1 mutant is a narrow leaf mutant with pale colour and decreased cell numbers in both leaf epidermis and palisade cell layers (Fleury et al. 2007. Plant Cell). Microarray analysis of HUB1 misexpression lines revealed that HUB1 is involved in transcriptional regulation of cell cycle and circadian clock and downstream pathways such as photosynthesis. These data suggest that HUB1 plays a critical role in regulation of the basic processes for plant development. The positioning of Elongator and HUB1 in molecular and genetic networks by double mutants, transcriptome analysis and protein-protein interactions are discussed.

S01-02 Dissecting the flexibility of histone methylation during Arabidopsis development
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During the lifetime of plants and animals, specific patterns that have been established in development need to be maintained throughout the lifecycle. Often, cell determination and gene expression patterns have to be stable through cell divisions, but need to be reset in each generation or during regeneration. In addition, genes may be stably and heritably repressed in early development but need to be activated during later developmental phases. Therefore, it is likely that epigenetic mechanisms play a major role in the control of cell fate. Important epigenetic regulators of development in plants are the Polycomb-group (Pc-G) proteins which control Histone H3 Lysine 27 tri-methylation (H3K27me3). We study the dynamics of epigenetic gene regulation in plant development by performing tissue-specific, genome-wide ChiP-on-chip analyses with antibodies against H3K27me3. When comparing the profile of meristem-enriched fractions with young leaves we found a surprising high number of genes with differential methylation patterns. This suggests very dynamic regulation of epigenetic gene regulation during differentiation and might reveal many more Pc-G targets than discovered in previous analyses. To identify novel factors regulating this flexibility we performed a reverse genetics screen on histone demethylases and found a member which maintains the switch from vegetative to reproductive growth. Thus, both histone methylation and its removal ensure proper differentiation in plant.
Intros are removed from the nuclear pre-mRNA of higher eukaryotes by a system that recognizes rather short and degenerate splice-site sequences at the intron/exon boundaries. Several such short sequence elements required for splice-site recognition have been identified. In the classic U2-type splicing, the consensus sequences of the 5′ and 3′ splices sites, AG/GTAAGT and TGGCAG/G are highly conserved, while a minor class of nuclear premRNA introns, referred to as U12-type, frequently start with AT and terminate with AC. Here we report an unusual, short direct repeats (SDR)-mediated posttranscriptional processing that is completely different from the classic U2/U12 premRNA splicing patterns. SDR-mediated splicing confers the precise excision/insertion of the exonic sequences that is well associated with the paired presence of the GC-rich SDRS distributed in the coding sequences. Expression analysis of mini-gene constructs demonstrates SDRs are necessary but insufficient for the unusual posttranscriptional splicing. This form of splicing has been detected in a large number of plant genes involved in stresses responses, developmental control and signal transduction. An attempt to isolate the protein factors that possibly interact with SDRs has been initiated.

**S01-04 Phytochrome A signaling: do we underestimate the role of very low fluence Responses?**

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Light serves as an important cue for plants to evaluate their living conditions and monitor their environment to ultimately trigger adaptive growth responses. Photoreceptors, such as phytochromes, have developed in higher plants. In Arabidopsis five phytochromes can be differentiated according to their stability in white light: phyA is unstable, whereas phyB-E are light-stable. Accordingly, phyA plays a major role in morphological responses of seedlings during deetiolation. However, it is also important during the adult life of a plant. Two different light intensities can activate phyA, the very-low-fluence rates and high-irradiance, which requires prolonged irradiation with higher fluence rates of far-red (FR) light. We could show that small changes introduced in the N-terminal domain of the phytochrome A photoreceptor itself leads to a differential response to very low fluence, high irradiance of far-red light and even red light. So far, several components of the high-irradiance response (HIR)-signaling pathway have been identified, but only few that are involved in very low fluence response (VLFR). We identified the first protein specifically involved in the VLFR, as the mutant, jdp1, is not impaired in the HIR. Physiological characterization of this mutant has revealed that jdp1 fails to germinate after exposition to far-red light, exhibiting a diminished inhibition of hypocotyl elongation and an increased gravitropic growth when exposed to pulses of far-red light.

**S01-05 Evolution of growth regulation**

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Plants coordinate growth and development with the environment through regulatory signalling networks. Our aim is to understand how such coordinating mechanisms evolved during land plant evolution. For example, the growth of angiosperms is regulated by DELLA proteins (DELLAs), which repress growth in response to various phytohormones and environmental stimuli. Recently, angiosperm DELLA were shown to interact with a gibberellin (GA) receptor, GID1, in the presence of GA. Here we show that the DELLA-GID1 interaction does not operate in bryophytes, which represent the earliest land plants (430 million years ago (MYA)). Bryophyte DELLA from ‘Italics start’Phycomitrella patens ‘Italics end’ ( moss) lack the ability to interact with GID1 and do not repress moss growth. We show that it was after the bryophyte divergence but before the lycophyte divergence (400 MYA) that the DELLA and GID1 proteins evolved to interact in a GA dependent manner, Interestingly, the lycophytes do not exhibit growth responses to GA, suggesting that while GA regulates DELLA, DELLA does not regulate growth in the lycophytes. However, both bryophyte and lycophyte DELLA are capable of repressing angiosperm growth when expressed in Arabidopsis. This may suggest that the recruitment of DELLA into growth regulation involved changes in downstream of DELLA rather than changes within the DELLAs themselves. It was, therefore, a step-by-step process that led to the evolution of growth regulation by GA through DELLA.

**S01-06 MAPK-mediated defence signalling – How Agrobacterium tumefaciens turns the table**

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The soil-borne pathogen Agrobacterium tumefaciens induces tumour growth in plant tissue. This manipulation relies on the nuclear import of the agrobacterial transfer DNA (T-DNA) into the plant cell nucleus, where it integrates into the plant genome. VIP1 has been shown to serve as adapter between the agrobacterial virE2/T-DNA complex and the host karyoperin thereby allowing T-DNA to enter the nucleus. MAPK cascades constitute a conserved module for signal transduction in eukaryotes. Arabidopsis MPK3 has been implicated in the response to a number of biotic and abiotic stresses. However, its targets so far have been unknown. We isolated VIP1 in a screen for interactors of MPK3. As revealed by in planta experiments, phosphorylation of VIP1 by stress-activated MPK3 results in the translocation of VIP1 from the cytoplasm to the nucleus. Only phosphorylated VIP1 enhances the efficiency of agrobacterial transformation of plant cells. Apparently, Agrobacterium has developed a neat way to take advantage of being recognized as pathogen by abusing the relocation of VIP1 as a nuclear shuttle of its T-DNA complex. A bZIP domain is located in the C-terminal region of VIP1. Latest findings give evidence for VIP1 to be a functional transcription factor. We identified a single residue within the bZIP domain that is crucial for VIP1’s transactivating function. A combination of in vitro and in vivo approaches has led to the identification of a DNA element targeted by VIP1.
In their natural environment plants are exposed to simultaneous abiotic and biotic hazards. Here we show that local and systemic acclimation in Arabidopsis leaves in response to excess excitation energy (EEE) is associated with cell death and is controlled by specific redox changes of the photosynthetic electron transport carriers, [for example, in plastoquinone pool, (PQ)]. In the Arabidopsis LESION SIMULATING DISEASE1 null mutant (lsd1) that is deregulated for EEE acclimation responses, propagation of programmed cell death depends on the plant defence regulators ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4). We find that this response and formation of the lysozymes aerenchyma depends on the plant defence regulator LSD1, EDS1 and PAD4 that operate upstream of ethylene and ROS production. Our results indicate that programmed cell death of lysozymes aerenchyma in hypocotyls occurs in a similar but independent manner from the foliar programmed cell death. Our resent results suggest that light induced electrical signals play an important role in systemic acquired acclimation. We observed different electrical signaling for light on and off and for light quantity and quality. We concluded that light acclimatory responses are signalized by a complex system of electrical ROS and hormonal signaling and that plant cells during light acclimatory responses performed complicated quantum computation.

S01-08 The plant circadian clock: mechanism and entrainment
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Proper plant growth and development requires a robust detection of the diurnal environment. This occurs through a coupling mechanism of light detection and the circadian clock. We recently took a genetic approach to confirm the core of the circadian oscillation in Arabidopsis leaves in response to excess excitation energy. Proper plant growth and development requires a robust detection of the light/dark transition, humidity change, calcium ions, hydrogen peroxide, and metabolites across guard cell membranes. Stomata also restrict the entry of ozone (an important air pollutant), global carbon fixation and climate change. Activation of guard cell anion channels has been proposed to be an essential step in mediating stomatal responses to physiological and stress stimuli. However, genes encoding membrane proteins mediating guard cell anion efflux have not been identified thus far. Here we report the mapping and characterization of an ozone-sensitive Arabidopsis thaliana mutant, slac1. We show that SLAC1 (SLOW ANION CHANNEL-ASSOCIATED 1) is preferentially expressed in guard cells and encodes a distant homologue of fungal and bacterial dicarboxylate/malic acid transport proteins. The plasma membrane protein SLAC1 is essential for stomatal closure in response to carbon dioxide, abscisic acid, ozone, light/dark transitions, humidity change, calcium ions, hydrogen.
peroxide and nitric oxide. Mutations in SLAC1 abolish slow (S-type) anion channel currents that are activated by cytosolic Ca 2+ and abscisic acid, but do not affect rapid (R-type) anion channel currents or Ca 2+ channel function. A low homology of SLAC1 to bacterial and fungal organic acid transport proteins, and the permeability of S-type anion channels to malate suggest a vital role for SLAC1 in the function of S-type anion channels.

PARALLEL SESSION 02: ORGANELLES

S02-01 Protein and metabolite transport in chloroplast J. Soll LMU Munich, Plant Biochemistry, Germany e-mail: soll@lrz.uni-muenchen.de

Chloroplasts originated from an ancient cyanobacterial endosymbiont. The arising organelle had to be integrated into the newly developing compartmentalized eukaryotic cell and its metabolic and signalling network. Carrier proteins and ion channels were integrated into the outer and inner envelope of the endosymbiotic organelle to facilitate solute and protein transport. Today more than 95% of the chloroplast constituent proteins are imported from the cytosol by two in principal independent transloci, the Toc and the Tic complex. The Toc machinery catalyses the recognition and translocation of preproteins across the outer envelope in a highly regulated ATP- dependent manner. The Tic complex completes the import process in a chaperone and ATP dependent fashion. The Tic complex is associated with a redox-regulon which can influence the translocation efficiency. Besides protein import the biosynthetic capacities of chloroplasts require a massive exchange of solute and metabolic intermediates with the parent cell. Like their gram-negative like progenitors chloroplasts have retained multiple ion-channels in the outer envelope in addition to specific transporters in the inner envelope. Surprisingly the outer membrane ion channels and the inner membrane anion channels exert an unexpected control over the metabolic flux between compartments. Therefore we need to revise our current thinking on metabolic exchange between plastids and the cytosol.

S02-02 The mechanism of plastid division
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Plastids are not formed de novo but arise by division in the cytosol. Plastid division is mediated by the coordinated action of a prokaryote-derived division system(s) and a host eukaryote-derived division system(s). The evolutionary conserved prokaryote-derived system comprises several nucleic-encoded proteins: a stromal ATPase MinD (AtMinD1) and a topological specificity factor MinE (AtMinE1). We have shown that the Arabidopsis AtMinE1 protein acts as a topological specificity factor during plastid division and that AtMinE1 forms homodimers and heterodimers with AtMinD1. AtMinD1 acts as an ATPase during division and its activity is stimulated by its interaction with AtMinE1. Further, we have shown that the FtsZ (Z)-ring in Arabidopsis consists of specific protein complexes: (1) AtFtsZ1-1 interacts with itself and with AtFtsZ2-1 which also homodimerizes, (2) the J-domain plastid division protein ARC6 interacts specifically with FtsZ2-1 within the Z-ring, and (3) the stromal plastid division protein ARC3 interacts specifically with AtFtsZ1-1. ARC3, with its FtsZ-like domain and MOR2 repeat domains, act as an FtsZ accessory protein with possible MinC-like properties. Our data demonstrates that AtMinE1 and AtMinD1 act in concert during division and that the FtsZ-ring has several accessory proteins vital for correct plastid division.

S02-03 Unique mechanisms of translation in higher plant chloroplasts
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The chloroplast genome of higher plants contains about 80 protein-coding genes and their expression is mainly controlled at the translation level. Chloroplast ribosomes include several additional ribosomal proteins not found in E. coli, and two-thirds of the chloroplast mRNAs contain no Shine-Dalgarno-like sequences at proper positions. Therefore, the mechanism of translation in chloroplasts is not the same as that in E. coli. To study molecular mechanisms of translation unique to chloroplasts, we developed an in vitro translation system from tobacco chloroplasts, and recently improved it using an mRNA for GFP instead of 35S-methionine. The improved method is 100-fold more active than the original one, extremely low in background and requires no additional tRNAs. The rate of translation initiation from a variety of mRNAs can be measured by monitoring the fluorescence intensity of synthesized GFP. Using this system, we analyzed translation efficiencies of various 5′-UTRs of tobacco chloroplast mRNAs and effects of mutations in 5′-UTRs on translation. Several cis-elements for translation were also identified. Interestingly, we found that translation of the downstream mRNA is dependent on the upstream stop codon in an overlapping bicistrionic mRNA. We devised an in vitro assay to measure translation efficiencies of synonymous codons, and found that translation efficiencies of synonymous codons are not always correlated with the codon usage in tobacco chloroplasts.

S02-04 Solute carriers in the plant vacuole
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The plant vacuole plays a major role in the storage of a large diversity of solutes. We have identified a first vacuolar dicarboxylate carrier AtTDT (Emmerich et al. 2003, Hurr et al. 2005) and a first vacuolar monosaccharide carrier AtTMT (Wormit et al. 2006). To study molecular mechanisms of AtTDT gene is strongly expressed in guard cells and corresponding knockout mutants show impaired stomatal movement and altered drought resistance. These observations indicated that AtTDT is actively involved in guard-cell anion homeostasis. The AtTMT gene is induced by low temperatures and knockout mutants exhibit altered metabolite levels upon cold induction. Interestingly, AtTMT overexpression lines show no sugar induced repression of development (after growth on glucose) indicating altered sugar sensing. In sum, our data provide clear evidence that controlled accumulation and release of primary metabolites in plant vacuoles is critical for optimal plant performance.

Physol. Plant. 133, 2008
Abstracts

Emmerich et al. (2003) The plant homolog to the human sodium/ dicarboxylic cotransporter is the vacuolar malate carrier. Proc Nall Acad Sci USA 100: 11122–11126


S02-05 Deletion of an organellar peptidosome PreP affects early development in Arabidopsis thaliana

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We have characterized a novel peptidosome, Presequence Protease PreP, in mitochondria and chloroplasts that is responsible for degradation of targeting peptides as well as other unstructured peptides that might be harmful to organellar functions (Glaser et al. 2006, Biol Chem, Johnson et al. 2006, EMBO J, Moberg et al. 2003, Plant J, Stähli et al. 2002, J Biol Chem). In A. thaliana there are two PreP paralogs, AtPreP1 and AtPreP2, both are dually targeted to mitochondria and chloroplasts and have overlapping substrate specificity (Bhushan et al. 2003, EMBO Rep, Stähli et al. 2005, J Mol Biol). Furthermore, PreP has been implicated in Alzheimer disease as it degrades amyloid-beta peptide (Falkevall et al. 2006, J Biol Chem). Furthermore, PreP has been implicated in Alzheimer disease as it degrades amyloid-beta peptide (Falkevall et al. 2006, J Biol Chem). Particularly, PreP mutants show stunted growth, delayed flowering and age-dependent formation of cell death lesions when grown under moderate growth light intensity. Intriguingly, all these characteristics become less pronounced when the pp2a-b mutants plants grow under high light intensity or at low temperature. Under moderate light conditions, the cell death phenotype of pp2a-b mutant plants is accompanied by enhanced sensitivity to methyl viologen-induced photo-oxidative stress, increased accumulation of hydrogen peroxide, and constitutive activation of defence-related genes, including PR1 and PR5. Accordingly, the pp2a-b mutants also show enhanced resistance against the virulent bacterial plant pathogen Pseudomonas syringae pv tomato DC3000 in the leaves. Taken together, our results suggest that a chloroplastic PP2A is involved in the cross-talk of signaling pathways that modulate light acclimation and defense responses in plants.

S02-07 Global monitoring of chloroplast mRNAs highlights connections between Arabidopsis chloroplast transcription, splicing and translation

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We analysed Arabidopsis mutants by quantitative RT-PCR to measure the accumulation and splicing of 80 plastid transcripts encoding all the characterised ORFs. We compared the patterns to those in wild-type seedlings and seedlings treated with spectinomycin to block plastid translation. Plastids encode both the major RNA polymerase (PEP) and the putative MatK splicing maturase, and we found that blocking translation led to a decrease in PEP transcripts, major increases in NEP transcripts and alterations to processing and splicing pattern, especially for the ndhA, petB and petD transcripts. The mutants ptac2 and chb19, missing specific pentatricopeptide repeat (PPR) proteins, show transcript patterns almost identical to spectinomycin-treated plants suggesting a specific lack of PEP activity and strong links between transcription and subsequent processing. In the course of this work, we discovered two new mutants affected in splicing of specific plastid introns: op531, defective for splicing of ycf3 intron 2, and op572, defective for splicing of atpF and trnV. Other mutants including potential plastid translation mutants are under investigation to get a better understanding of the interplay between transcription, splicing and translation in chloroplasts.

S02-06 Knock-down of a putative chloroplast protein phosphatase 2A regulatory subunit B modulates light acclimation and pathogen resistance in Arabidopsis

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Serine/threonine protein phosphatase 2A (PP2A) family members play crucial roles in regulation of disease responses and cell death in animals and plants. PP2A consists of a catalytic subunit C, a scafold subunit A, and a highly variable regulatory subunit B, which modulates the activity and substrate specificity of the PP2A holoenzyme. We have explored the functional role of a putative chloroplastic PP2A regulatory subunit B’ (PP2A-B’) in the light tolerance and stress signaling in Arabidopsis thaliana. Knock-down pp2a-b mutants show stunted growth, delayed flowering and age-dependent formation of cell death lesions when grown under moderate growth light intensity. Intriguingly, all these characteristics become less pronounced when the pp2a-b plants grow under high light intensity or at low temperature. Under moderate light conditions, the cell death phenotype of pp2a-b mutant plants is accompanied by enhanced sensitivity to methyl viologen-induced photo-oxidative stress, increased accumulation of hydrogen peroxide, and constitutive activation of defence-related genes, including PR1 and PR5. Accordingly, the pp2a-b mutants also show enhanced resistance against the virulent bacterial plant pathogen Pseudomonas syringae pv tomato DC3000 in the leaves. Taken together, our results suggest that a chloroplastic PP2A is involved in the cross-talk of signaling pathways that modulate light acclimation and defense responses in plants.

S02-08 Hunting low-abundance proteins of plant peroxisomes: a combination of proteomic and computational approaches

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We have characterized a novel peptidosome, Presequence Protease PreP, in mitochondria and chloroplasts that is responsible for degradation of targeting peptides as well as other unstructured peptides that might be harmful to organellar functions (Glaser et al. 2006, Biol Chem, Johnson et al. 2006, EMBO J, Moberg et al. 2003, Plant J, Stähli et al. 2002, J Biol Chem). In A. thaliana there are two PreP paralogs, AtPreP1 and AtPreP2, both are dually targeted to mitochondria and chloroplasts and have overlapping substrate specificity (Bhushan et al. 2003, EMBO Rep, Stähli et al. 2005, J Mol Biol). Furthermore, PreP has been implicated in Alzheimer disease as it degrades amyloid-beta peptide (Falkevall et al. 2006, J Biol Chem). Particularly, PreP mutants show stunted growth, delayed flowering and age-dependent formation of cell death lesions when grown under moderate growth light intensity. Intriguingly, all these characteristics become less pronounced when the pp2a-b mutants plants grow under high light intensity or at low temperature. Under moderate light conditions, the cell death phenotype of pp2a-b mutant plants is accompanied by enhanced sensitivity to methyl viologen-induced photo-oxidative stress, increased accumulation of hydrogen peroxide, and constitutive activation of defence-related genes, including PR1 and PR5. Accordingly, the pp2a-b mutants also show enhanced resistance against the virulent bacterial plant pathogen Pseudomonas syringae pv tomato DC3000 in the leaves. Taken together, our results suggest that a chloroplastic PP2A is involved in the cross-talk of signaling pathways that modulate light acclimation and defense responses in plants.
To comprehensively understand interorganellar metabolic and signalling networks, low-abundance proteins of organelles must be identified. To this end, we are applying two complementary approaches, experimental proteomics and computational protein prediction. We established an innovative protocol for the isolation of highly pure leaf peroxisomes from Arabidopsis and analyzed the proteome gel-based and gel-free approaches. In collaboration with the Arabidopsis 2010 peroxisome project more than 70 novel proteins have been identified by now, many of which carried novel peroxisome targeting signals (PTS) type 1 or type 2. Peroxisome targeting has been confirmed for many novel proteins in vivo. Generation of an EST sequence set deriving from 1200 plant PTS1 proteins allowed the recognition of ten previously unrecognized PTS1 peptides. When fused to EYFP and expressed transiently in onion epidermal cells, all predicted novel PTS1s were shown to be functional. The recognition of the novel PTS1 peptides allows a significant extension of the plant peroxisomal protein database AraPerox. Algorithms have been deduced to predict the peroxisome targeting probability of unknown proteins from their primary sequence and are being optimized by site-directed mutagenesis combined with subcellular targeting analysis. The combination of proteomics and computational studies is thus suitable to comprehensively describe the low-abundance proteome of plant peroxisomes.

**PARALLEL SESSION 03: NATURAL VARIATION AND ADAPTATION**

**S03-01 Genetic hybrid incompatibilities among wild strains of Arabidopsis thaliana**

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Post-zygotic hybrid incompatibility or weakness, in which offspring of two parents show reduced viability or fertility, has been reported in many species. In most cases, following the theoretical model of Bateson-Dobzhansky-Muller, two or three interacting genes are involved. Despite extensive research, only a few causal genes have been identified so far, mostly in Drosophila. Recently, a gene causing hybrid necrosis, a type of post-zygotic hybrid incompatibility in plants, was isolated in Arabidopsis thaliana. This gene encodes a pathogen response gene and leads to environmentally regulated necrosis-like symptoms in F1 hybrids. A survey of hybrid necrosis in Arabidopsis accessions revealed that around 2% of the F1 hybrids show different degrees of necrosis, and in all tested cases, pathways implicated in pathogen response are induced. This suggests that autoimmunity could be a common mechanism for hybrid necrosis, which is a genetic barrier known from many plant species. To further characterize hybrid incompatibility in Arabidopsis thaliana, we are studying additional cases of F1 incompatibility. In addition, a survey of F2 hybrid incompatibility was performed to explore the different mechanisms that underlie hybrid weakness and could lead to gene flow barriers within species, which may be a first step leading to reproductive isolation in nature.

**S03-02 In search for salinity tolerance genes in the red alga Furcellaria lumbricalis**

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All organisms inhabiting the Baltic Sea are challenged by its profound south-north salinity gradient combined with an array of other environmental factors. The red alga Furcellaria lumbricalis originates from the marine rocky shores of the northern Atlantic Ocean (salinity 33 practical salinity units, psu) but can also be found in the Baltic Sea down to salinities of about 3.6 psu. Our aim is to link genetic variation, present in natural macroalgal populations, with a specific environmental factor (salinity) in order to discover genes important to salinity tolerance, to analyse population genetic variation in those genes, and to compare the evolution of neutral and adaptive genomic regions. Previous knowledge of neutral genetic variation in F. lumbricalis populations already exists. Therefore, an EST-library was constructed via high-throughput sequencing of cDNA prepared of two algal populations, one originating from the Atlantic Ocean and another from the Baltic Sea, subjected to extremely low salinity (6 psu) for 3 days in laboratory conditions. In this presentation, we show how the obtained gene expression data are being used for gene expression mining and marker gene discovery in F. lumbricalis.

**S03-03 Starch is a major integrator in the regulation of growth in Arabidopsis thaliana**

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Deeper understanding of the genetic, molecular and physiological determinants of plant growth is required to support breeding for higher plant growth rates. In this study, we investigated the relationship between growth and metabolism in the reference plant species Arabidopsis by profiling 48 metabolites and 24 enzyme activities involved in the primary metabolism across 94 genotypically-diverse natural accessions. Most metabolites and enzymes were negatively and positively correlated with growth, respectively. The strongest correlation (R = -0.54) and most significant (P = 2.3E-08) was for starch. PLS regression with cross-validation using the full set of metabolites including starch gave only a small improvement for the prediction of biomass (R = 0.57, explaining 32.5% of the total variance) compared to the result obtained with starch alone. Adding enzymes activities did not allow a real improvement for the prediction of growth, most likely because they were highly correlated each others, constituting a very dense network, and then could not add additional information to the model. Using metabolites to predict starch and biomass revealed that the best metabolic predictors were almost identical, suggesting that starch integrates the overall metabolic response in the aerial rosette and then constitute the main determinant for the regulation of growth. Such result suggests that altering carbon partitioning in plants could lead to an improvement of growth rates.
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S03-04 Gibberellins in the control of shade-avoidance response in *Brassica juncea* (L) Coss.: hypocotyl elongation and population genetic variability
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There is a number of photosensory systems in plants that allow them to sense neighbours and compete for light. First, plants respond to the changed spectral quality of the filtered/reflected light within coenosia. Gibberellins (GAs) are ubiquitous diterpenoid phytohormones required for the transduction of photomorphogenetic signals. Series of GAs which belong to either the early-13-hydroxylation pathway or early-non-hydroxylation pathway were identified by combined gas chromatography – mass spectrometry in the hypocotyls of *Brassica juncea* (L.) Coss., plants, suggesting that GA1 and GA4 are endogenous physiologically active forms. Light conditions imitating shade environment (low red:far-red ratio) triggered the dramatic increase of GA4 content in the seedlings, while GA1 and GA20 (precursor of GA1) contents were independent of light conditions. In hypocotyl elongation bio-assay, an increased plant sensitivity to exogenous GA4 was observed as compared to GA1. These data provide evidence for the crucial role of GA4 in the control of initial shade-avoidance response. Determinations of GA content and hypocotyl growth responsiveness to exogenous GAs in the plants of self-pollinated lines representing biotypes from several *B. juncea* varietal populations have shown their significant variability. This provides population heterogeneity and biotype biochemical adaptation to the changing environment resulting in their developmental rhythm plasticity.

S03-05 Ecophysiological factors of survival of herbaceous species during the winter period
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During the last 40 years the wintering perennial plants (representatives of family Poaceae, genera *Medicago*, *Tulipa*, and others) in the central regions of Russian Federation are subjected to severe low temperature stresses due to the changes in global and local ecological factors. The dynamics of morphological and physiological state of mint rhizomes (*Mentha arvensis* and *Mentha piperita*), tulip bulbs (*Tulipa biflora* Vved.) and tillering nodes of cereals (*Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum aestivum* and *Triticum vulgare* cv. *Triticale* and *Triticum vulgare*) during the winter period was studied. The significance of the preservation of shoot and root apical meristems in viable state and the role of epigenetic factors for plant survival was shown. The high activity of carbohydrate and phytohormone metabolism in the subterranean organs of these herbaceous species was shown. The dynamics of fructan pools, cytokinin/abscisic acid and soluble carbohydrate/starch ratios reflected the thaw phenomena during the winter. The results of evaluation of tolerance to winter stresses and snow mold of plastid apparatus of perennial wheat varieties will be presented. The selected intergeneric hybrids of crop species possessing a high tolerance to abiotic and biotic stresses during their life cycles will be characterized. This work was supported by Department of Biological Sciences of RAS (Program ‘Fundamental bases of management of biological resources’).

S03-06 Global patterns in photosynthesis: an adaptation to growing season length – story based on *Populus*
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The cline towards increased photosynthesis with latitude may be a generalized phenomenon among deciduous trees in northern hemisphere. Guy et al. (2008) recently reported that light-saturated photosynthetic rates increased with latitude of origin in *Populus trichocarpa*. This display of ‘latitudinal compensation’ in tree populations may reflect adaptive, genotypic changes, but careful experimental work is necessary to distinguish genetic differences from other effects such as acclimatization. Using *Populus balsamifera* populations’ native to North America, pre-adapted to different climatic conditions: we demonstrate that northern provenances have inherently high photosynthetic rates to compensate for shorter growing seasons. Work is in progress in *Populus tremula* originating from different provenances across Sweden. Carbon isotopic composition in leaf dry matter and leaf nitrogen, were measured, along with photosynthesis, to study the variability of primary productivity using twenty-one populations of balsam poplar. Indeed, under an extended photoperiod in the greenhouse, where free growth is maintained, the fastest growing balsam poplar is from the far north. During free growth in the greenhouse, plant height was positively correlated with latitude of origin. Although trees representative of northern populations generally do not grow as much as those from the south over any given summer, they in fact possess higher photosynthetic rates if measured at the height of summer.

S03-07 Molecular evolution of inflated calyx syndrome (ICS) in Withania
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The project deals with the evolution of morphological novelties in Withania, a member of Solanaceae. In the genera Withania and Physalis, sepalcs resume growth after pollination to encapsulate the mature fruit, thus forming the ‘Chinese lantern’, a trait also termed inflated-calyx syndrome (ICS). Formation of ICS and Physalis, sepals resume growth after pollination to encapsulate the mature fruit, thus forming the ‘Chinese lantern’, a trait also termed inflated-calyx syndrome (ICS). Formation of ICS require heterotic expression of MADS-box transcription factor MPF2 as well as signals that are dependent upon pollination. Tubocapsicum a sister to Withania is devoid of ICS. Hormones, like gibberellins and cytokinins, have been shown to trigger ICS formation on de-pistillated flower buds, even prior to pollination in Withania. Phylogenetic analyses have revealed a duplication of MPF2-like gene in Withania. Not only the coding sequence but also the promoter sequences are highly divergent in Withania duplicates and Tubocapsicum. In addition, a...
300 bp conserved region has been identified in the first intron of these genes with 8 bp auxin response factor binding (ARF) site. Promoters with conserved intronic sequences have been tested for functionality with Promoter::GUS and Promoter::YFP fusion constructs transiently in Nicotiana and stably in Arabidopsis. To study their functional divergence, both the genes have been over expressed in Arabidopsis.

The origin of morphological novelty is a long-standing question in biology. The Solanaceae plants feature richful calyx diversity. Some of these plants have one inflated calyx syndrome (ICS). The MADS-box protein MPF2, together with the plant hormones cytokinin and gibberellin, has been shown to be responsible for this trait in Physalis floridana. Here we investigated the diversity of ICS kinin and gibberellin, has been shown to be responsible for this trait in Physalis floridana. We showed that the ICS trait seems to be of multiple origins both within the Solanaceae and the Physaleae. Surprisingly, expression of MPF2-like genes in floral organs appears to be plesiomorphic in both the Physaleae and the Capsiceae. Some species in these tribes that show neither ICS nor calyx accrescence fail to express the MPF2-like gene in floral organs. All those species (including at least Capsicum, Lycianthes, Tubocapsicum, Witheringia and Vassobia) that do express this gene in the calyx are forming small calyces but not responding to external hormones. The plesiomorphic nature of MPF2-like gene expression in the calyx of the Physaleae and Capsiceae raises the possibility that originally ICS also was actually a plesiomorphic character in these 2 groups. However, by comparing the MPF2 and its homologous genes we could show that gene duplication via polyploidization and hereafter gene loss and recombination that possibly leading to a promoter switch could also play essential roles during the evolution of ICS.

S03-08 Molecular evolution of the inflated calyx syndrome (ICS) in Solanaceae
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The recent completion of the sequencing of the genome has opened the way for detailed study of the functions of genes involved in both development and metabolism.

S04-02 The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants
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We report the draft genome sequence of the model moss Physcomitrella patens and compare its features with those of flowering plants, from which it is separated by more than 400 million years, and unicellular aquatic algae. This comparison reveals genomic changes concomitant with the evolutionary movement to land, including a general increase in gene family complexity; loss of genes associated with aquatic environments (e.g. flagellar arms); acquisition of genes for tolerating terrestrial stresses (e.g. variation in temperature and water availability); and the development of the auxin and abscisic acid signaling pathways for coordinating multicellular growth and dehydration response. The Physcomitrella genome provides a resource for phylogenetic inferences about gene function and for experimental analysis of plant processes through this plant’s unique facility for reverse genetics.

S04-03 Gene targeting
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Gene targeting is the delivery of transforming DNA to a predetermined site in a genome. The integration of transgenes occurs via mechanisms utilized for the repair of double-strand breaks in DNA, and the nature of transgene integration thus reflects the organism’s preferred pathway for DNA repair. There are two principal routes for DNA repair: the non-homologous end-joining (NHEJ) pathway and the homology-dependent pathway (commonly termed homologous recombination: HR). Uniquely among model plants, Physcomitrella patens uses the HR-DNA repair pathway resulting in efficient targeted integration of transforming DNA containing short homologous targeting sequences (up to 100% of stable transformants). Physcomitrella thus enables ‘reverse genetic’ analysis of plant genes either by construction of ‘gene knockouts’ or by more sophisticated genome engineering, including the construction targeted ‘knock-in’ mutants, incorporating in-frame reporter genes or epitope tags, and site-directed mutagenesis of specific loci to alter as little as a single base-pair. By virtue of its preference for HR-mediated transgene integration, Physcomitrella is a powerful model to study the mechanism of homology dependent DNA repair in plant cells. The analysis of targeted knockouts demonstrates the requirement for conserved DNA repair genes, whilst the potential for mutagenic interrogation of the DNA repair process should enable the identification of as-yet uncharacterised plant-specific components.

Abstracts
Abstracts

S04-04 Four distinct classes of small RNAs in Physcomitrella
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MicroRNAs have been extensively documented in multiple plant species. However, the vast majority of expressed small RNAs in plants are not microRNAs. These other small RNAs are much less well understood, especially outside of Arabidopsis thaliana. In Physcomitrella patens we found that many of the most prolific small RNA producing loci made 21, 23, and 24nt siRNAs in a strikingly consistent ratio. These predominantly intergenic loci were significantly enriched in transposon content. Similar to 24nt siRNA loci in A. thaliana, these loci had large sizes, a depletion in overlap with genes, and dense concentrations of 5-methyl cytosine. Similar small RNA producing loci were not apparent in the unicellular green alga Chlamydomonas reinhardtii. These data suggest that a specialized class of siRNAs directed against diverse intergenic regions and correlated with methylated cytosine is specific for land plants but absent in C. reinhardtii. It is likely that this intergenic siRNA system largely functions to repress inappropriate transcription. Combined with the previously described microRNAs, trans-acting siRNAs, and a heterogenous class of 21nt-producing small RNA loci, we have described a total of four distinct classes of small RNA genes in P. patens.

S04-05 Photoreceptors and their downstream targets for chloroplast movement in Physcomitrella patens
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Red light-grown protonemata of Physcomitrella patens show chloroplast accumulation along cross walls between two protonemal cells when irradiated with polarized light vibrating parallel to the cross walls or at an area irradiated with a microbeam of red or blue light (Kadota et al. 2000). The blue light receptors are found to be phototropins (photA1, A2, B1 and B2). PhotA involves mainly for the avoidance response but phot B and partly photA involved accumulation response (Kasahara et al. 2004). Simultaneous irradiation with red and far-red light nullified chloroplast movement, indicating the involvement of phytochrome as a red light receptor (Kadota et al. 2000). The knockout lines of photA1, photB1 and photB2 lack red light-induced chloroplast movement as well as blue light induced one, suggesting that the red light signal emitted by phytochrome must be transferred to a phototropin signal transduction pathways (Kasahara et al. 2004). Recently phytochromes mediating chloroplast avoidance response were identified as phy1, phy2 and phy3, but not phy4 using protoplast cells obtained from white light-grown protonemata (Uenaka and Kadota 2007). Unfortunately, accumulation response could not be induced in this system, so that molecular species of phytochrome for accumulation response are not yet identified. Downstream of the signal transduction pathways will be discussed.

S04-06 Evolution of land-plant growth regulatory mechanisms
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The DELLA proteins (DELLAs) are a subfamily of the plant-specific GRAS family of putative transcriptional regulators that regulate angiosperm growth in response to the phytohormone gibberel-lin (GA). The DELLAs restrain growth, and GA promotes growth by opposing DELLA function. Essentially, GA binds to a specific GA-receptor protein (GID1), thus stimulating a GID1-DELLA protein-protein interaction. This interaction itself promotes specific targeting of DELLAs for destruction in the proteasome via the SCFSLY1 E3 ubiquitin ligase. Additional signalling pathways, such as those associated with phytohormones other than GA, and environmental variables such as light, temperature and nutrient status, also influence angiosperm growth via effects on the GA-DELLA growth-regulatory mechanism. The concept that the DELLAs are integrators of multiple angiosperm growth-regulatory signalling inputs will be explored, and, using example studies from the bryophyte Physcomitrella patens and the lycophyte Selaginella kraussiana, the question of how the GA-DELLA growth-regulatory mechanism arose during land-plant evolution will be explored. Recent publications: Achard et al. (2006), Science 311: 91–94, Achard et al. (2007), Plant Physiol 143: 1163–1172, Achard et al. (2007), Proc Natl Acad Sci U S A 104: 6484–6489, Yasumura et al. (2007),Curr Biol 17: 1225–1230.

PLINARY LECTURES ON TUESDAY, 19 AUGUST 2008

L07 Auxin transport – developmental output of subcellular dynamics
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Auxin is a prominent intercellular signal in plants. Directional, active, cell-to-cell auxin transport mediates local auxin gradients, which are required for various patterning processes including apical-basal axis formation, organogenesis and tropisms. The chemiosmotic hypothesis postulates that auxin transport is accomplished by the action of auxin influx and efflux carriers, which are localized at the plasma membrane of transporting cells. Genetic approaches in Arabidopsis thaliana identified candidate genes coding for regulators of auxin influx including plant-specific plasma membrane PIN proteins. PIN proteins show dynamic asymmetric subcellular localization, which correlates with and determines direction of auxin flow. In turn, auxin distribution itself is regulating PIN occurrence at the plasma membrane and PIN polarity. In addition, other internal as well as external signals can modulate endocytic recycling-based PIN localization and thus directional auxin signalling. We will provide new insights into the mechanism of auxin transport, PIN polar targeting and auxin-dependent regulation of plant development. Supported by VolkswagenStiftung, EMBO Young investigator program and Odysseus program of FWO.

L08 The mechanics behind plant development, a pluridisciplinary view
Y. Couder†, M. Heisler‡, H. Jönsson§, O. Hamant*, E. Meyerowitz* and J. Traas†
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During the development of multi-cellular organisms the regulators of growth and patterning must somehow interfere with physical processes to generate specific shapes. How this is achieved, i.e. how molecules assemble into complex systems with a particular form is not known in any organism. Here, we address this central issue in developmental biology using the shoot apical meristem in the higher plant Arabidopsis. The shoot apical meristem is a population of stem cells which continuously generates aerial organs and to do so undergoes complex shape changes. Using a combination of physical, mathematical and biological approaches we provide evidence for a model where molecular networks would be uncoupled from the control of overall differential growth patterns, associated with the rapid outgrowth of organs at particular location. Using mechanical models we show that this hypothesis is sufficient to explain all morphogenetic processes observed at the shoot meristem.

L09 Early events in gibberellin signaling
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Plant hormone gibberellin (GA)-induced growth and development is modulated by DELLAs, proteins which are major repressors of GA signaling. Recent studies demonstrated that GA, upon binding to its receptor, de-represses its signaling pathway by targeting DELLAs for rapid degradation, via the ubiquitin-proteasome pathway. The nuclear-localized DELLAs may function as transcriptional regulators, which control target gene expression via interaction with other transcription factors. Several DELLA targets in Arabidopsis were identified by microarray and chromatin-immuno-precipitation studies.

L10 The molecular basis of vernalization in Arabidopsis
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The Dean laboratory is studying the importance of prolonged cold (winter) for flowering, a process known as vernalization. We have used a molecular genetic analysis in the model plant Arabidopsis thaliana to identify genes involved in determining both the ability to vernalize and the need for vernalization. The pathways we study share a common downstream target, a gene encoding the repressor of flowering, FLC. Mutants attenuating the vernalization response revealed FLC expression is silenced during the cold and this repression remains epigenetically stable through the rest of the plant life-cycle. A Polycomb-based chromatin regulation involving conserved regulators and PHD finger proteins mediates this epigenetic silencing. The autonomous floral promotion pathway affects the need for vernalization. Recent work suggests this pathway links RNA processing, RNAi machinery and chromatin regulation to cause the down-regulation of FLC. It has also been shown to play widespread roles in the epigenetic silencing of the Arabidopsis genome. Functioning antagonistically to both the autonomous pathway and vernalization, FRIGIDA (FRI) causes plants to overwinter in the vegetative state by up-regulating FLC expression. The talk will describe our current understanding of these pathways and how they have changed in Arabidopsis variants adapted to different climates.

L11 Monitoring and manipulating information flow at the host/pathogen interface
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Plant pathogens use small molecules and also proteins to render their hosts susceptible. Several pathogens either make plant hormones, or perturb host hormone signalling networks by other means. To counteract pathogen activation of auxin signalling, plants induce microRNA miR393 that targets the TIR1 auxin receptor, to attenuate auxin sensitivity. Furthermore, gibberellin made by fungal necrotrophic pathogens leads to attenuation of the defence response to necrotrophs by interference with jasmonic acid signalling. In addition, many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial T3SS secretion system to investigate effectors from filamentous pathogens such as oomycetes. I will report recent data on oomycete effector functions and the use of the Solexa/Illumina sequencing instrument to advance oomycete genomics.

L12 Biochemical, genetic, and genomic dissection of defense signaling pathway
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Induction of systemic acquired resistance (SAR) involves salicylic acid (SA)-mediated transcriptional reprogramming. Genomic and genetic analyses performed in Arabidopsis showed that this process requires the function of the master regulator NPR1 protein. Similar to the mammalian immuno-regulator NF-kB, the NPR1 protein is nuclear translocated upon induction. The translocation of NPR1 is regulated by cellular redox changes triggered by the defense signal molecule SA. In the nucleus, NPR1 acts as a transcription cofactor affecting the expression of over 2000 genes. Using biochemical, genetic and genomic approaches, we study the regulation of NPR1 cytoplasmic/nuclear partition, the transcriptional activity as well as the target genes of NPR1. Progress in these studies will be reported.
Abstracts

PARALLEL SESSIONS ON TUESDAY,
19 AUGUST 2008

PARALLEL SESSION 05: STRESS AND ACCLIMATION; BIOTIC

S05-01 Role of protein quality control in the ER for MAMP-triggered immunity in Arabidopsis

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Plants detect microbe-associated molecular patterns (MAMPs) such as flagellin and EF-Tu (their derived peptides flg22 and elf18, respectively) and trigger innate immune responses, designated MAMP-triggered immunity (MTI). Perception of flagellin and EF-Tu requires their cognate receptors FLS2 and EFR, respectively. We have observed that sucrose/UVB-induced flavonoid accumulation, a characteristic response to these abiotic stresses, is abolished in young seedlings in the presence of flg22 or elf18. Using this readout, we have taken a genetic approach to identify components required for MTI and repression of abiotic stress-induced flavonoid accumulation. Our mutational screens have revealed ‘priority in sweet life’ (psl) mutants that show sucrose/UVB-induced flavonoid accumulation in the presence of elf18, but not flg22. This indicates separate genetic requirements for the FLS2- and EFR- signaling pathways. These psl mutants are classified into at least 5 independent complementation groups, of which one comprises novel efr alleles. The mutants are impaired in characteristic MTI-associated events e.g. a rapid oxidative burst, MAPK activation and callose deposition, and show super-susceptibility upon challenge with virulent Pseudomonas syringae. Cloning of PSL genes has defined components of the endoplasmic reticulum (ER) chaperon system that ensures proper folding of glycoproteins as critical factors for EFR function. We will further present an overview of our genetic studies.

S05-02 Control of EFR function by a set of ER proteins in plant innate immunity

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The Arabidopsis leucine-rich repeat receptor kinase (LRR-RLK) EFR is the pattern recognition receptor (PRR) which recognizes the bacterial pathogen-associated molecular pattern (PAMP) EF-Tu. EFR activation triggers a set of rapid defence responses including production of reactive oxygen species (ROS), deposition of callose, expression of numerous defence-related genes, and contributes to disease resistance against bacteria (Kunze et al. 2004, Zipfel et al. 2006). Little is known about PRR signal transduction in plants. Although many phenomena have been correlated with PAMP treatment, the order of events and the mechanisms required have not been subjected to genetic analysis. Here, we report the identification by forward genetic approach of three ER-localised Arabidopsis proteins required for EF-Tu responses. We will present a detailed physiological and biochemical analysis of the corresponding mutants and discuss the potential role of these genes in controlling EFR function. This work is supported by the ERA-PG program and the Gatsby Charitable Foundation.


S05-03 The plant innate immune system, role of phospholipid and oxylipin

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Bacterial pathogens deliver type III effector proteins into the plant cell during the course of infection. In susceptible (r) hosts, type III effectors contribute to virulence, but in the case of resistant host-plant (R), they betray the pathogen to the plants surveillance system. Recognition induces a complex suite of cellular and molecular events comprising plants inducible defense. As plant recognition of bacterial Avr proteins occurs in the cytosol, the response can be mimicked using a transgenic system. The gene encoding the bacterial type III effector AvrRpm1 of Pseudomonas syringae was introduced as a chemically inducible construct into Arabidopsis. Recognition of AvrRpm1 was found to cause the sequential activation of two phospholipases, C and D. Inhibition of the phospholipases inhibited defence responses. Recognition of AvrRpm1 also caused oxylipin accumulation. The 13-LOX products 12-oxo-phytodienoic acid (OPDA) and dinor-oxo-phytodienoic acid (dino-OPDA) were the most prominent oxylipins. Interestingly the majority of the OPDA and dino-OPDA (>90%) were found to be esterified a novel galactolipid, Arabidopside E. This substance accumulated to surprisingly high levels, 7–8% of total lipid content after recognition of AvrRpm1 and was shown to inhibit growth of both bacterial and fungal pathogens in vitro. In summary, our data supports that PA as well as oxylipins are integral and necessary components of the plant innate immune system.

S05-04 Role of pectin degradation in plant-pathogen interactions

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The cell wall is the first barrier opposed by plants to invading microorganisms, and plant pathogens have evolved a wide array of enzymes to degrade cell wall structural components. Among these enzymes, polygalacturonases (PGs), which hydrolyze homogalacturonan (HG), a major component of primary cell wall, are major virulence factors in several fungal pathogens. Oligogalacturonides (OGs), fragments derived by the partial hydrolysis of HG, are able to activate defense responses in plants. In Arabidopsis, OGs induce defense gene expression and increase resistance to B. cinerea independently of the signaling molecules salicylic acid (SA), jasmonic acid (JA) or ethylene, suggesting that other signals mediate elicitor-activated responses. We have analyzed the role of the oxidative burst in OG-dependent early and late responses. H2O2 and callose accumulation in response to OGs require the NADPH oxidase AtbohD. However, both early gene expression and induced resistance to B. cinerea are independent of the oxidative burst. To further investigate the role of pectin degradation in plant defense, we have also overexpressed a fungal PG in tobacco and Arabidopsis plants. These plants have reduced HG content, constitutively express defense responses and are more resistant to B. cinerea, suggesting that modification of pectin integrity is perceived by plant cells as a warning signal during pathogen infection.

**S05-05 Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph Ustilago maydis**


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Ustilago maydis, the causal agent of corn smut disease, establishes a complex biotrophic interaction with its host plant maize. Hallmarks of the disease are large plant tumors in which fungal proliferation occurs. Confocal microscopy, global expression profiling and metabolic profiling showed that U. maydis is recognized early and triggers massive defense responses. With beginning of the biotrophic phase, many of the early response genes are down-regulated whereas several genes associated with cell death suppression are induced. The interplay between fungus and host involves changes in hormone signaling, induction of antioxidant and secondary metabolism, as well as the prevention of source leaf establishment. To compare the compatible U. maydis/maize interaction with a nonhost-interaction, we also performed expression profiling of maize leaves after infected with the barley pathogen Ustilago hordei. For a rapid functional characterization of the candidate genes identified in our expression studies, we have established virus based systems for systemic silencing and over-expression in association with U. maydis infection. The presented, complementary approaches allow description of maize expression programs that are altered by fungal pathogens and are expected to enable identification of pivotal components for the establishment of a biotrophic interaction.

**S05-06 Effects of a plant growth-promoting rhizobacteria (PGPR) strain on Arabidopsis thaliana N nutrition**

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PCPR may, a priori, enhance the growth rate of a plant either directly (phytostimulation) or indirectly via improved mineral, especially N, nutrition. Conversely, the changes in plant growth would affect N assimilation, so that it is difficult to identify the primary effects of PCPR. Phyllobacterium brassicaeae STM196, a PGPR strain isolated from field-grown canola, was not able to rescue nitrate reductase (nrt1/nit2) mutants when grown on a nutrient medium where nitrate was the unique N form, indicating that this PGPR did not provide significant amounts of ammonium to plants. Transcriptome analysis using CATMA microarrays allowed us to identify STM196-responsive genes at low to high external nitrate concentration. Expression level changes of genes selected for their role in N assimilation were confirmed using quantitative RT-PCR. Knock-out mutants’ analysis showed that neither of the putative nitrate transporters NRT2.5 and NRT2.6 plays a role in nitrate uptake or root to shoot transport. Both of them are likely involved in nitrate-mediated regulatory processes of N metabolism (N pools, N assimilation genes expression) and root development (lateral root growth, root hair elongation). All together, the effects of PGPR on plant N nutrition and development appear very intricate, involving probably the elicitation of common signaling pathways.

**S05-07 Polyphenolic compounds on leaves limit iron availability and affect bacterial epiphytic growth**

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Polyphenolic compounds produced by plants can chelate iron, reducing its bioavailability to plant-associated bacteria. To acquire iron from limited sources, bacteria may produce siderophores. Concentration of phenolics in methanolic leaf extracts varied appreciably among plant species (nearly 5-fold greater in Pelargonium hortorum compared to Phaseolus vulgaris). Tannin concentration was generally proportional to the leaf phenolic content, amounting to 85% in species with high total phenolics. Both, stimulation of siderophore production in Pseudomonas syringae strain B728a and inhibition of growth of isogenicould strain deficient in pyoverdine production, were associated with plants of high phenolic concentration. Both strains exhibited similar growth on P. vulgaris leaves, while populations of I-1 were much lower than of B728a.
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on detached leaves of plants with high tannin content. Application of FeCl3 on P. hortorum reversed growth deficiencies of strain 1-1, while application of tannin on P. vulgaris inhibited growth of 1-1 but not of B728a. This provides evidence that ample iron availability counterbalances the inhibitory effect of tannins, otherwise contributing to limitation of growth of siderophore deficient strains. Consequently, the ability to acquire iron from insoluble sources such as tannin-iron complexes may be a prerequisite for microflora to inhabit plants with substantial tannin concentrations, thus imposing another limiting factor on plant-microbe interplay.

S05-08 Syntaxin has opposing regulatory functions in pathogen defence
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Plant disease resistance is the result of the collective activity of separate defence mechanisms. We have previously discovered that the syntaxin gene SYPI21 (PEN1) in Arabidopsis is required for penetration resistance to powdery mildew fungi. SYPI21 is necessary for vesicle trafficking leading to formation of papillae, which are local cell wall appositions functioning as barriers against fungal penetration. In an attempt to understand SYPI21’s function more precisely, we have applied vesicle trafficking inhibitors and discovered an importance of endocytosis in penetration resistance. Interestingly, the data indicate that endocytosis-mediated resistance is SYPI21-dependent.

SYPI21 has a separate function as negative regulator of other defences, which it shares with SYPI22. This is reflected in the syntaxin double mutant syp121 syp122 that has a lesion mimic phenotype. Introducing knock-out mutations in a number of well-known defence pathways partially rescues the lesion mimic phenotype. This shows that these pathways are active in syp121 syp122. Reversal of the syp122 mutation has led to isolation of a large number of triple mutants with improved performance. The third mutations that have occurred in SUPPRESSOR OF SYNTAXIN-RELATED DEATH (SSD) genes, a number of which we have positionally cloned. The phenotype of quadruple mutants, combining pairs of ssd mutations in syp121 syp122, allows us to study genetically how defence signalling genes interact in a network.

S05-09 Nuclear localization of the Arabidopsis immune receptor RPS4: exploring a link to defense gene activation
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Intracellular resistance (R) proteins of the TIR-NB-LRR class recognize pathogen effector proteins or the consequence of their actions inside the host cell. Effector recognition typically leads to generation of reactive oxygen species, localized programmed cell death and restriction of pathogen colonization. TIR-type NB-LRR receptors specifically require the defense regulator EDS1 to confer resistance. Arabidopsis EDS1 protein localizes to the cytoplasm and the nucleus and functions together with salicylic acid (SA) to induce local and systemic immunity. We focus on the TIR-NB-LRR receptor RPS4 that recognizes the Pseudomonas syringae effector AvrRps4 in order to understand how TIR-type receptors molecularly connect to the activation of EDS1/SA signalling. RPS4 distributes between a non-nuclear membrane compartment and the nucleus but requires nuclear localization for defense gene activation (Wirthmüller et al. 2007). A recently discovered interaction between an NB-LRR receptor and a WRKY transcription factor (Shen et al. 2007) indicates that some R proteins associate with components of the transcriptional machinery. We report that although RPS4 lacks conserved DNA binding domains the protein can be readily cross-linked to DNA by formaldehyde. Preliminary results suggest a positive correlation between RPS4 activity and the amount of DNA-associated RPS4. We will report on our analysis of nuclear RPS4 protein (complexes) and a screen for putative DNA target regions.

S05-10 Two sides of a leaf blade: Blumeria graminis needs chemical cues in cuticular waxes of Lolium perenne for germination and differentiation
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The lower, abaxial Lolium leaf surface is extremely glossy and wettable compared to the glaucous and more hydrophobic upper, adaxial surface. Earlier investigations have demonstrated that the abaxial leaf surface was rarely infected by powdery mildew (B. graminis) even when the adaxial surface was densely colonized. This led to the assumption that components of the abaxial epicuticular leaf wax might contribute to the impairment of growth and development of B. graminis conidia on abaxial surfaces of several Lolium species (Carver et al. 1990). To reassess this hypothesis, we analyzed abundance and chemical composition of L. perenne ab- and adaxial epicuticular wax fractions. While the adaxial epicuticular waxes were dominated by alcohols and esters, the abaxial fraction was mainly composed of n-alkanes and aldehydes. However, the major germination and differentiation inducing compound, the C26-aldehyde hexacosanal, was not present in the abaxial epicuticular waxes. Spiking of isolated abaxial epicuticular Lolium waxes with synthetically produced hexacosanal allowed reconstituting germination and differentiation rates of B. graminis i.e. the rorhléi was studied in an in vitro germination assay using wax covered glass slides. Hence, it appears to be primarily the absence of hexacosanal from the abaxial leaf surface that is responsible for the failure of normal germination development of B. graminis on the lower leaf surfaces of L. perenne. Implications of surface hydrophobicity will be discussed.

PARALLEL SESSION 06: DEVELOPMENT; VEGETATIVE

S06-01 Regulation of auxin transport
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Active transport of the essential signaling molecule auxin is essential for plant physiology and development. Many aspects of these
are controlled by cell-to-cell or polar auxin transport, which is primarily determined by auxin efflux complexes, characterized by PIN and ABCB (PGP/MDR) auxin exporters. Here, we will summarize recent biochemical and genetic studies indicating that both types of proteins appear to act independently but— at least in certain cell files—perform specific interactions that determine the specificity and direction of auxin efflux. Moreover, we summarize recent progress of ABCB interaction with immunophilin-like FKBP42, TWISTED DWARF1, which functions as a sensor in ABCB-mediated auxin transport. Our data suggest that a combined action of several components forming an auxin efflux complex is needed for the establishment and control of asymmetric auxin fluxes.

S06-02 A repressor of auxin biosynthesis modulates gradient-directed planar polarity in Arabidopsis roots
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During development of multicellular organisms, the polarities of single cells commonly need to be coordinated within the plane of a single tissue layer. This phenomenon is referred to as planar polarity, though underlying mechanisms differ between plant and animal tissues. The root epidermal layers of different plants including Arabidopsis display a plant-specific planar polarity of hair positioning. Root hairs emerge close to basal ends of hair-forming cells directed towards a concentration maximum of the hormone auxin in the root tip. How regulation of this gradient coordinates planar polarity is not well understood. Here, we unequivocally demonstrate that the Raf-like kinase CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) acts as a dosage-dependent repressor of a local auxin biosynthesis maximum that modulates planar polarity over long distances. By employing mutations differentially affecting CTR1 kinase activity, combined with multiple mutant analyses of auxin biosynthesis, transport and efflux carrier trafficking mutants, we show that planar polarity relies on influx and efflux carrier-dependent auxin redistribution from the biosynthesis maximum. Direct auxin-biosynthesis-rate and concentration-gradient measurements in diverse mutant combination support a model in which CTR1 acts as an endogenous dosage-dependent repressor of auxin biosynthesis modulating long-range auxin action on planar polarity.

S06-03 Control of root vascular patterning: an interplay between PHABULOSA, SHORT-ROOT and cytokinin signalling
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The arrangement of conductive tissues, the vascular pattern, in plants is under the regulation of a group of transcription factors and plant hormones. The spatial domain of cytokinin hormone action is specified by interaction with the negative regulator AHP6 and the activity domain of class III HD-ZIP transcription factors is specified by a set of microRNA species (miR165/166). Until now little is known about how these miRNA and hormonal pathways are spatially regulated and how they are interacting. We show that class III HD-ZIP genes are required for cell fates at the epicenter of the root. Ectopic expression of one of them, PHABULOSA (PHE), results in ectopic central cell fates at the periphery of the root vascular bundle. Conversely, loss of function results in ectopic peripheral vascular cell types in the centre. The peripheral cell fates are achieved by down regulating PHB through miRNAs, the expression of which requires another transcription factor, SHORT-ROOT (SHR), active outside the vascular bundle. Furthermore, we show that the HD-ZIP IIIIs are required to restrict the spatial domain of AHP6 thus integrating with cytokinin regulation of vascular development. Our results reveal a network of miRNA, transcriptional and hormonal factors specifying morphogenetic gradients regulating vascular cell fates.

S06-04 Regulation of xylem cell death by polyamines and ethylene
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The final stage of xylem development is the programmed cell death (PCD). We have identified some of the typical morphological hallmarks of PCD, such as DNA degradation and vacuolar rupture, in the xylem elements of poplar trees and in the in vitro induced vessels of Zinnia elegans by the DNA diagnostic methods TUNEL and COMET and by electron microscopy. Analysis of the xylem PCD transcriptome showed that the poplar spermine synthase ACL5 was specifically expressed in the early differentiating xylem vessels. A functional analysis revealed excessive formation of protoxytem-like vessels in the Arabidopsis acl5 mutant. Our results in both Arabidopsis and Zinnia suggest that the overproliferation of the protoxylem elements is due to inappropriate control of the lifetime of the cells and that the function of ACL5 is to delay cell death to allow appropriate maturation of the xylem vessels. We also obtained evidence for a critical role of ethylene in regulation of xylem maturation. In the Zinnia elegans in vitro system, inhibitors of ethylene signaling blocked both the secondary cell wall formation and the cell death of the vessel elements. Similarly in Arabidopsis, defective xylem maturation was apparent in xylem fibers of the dominant ethylene-insensitive ein4-1 mutant. We have also identified some of the target genes possibly involved in the ethylene-mediated xylem maturation in subtracted cDNA libraries of Zinnia.

S06-05 Molecular control of secondary growth initiation in the Arabidopsis shoot
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One of the most important renewable energy resources is wood. Plants produce wood based on the activity of a cylindrical meristem, the vascular cambium, located at the periphery of growth axes. Arabidopsis generates a vascular cambium in the shoot by establishing meristematic activity between primary vascular bundles. The resulting interfascicular cambium connects fascicular cambia of vascular bundles, generating a closed cambial cylinder. Surprisingly, in spite of its significance for plant development and the production of biomass, knowledge about the molecular control of secondary growth initiation is very limited.

Here, we study the regulation of auxin accumulation in interfascicular regions, a process essential for the initiation of cambial activity. Genetic and in vitro analyses suggest that auxin transported basipetally along the stem accumulates at the stem base due to limitations of auxin transport capacities and, subsequently, cell divisions are initiated. PIN3, an auxin efflux facilitator, contributes to the basipetal auxin transport and is specifically expressed in the starch sheath of primary stems. We show that PIN3 protein levels are reduced in the starch sheath during the transformation to secondary growth and that this reduction is based on a post-transcriptional regulation of PIN3 protein abundance. This suggests that auxin accumulation and secondary growth initiation can be positively regulated by a reduction of PIN protein levels in Arabidopsis shoot.

PARALLEL SESSION 07: PLANT BIOTECHNOLOGY

S07-01 Cost-effective production of a vaginal protein microbicde to prevent HIV transmission
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Almost 40 million HIV-infected people were reported in 2006. Novel therapeutic strategies are urgently needed for use with conventional strategies to prevent HIV spread. A series of small-molecule microbicides developed for vaginal delivery to prevent heterosexual HIV transmission have had disappointing results in human clinical trials. Protein-based microbicides, such as HIV-specific monoclonal antibodies, have been considered as an alternative approach. Despite their promising safety profile and efficacy, the major drawback of such molecules is the economy of large-scale production in mammalian cells, the current system of choice. Here we present an alternative biomanufacturing platform for one of the most promising anti-HIV antibodies, 2G12. We show high-level, endosperm-specific production of 2G12 in maize at 75 µg/g dry seed weight in T0, T1 and T2 transgenic plants; and to >100 µg/g following a dedifferentiation-regeneration cycle using immature zygotic embryos. The N-terminal protein sequences of purified maize 2G12 were identical to its CHO equivalent; although glycan structures were distinct, the two intact antibodies were almost indistinguishable in their antigen-binding activity with the maize 2G12 being at least as efficacious, if not superior, in HIV-neutralization assays. We conclude that this production system may provide a means to achieve microbicde ingredient manufacture at costs that would allow product introduction and manufacture in the developing world.
S07-02 Higher lysine or threonine levels affect the methionine level in higher plants

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Lysine, threonine and methionine are three essential amino acids whose levels limit the nutritional quality of cereals and legume plants. These amino acids synthesized through the aspartate family biosynthesis pathway. To elucidate the relationship between these amino acids and to study the factors that regulate the methionine synthesis, we crossed between transgenic tobacco plants overexpressing Arabidopsis cystathionine \( f-x \)-synthase (AtCGS), the first unique enzyme of methionine biosynthesis, which exhibits higher levels of methionine and two different lines. The first line overexpressed feedback-insensitive bacterial enzyme dihydrodipicolinate synthase (bDHPS) that contains a significantly higher lysine level, and the second line overexpressed the feedback-insensitive bacterial enzyme aspartate kinase (bAK).

The results of the analysis of the progenies of plants expressing bDHPS/AtCGS together with the analysis of feeding plants demonstrated that lysine reduced the expression level of S-adenosylmethionine (SAM) synthase. As a result, the methionine level was significantly increased. Testing the second set of crosses (AtCGS/bAK), we next found that plants co-expressing both foreign genes have significantly higher methionine and threonine levels. The results of this study suggest new ways of producing transgenic crop plants containing increased methionine and lysine levels, as well as methionine and threonine levels, and consequently having improved nutritional quality.

S07-03 Control of stature of ornamental plants by ectopic expression of a GA 2-oxidase gene

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The application of growth retardants to inhibit stem elongation is a common commercial practice in ornamental plant production, to produce compact plants that are suitable for pot cultivation. However, the cost of chemical growth retardants and the general concern regarding such applications, leads to the search for alternative ways of controlling plant stature. The effects of growth retardants are similar to those found in gibberellin (GA)-deficient mutants. Most of the genes involved in the GA biosynthesis pathway have been identified, including the GA 2-oxidase (GA2ox) genes, which encode GA-deactivating enzymes.

The runner bean (Phaseolus coccineus) GA 2-oxidase gene (PcGA2oxI) was ectopically expressed in Nicotiana sylvestris, Solanum nigrum and Petunia under the control of the CaMV 35S promoter. In addition, the feasibility of tissue-specific expression of the PcGA2oxI gene (mainly in stems) was also investigated.

Our results show that it is possible to reduce the stature of ornamental plants by ectopic expression of PcGA2oxI. Furthermore, it is possible to induce dwarfism without affecting flower morphology. Such an approach could be exploited in commercial production of ornamental plants, since dwarf plants may have considerable consumer appeal.

S07-04 Ethylene is an endogenous stimulator of cell division in the vascular cambium

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Exogenous application of the plant hormone ethylene stimulates cambial growth and xylem formation. Earlier reports have also shown correlations between gravity-induced tension wood formation and ethylene emission. These observations led us to generate transgenic poplars to explore the detailed biological function of endogenous ethylene during wood formation. Ethylene responses are mediated through a large family of ERFs (ethylene response factors) that are the key players in understanding the role of ethylene in wood development. We identified 173 putative ERFs in the black cottonwood genome, and detected candidate ERF genes with high expression in response to ethylene. We are using a transgenic approach to elucidate their function. Additionally, we have generated ethylene-insensitive poplar lines expressing the mutated ethylene receptor, Arabidopsis etr1-1, under three different promoters giving a dominant negative ethylene response, and generated lines with enhanced ethylene production by over-expressing ACC oxidase. Our results show that ethylene inhibits height growth, stimulates xylem growth, and inhibits vessel and fibre radial expansion. Significantly, we found that the localized growth stimulation normally observed in the tension wood response was inhibited in ethylene-insensitive trees providing conclusive evidence for a role of ethylene in the vascular cambium for the first time.

S07-05 Arabidopsis thaliana MYB75/PAP1 transcription factor induces anthocyanin production in transgenic tomato plants

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Tomato (Solanum lycopersicum L.) cv. Micro-Tom plants were transformed with the Arabidopsis thaliana (L.) Heynh MYB75/PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1) gene. This gene codes for a transcription factor which is involved in anthocyanin production and is modulated by light and sucrose. The transgenic tomato plants expressing AtMYB75 were characterized by a significantly higher anthocyanin production under normal growth conditions in leaves, stems, roots, flowers and, interestingly, in fruits. In the vegetative organs of the transgenic plants, where AtMYB75 overexpression was determined, a clear up-regulation of all the main genes involved in flavonoid pathway was also detected. On the contrary, no effect was produced on the expression of the tomato MYB-gene ANT1 (ANTHOCYANIN1) that had previously been identified as a transcriptional regulator of anthocyanin biosynthesis. Additionally, induction of many but not all the structural genes of the biosynthetic pathway was observed in the fruits. The higher basal content...
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of anthocyanins in the leaves of the transgenic plants could be further increased in the presence of high light conditions and contributed to mitigate photobleaching damages under high irradiance. Transformations of Anthocyanin fruit (Af) and Ailsa Craig tomato genotypes were also performed obtaining similar results. Molecular characterizations of these transgenic plants are in progress.

PARALLEL SESSION 08: PHOTOSYNTHESIS AND RESPIRATION

S08-01 Mitochondrial contributions to dark-induced leaf senescence in Arabidopsis thaliana

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Dark-induced leaf senescence was studied in Arabidopsis thaliana by comparing whole darkened plants (DP) and individually darkened leaves (IDL). In DP chloroplasts and mitochondria were retained after a 6-day dark treatment and photosynthetic capacity was maintained while respiration decreased. In IDL darkened for the same time the photosynthetic capacity severely decreased connected to chloroplast degradation while a high mitochondrial respiration was maintained. Leaf senescence also resulted in drastic modifications of microtubular structure and mitochondrial movements. The process was further characterized by metabolic and transcript profiling. After 6 days in darkness, leaves from DP showed low levels of carbohydrates whereas many amino acids were abundant. In contrast to DP, IDL had low amino acid content possibly linked to transport of N from the senescing leaf. In general the transcript profiles showed big similarities between the two dark-treatments, however, about 300 genes had significantly different expression between DP and IDL. The transcriptomics results are discussed in relation to the metabolic profiles determined. Based on the changes observed we suggest that leaves of DP enter a stand-by metabolic state of low respiratory activity with cell wall and membrane components as important carbon sources. In contrast, IDL show high respiratory activity with active mitochondrial contribution to retrieval of nutrients from senescing leaves.

S08-02 Arabidopsis lacking mtComplex I: linking changes in photosynthesis, leaf morphology and stress tolerance to alterations in primary metabolism

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Complex I is the largest complex of the respiratory chain but most of its subunits are of unknown function, making it the last frontier in defining the respiratory process. In Arabidopsis it has a unique composition of 44 subunits encoded either by nc or mt genes, remarkably over 20% of its subunits are plant-specific Meyer et al. (2008). We target both the nc and mt components of the complex, using mutants in nc genes encoding complex I subunits (ndufs4 and ndufa1) or in components involved in the expression of mt complex I genes de Longevialle et al. (2007). In isolated mutant mitochondria, Complex I abundance and activity are highly reduced, O2 consumption is rotenone-insensitive and deaminoNADH, which is selective for complex I, is not a respiratory substrate. Differential proteomics shows decreases in complex I subunits and subtle changes in other mt proteins. The metabolome in mutants shows elevated organic and amino acids and a modified sugar composition. The mutants have a more persistent root growth following cold and mannitol treatments, a pronounced curly leaf phenotype, contain more chlorophyll and preliminary data shows up-regulation of nc genes involved in photosynthesis. Photosynthesis may be induced to complement a lower mitochondrial ATP production and induction of the alternative respiratory pathways to bypass complex I may be enhancing the tolerance of the mutants to abiotic stress.

Meyer EH et al. (2008) J Proteome Res 7: 786–794

S08-03 Engineering the photorespiratory pathway to enhances carbon assimilation in C3-plants

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Photosynthetic CO2 assimilation in C3-plants is limited by environmental variables and can be attributed to the catalytic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO). O2 compete with CO2 for binding to the active site of RubisCO. The oxygenation of ribulose 1,5-bisphosphate (RuBP) yields only one molecule of glyceraldehyde-3-P, and the remaining two carbons form glycolate 2-P. This glycolate 2-P is the initial substrate of the C2-oxidative photosynthetic cycle (the photorespiratory cycle) leading to the loss of CO2. To increase the CO2 concentration directly within the chloroplast and thereby decrease the oxygenase activity of RubisCO, a complete glycolate catabolic cycle was established in chloroplasts of the C3-model plant Arabidopsis thaliana. As a result of this cycle, one molecule of glycolate is completely oxygenized to two molecules of CO2, and reducing power in the form of NADPH and NADH is generated. Transgenic lines expressing the novel pathway produced more leaves, had a higher fresh and dry weight, displayed higher photosynthetic capacities, and showed less negative carbon isotope ratio values and glycine/serine ratios than the wild-type. In this way, a cycle was created which resulted in an attenuation of photorespiration, increased efficiency of CO2 assimilation and, consequently, faster biomass production.

S08-04 Molecular evolution of cell-specific gene expression in C4 photosynthesis

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C4 photosynthesis is characterised by a division of labour between two different leaf cell types, mesophyll and bundle sheath cells. The photosynthetic, but also other metabolic activities of these two cells are highly integrated and based on differential gene expression. The cell-type specific expression of genes is mostly controlled by transcription; however, post-transcriptional regulation has been reported, too. The C4 photosynthetic pathway is of polyphyletic origin and has arisen many times independently during the evolution of angiosperms. This indicates that from the genetic perspective it must have been relatively easy to evolve C4 plants from C3 ancestral species. Using the genus Flaveria as a model system we are investigating the differentiation of mesophyll and bundle sheath cells by pursuing an evolution-oriented approach. Flaveria was chosen for this analysis because the genus contains C4 and C3 plants and, in addition, a broad spectrum of C3-C4 intermediate species. To identify cis- and trans-regulatory factors responsible for mesophyll a bundle-sheath specific expression the genes encoding the C4 isomers of phosphoenolpyruvate carboxylase (ppcA) (Gowik et al. 2004, Plant cell, Akyildiz et al. 2007, Plant Cell) and the P subunit of glycine decarboxylase (GLDPA) (Engelmann et al. 2008, Plant Physiol) of the C4 species F. trinervia are currently being studied. Supported by the Deutsche Forschungsgemeinschaft.

**S08-05 Assembly and repair of photosystem II**

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Photosynthetic organisms are subjected to photo-oxidative stress when more light energy is absorbed than used in photosynthesis. Photosynthetic organisms are subjected to photo-oxidative stress when more light energy is absorbed than used in photosynthesis. Under strong light, highly reactive singlet oxygen can be produced via triplet chlorophyll formation in the reaction centre of photosystem II. In the reaction centre 1O2 is formed via charge recombination of the light-induced charge pair. Changes in the midpoint potential of the primary quinone acceptor in photosystem II modulate the pathway of charge recombination in photosystem II and influence the yield of singlet oxygen production. Changes in the midpoint potential of the primary quinone acceptor QA by different herbicides (DMCU and phenolic herbicides), by point mutations in the QA binding pocket and by inactivation of the water-splitting complex on the midpoint potentials can be used as a tool to investigate the charge recombination pathways in PSII. The yield of 1O2 production is correlated with the midpoint potential of QA. Upregulation of expression of nucleus-encoded genes in response to 1O2 produced by PSII was studied by following the expression level of a 1O2 responsive reporter gene construct or the glutathione peroxidase homologous gene from Chlamydomonas reinhardtii.

Recently, we have shown that PSII-generated 1O2 is able to diffuse out of the thylakoid membranes and even out of the chloroplast by spin trapping EPR spectroscopy and fluorescence microscopy. The role of 1O2 in signalling will be discussed.

**S08-06 Singlet oxygen production in photosystem II and its role in signalling**

A. Krieger-Liszkay, B. B. Fischer and E. Hideg
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Photosynthetic organisms are subjected to photo-oxidative stress when more light energy is absorbed than used in photosynthesis. Under strong light, highly reactive singlet oxygen can be produced via triplet chlorophyll formation in the reaction centre of photosystem II. In the reaction centre 1O2 is formed via charge recombination of the light-induced charge pair. Changes in the midpoint potential of the primary quinone acceptor in photosystem II modulate the pathway of charge recombination in photosystem II and influence the yield of singlet oxygen production. Changes in the midpoint potential of the primary quinone acceptor QA by different herbicides (DMCU and phenolic herbicides), by point mutations in the QA binding pocket and by inactivation of the water-splitting complex on the midpoint potentials can be used as a tool to investigate the charge recombination pathways in PSII. The yield of 1O2 production is correlated with the midpoint potential of QA. Upregulation of expression of nucleus-encoded genes in response to 1O2 produced by PSII was studied by following the expression level of a 1O2 responsive reporter gene construct or the glutathione peroxidase homologous gene from Chlamydomonas reinhardtii.

Recently, we have shown that PSII-generated 1O2 is able to diffuse out of the thylakoid membranes and even out of the chloroplast by spin trapping EPR spectroscopy and fluorescence microscopy. The role of 1O2 in signalling will be discussed.

**S08-07 Investigation of the phenomenon of state transitions and the photosystem II megacomplex structure in the Arabidopsis thaliana koLhcb3 mutant**

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*Department of Biophysical Chemistry, University of Groningen, Netherlands
*Queen Mary, University of London, School of Biological and Chemical Sciences, UK
*Department of Molecular Biology and Biotechnology, University of Sheffield, UK
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Study of the Arabidopsis thaliana knockout mutant lacking Lhcb3 (koLhcb3) have revealed a close similarity to the wildtype plants. Growth rate, NPQ, qP, D1, circular dichroism spectra, pigment composition and content of LCHII trimers have been found to be unaffected by this mutation. The proteomic analysis have shown only some minor alterations in the stoichiometry of thylakoid proteins. However, the results have clearly revealed a considerable increase in the rate of the state 1 to state 2 state transition in the koLhcb3 mutant. None the less, the extent and regulation of the state transition appears identical to the wildtype. At this time the mechanism that cause this difference and similarity (respectively) remains unclear. Remarkably, peculiar alterations in the PSII megacomplex structure have been discovered. In koLhcb3, the M-trimer were found to be noticeably rotated leading to the alteration of the PSII unit cell topography. Localisation of the Lhcb3 protein within the PSII megacomplex and the thylakoid membrane with Ni-Au particles is under way and should enable the direct localisation of the subunit and help explain the observed state transition phenotype of the koLhcb3 mutant. In addition, since the Lhcb3 subunit does not
have the phosphorylation sites linked to state transition in Lhcb1 and Lhcb2, we are investigating if the altered phosphorylation site availability is the cause of the accelerated state transition.

S08-08 The thiol-disulfide state of the cytosolic translation repressor NAB1 controls the expression of light-harvesting antenna proteins

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The cytosolic RNA-binding protein NAB1 represses translation of LHCII light-harvesting complex of photosystem II mRNA in the green alga C. reinhardtii. Analysis of NAB1-RNA complexes in vivo revealed that NAB1 recognizes LHCII transcripts with different affinities, displaying a strong preference for the mRNA of isoform LHCBM6. The translation repressor activity of NAB1 has to be modulated in response to changing environmental conditions and protein expression studies indicated that this is achieved by post-translational modification rather than modulation of the cellular NAB1 amount. NAB1 contains two RNA-binding motifs, and the C-terminal RRM motif harbours two cysteine residues. These cysteines are reversibly oxidized in vitro and oxidation results in a switch-off of the RNA binding activity. Conformational changes caused by cysteine oxidation implied the formation of an intramolecular disulfide within the RRM domain. Analysis of the in vivo relevance of this cysteine modification demonstrated that this thiol-based switch is crucial for NAB1 activity regulation in the alga cell. Expression of different mutated NAB1 versions in a strain devoid of wild-type NAB1, in which single cysteines were replaced by serine, clearly showed that both cysteines are essential for NAB1 deactivation in vivo. Intramolecular disulfide formation is prevented in these mutant strains and the observed reduced P571 antenna size is caused by a permanent repression of LHCII mRNA translation.

S08-09 Dissection of the role of chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses

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Photosynthetic light reactions comprise a significant source of hydrogen peroxide in illuminated leaves. We have addressed the significance of chloroplast H$_2$O$_2$-detoxifying enzymes in stress tolerance and signaling in Arabidopsis thaliana. To this end, T-DNA insertion mutants tapx, sapx and tapx sapx, lacking the thylakoid-bound ascorbate peroxidase (tAPX), stromal ascorbate peroxidase (sAPX) or both, respectively, were characterized. The tapx sapx double mutant showed distinct susceptibility to short-term photo-oxidative stress, especially during germination. Moreover, the absence of tAPX and sAPX induced alterations in the transcriptomic profile of tapx sapx double mutants under quite optimal growth conditions, and these transcriptional alterations became more pronounced upon a short high-light illumination period. Intriguingly, after two-week acclimation to high light, none of the mutants exhibited enhanced stress symptoms. Immunoblot analysis revealed that high-light-acclimated tapx sapx double mutants compensated the absence of tAPX and sAPX by increasing the level of 2-cys peroxiredoxin. We conclude that tAPX and sAPX are functionally redundant, and crucial upon sudden onset of oxidative stress. After long-term acclimation to stress conditions, however, the function of chloroplast APXs becomes compensated by other components of the plant antioxidant system.

PLENARY LECTURES ON WEDNESDAY, 20 AUGUST 2008

L13 Multi-level regulation of photosynthesis
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Flowering plants regulate photosynthesis at several levels. This includes the control of energy allocation to their photosystems in response to illumination changes. In the short term, plastoquinone reduction induces phosphorylation of light-harvesting complex II (LHCBII) and state transitions. Longer-lasting light changes invoke a long-term response (LTR) by modifying gene expression in the nucleus and chloroplast ultimately altering thylakoid composition. Both responses require the thylakoid protein kinase STN7. Different environmental and metabolic conditions require the adjustment of ATP/NADPH ratios and a switch of electron distribution between the two photosystems. Therefore, flowering plants can switch between linear and cyclic electron flow. With the exception of PGR5, other components facilitating cyclic electron flow are so far unknown. Here, novel results in the field of the functional relationship of state transitions to longer-lasting photosynthetic acclimation and in the field of cyclic electron flow will be presented.

L14 The importance of gene and genome duplications for plant evolution
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Recent analyses of eukaryotic genome sequences have revealed that gene duplication, by which identical copies of genes are created within a single genome, has been rampant. The creation of extra genes by such duplication events has now been generally accepted as crucial for evolution and of major importance for adaptive radiations of species and the general increase of genetic and biological complexity. We have developed software to identify remnants of large-scale gene duplication events and, more recently, we have also developed mathematical models that simulate the birth and death of genes based on observed age distributions of duplicated genes. Applying our model to the model plant Arabidopsis shows that much of the genetic material in extant plants, i.e., about...
60% has been created by several genome duplication events. More importantly, it seems that a major fraction of that material could have been retained only because it was created through large-scale gene duplication events. In particular transcription factors, signal transducers, and regulatory genes in general seem to have been retained subsequent to large-scale gene duplication events. Since the divergence of (duplicated) regulatory genes is being considered necessary to bring about phenotypic variation and increase in biological complexity, it is indeed tempting to conclude that such large scale gene duplication events have indeed been of major importance for evolution.

L15 Genome meets epigenome: uncovering genetic and epigenetic variation in Arabidopsis
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Understanding of the relationship between genetic and epigenetic regulatory mechanisms that mediate control of transcription at multiple levels is critical to understanding how plants develop and respond to their environment. We combined generation sequencing by synthesis (SBS) technologies with novel methods for direct sequencing of the entire cytosine methylome (methylC-seq), transcriptome (RNA-seq), and the small RNA component of the transcriptome (smRNA-seq) to create a set of highly integrated epigenome maps for Arabidopsis thaliana for several accessions (Col-0, Ler, Cvi). At single-base resolution we discovered extensive, previously undetected, DNA methylation and sites of active demethylation, identified the context and level of methylation at each site, and found that local composition has effects upon DNA methylation state. Deep sequencing of the smRNAome exposed a direct relationship between the location and abundance of smRNAs and DNA methylation, perturbation of smRNA biogenesis upon loss of CpG DNA methylation, and a tendency for smRNAs to direct strand-specific DNA methylation in the region of RNA-DNA homology. Strand-specific RNA-seq revealed changes in the transcript abundance of hundreds of genes upon alteration of the DNA methylation state, and enabled the identification of numerous previously unidentified genes regulated by DNA methylation. Finally we have developed an open-source web-based application called Anno-J built to handle large amounts of data.

L16 Small RNAs and epigenetic regulation in abiotic stress resistance
J. K. Zhu
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The research in my lab is focused on the molecular mechanisms of salt, drought and cold stress signaling and resistance. Recently, we began to study the roles of microRNAs and small interfering RNAs in abiotic stress response pathways, the mechanisms of active DNA demethylation and small RNA-directed DNA methylation, and the contribution of these epigenetic mechanisms to stress resistance. Recent results concerning abiotic stress-regulation of small RNAs and DNA methylation in Arabidopsis will be presented.

L17 Cell-cell communication governing vascular tissue organization
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Multi-cellular organisms form their body by postnatal regulations through cell-cell communication as well as by genetic program. To understand cell-cell communication during development in plants, we have studied intercellular signaling machineries governing plant vascular tissues. Recently we identified a 12-amino acid peptide that suppresses tracheary element differentiation in a Zinnia xylogenic culture and designated TDF (Tracheary element differentiation inhibitory factor). TDF was encoded by the CLE41 and CLE44 genes in Arabidopsis. The promoter analysis of the two genes and immunohistochemical analysis of TDF revealed that TDF is expressed mainly in phloem cells and seconated toward procambium cells (vascular stem cells). On the other hand, we succeeded in the isolation of a gene for a putative receptor of TDF and designated TDR (putative TDF receptor). The localization of the TDR gene and its loss-of-function phenotype suggests that TDR functions in maintenance of vascular stem cells and in suppression of differentiation of vascular stem cells into xylem cells.

L18 A systems biology approach to understanding lignin biosynthesis
V. Chiang
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Wood is an essential component of our energy strategy for ethanol because it can be produced in very large scale and on marginal land. However, current wood has several barriers. Foremost is lignin, which limits the accessibility of cellulose. Substantial information has been generated that could implement biotechnological approaches to overcome the lignin barrier, however, much new information is needed. First, we are not yet certain about how many possible enzymes are involved in the metabolic pathway for lignin biosynthesis. Second, we do not yet have an adequate understanding of the robustness of this metabolic network. Metabolic robustness may increase insensitivity of lignin biosynthesis to biotechnological manipulations. Third, there is essentially no information on the abundance of the enzymes and the associated metabolites in the lignifying tissue, and about the effect of these concentrations on lignin composition and quantity. Trees are the only target energy crop that would allow investigations at the genome level for such information, because of the genome sequence of Populus trichocarpa. New quantitative tools and the feasibility of identifying and modifying practically every lignin pathway gene by genetic transformation now make the generation of such information possible. We envision the use of trees to establish systems-based genomic model
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where the lignin and the process are coherently designed together for an optimized and high efficiency ethanol production.

L19  FT: a mobile inductive signal for flowering and tuberization transition
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Short-days induce tuber formation in all potato varieties but are strictly required for andigena species. In these species a 15 min night break inhibits tuber formation in a similar manner as reported for the SD-dependent flowering species Pharbitis nil or rice. We had showed that over-expression of the Arabidopsis CO gene in andigena potatoes strongly delays tuber formation under SDs, suggesting that a genetic pathway like that controlling flowering may also be involved in day length control of tuberization. In this work, we show that expression of a rolC-Hd3a construct, encoding for rice FT, promotes tuberization of andigena plants in non-inductive (LD) conditions. The Hd3a-GFP protein but not the transgenic RNA was detected in the stolons of WT plants grafted with the transgenic scions, confirming phloem transport of the FT protein but not the RNA. Noteworthy, transgenic Hd3a stolons differentiated floral buds out of the SAM simultaneously to tuber enlargement of the subapical meristem region. Increased branching was also observed in the transgenic lines. These results indicate that FT is not only involved in flowering control but functions as a general daylength-regulated modulator of meristem differentiation, playing a role in floral and tuberization transition and lateral meristem activation by binding different partners in the SAM, subapical or lateral meristems. Progress to the characterization of the potato FT homologs and partners in stolon cells will be presented.

L20  Comparative genomics between Arabidopsis and Poplar: What genes make a tree into a tree?
O. Nilsson
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At the Umeå Plant Science Centre most groups work in parallel with the two fully sequenced plant model systems Arabidopsis and Poplar. Working with two complete genome sequences allows us to very efficiently address questions like ‘what genes make a tree into a tree?’ Three of the key differences between annual plants like Arabidopsis and a perennial tree like Poplar is the extremely long juvenile phase of trees before they are reproducitively mature, the cycling between growth and dormancy, and the extensive secondary growth of the tree trunk, allowing the tree to reach heights of up to a 100 m. I will show how the comparative functional genomics approach has allowed us to identify key molecular regulators of not only flowering and the length of the growing season in trees, but also the control of wood formation. All these genes must have been of evolutionary importance in allowing the tree growth and development strategy, and also have ecogenetic importance in adapting the growth of trees to different climates and locations.

PARALLEL SESSIONS ON THURSDAY, 21 AUGUST 2008

PARALLEL SESSION 09: STRESS AND ACCLIMATION; ABIOTIC

S09-01  ROS perception and signaling in plants
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Reactive oxygen species (ROS), formed in plants by several stresses, are involved in regulation of plant stress sensitivity/tolerance. Ozone (O3) is an air pollutant also used as a tool to induce the formation of ROS in the apoplast to identify components and processes regulated by apoplastic ROS. We have identified O3-sensitive rcd-mutants. Map-based cloning of the rcd-mutations has revealed new components in the cellular acclimatization mechanisms. RCD1 seems to be involved in processes that affect interplay between hormonal signaling cascades, acclimatization to oxidative stress and salt and osmotic stress, and are required for the proper growth and development of the plant. Yeast two-hybrid analysis identified RCD1-interacting transcription factors related to salt and osmotic stress, auxin, and surprisingly, to photomorphogenetic processes. The rcd1 mutant displays phenotypic deficiencies relating to the function of the interacting transcription factors.

S09-02  Identification of new factors involved in 1O2-mediated communication between the chloroplast and the nucleus in Arabidopsis
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In plants exposed to environmental stress factors rapid increase of reactive oxygen species (ROS) occur mainly within the chloroplast. The plant may perceive these ROS as signals that trigger changes in gene expression resulting in adaptation to environmental changes. In order to study the biological activity and signaling role of a specific ROS, we took advantage of the conditional flu mutant of Arabidopsis that generates specifically singlet oxygen (1O2) in plastids. Following the release of 1O2, dramatic changes in nuclear gene expression occur that reflect an intense 1O2-dependent communication between the chloroplast and the nucleus. We have designed a genetic screen that aims at identifying signaling components involved in the chloroplast-to-nucleus communication. A reporter line was created consisting of the 1O2-inducible promoter of AAA ATPase gene fused to the luciferase reporter gene. The reporter line was EMS-mutagenised and used for the isolation of mutants with altered expression of AAA ATPase. We have characterized 5 caa mutations that cause constitutive activation of AAA ATPase as well as three loss-of-function mutants that are no longer able to activate the AAA ATPase gene in response to 1O2. Recently the identity of three CAA mutated genes has been determined by map-based cloning approach. First characterization of the mutants confirms the success of our screen in identifying extraplasmic factors controlling the expression of 1O2-responsive genes.
**S09-03 Structure-function of nuclear and cytosolic pools of tomato ASR1, a water stress- and salt stress-regulated plant-specific protein**


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ASR1 (abscisic acid stress ripening) is a low molecular weight plant specific protein encoded by an abiotic-stress regulated gene. ASR1 protein is presumed to be a natively unfolded protein using a number of prediction algorithms. The degree of order of ASR1 was determined experimentally using non-tagged recombinant protein expressed in E. coli and purified to homogeneity. Purified ASR1 was shown to be unfolded using a number of biophysical methods. The protein was shown to be monomeric by analytical ultracentrifugation. The cytosolic pool of ASR1 is largely unfolded. The unfolded monomeric form of ASR1 has a chaperone-like activity, and can protect against inactivation of proteins by a number of causes. Chaperone-like activity of ASR1 acts synergistically with glycine betaine, an osmolyte known to be accumulated under abiotic stress conditions. The ASR1 protein also possesses a zinc-dependent DNA-binding activity. The DNA binding site was suggested to reside in the central part of the polypeptide by using truncated forms of the protein. Two additional zinc-binding sites were shown to be localized at the amino terminus of the polypeptide. Addition of zinc ions resulted in a global change in the ASR1 structure, from monomer to homodimer. Upon binding of zinc ions, the protein becomes ordered as shown by FTIR and microcalorimetry, concomitant with dimerization. Ordered zinc-bound nuclear ASR1 is involved in regulation of gene expression.

**S09-04 Energy signaling during the stress response: the central role of Arabidopsis KIN10 and KIN11**

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Different types of stress result in both specific and convergent responses that modulate plant growth and development. Large-scale metabolic and microarray studies have revealed that this is partly due to an extensive cross-talk among stress response pathways that were once considered linear. Different stress conditions induce similar alterations in carbon and nitrogen metabolism and lead to overlapping patterns of gene expression, with many genes being induced or repressed by multiple stimuli. As photosynthesis and respiration are often major targets of stress, decreased cellular energy levels are an obvious common consequence. Using a combination of cellular and systems approaches we have identified Arabidopsis KIN10 and KIN11 as central mediators of various stress conditions that impinge on cellular energy levels. Sensing and signaling stress-associated energy deprivation, these protein kinases trigger global gene expression reprogramming, implementing an ‘energy-saving’ program that promotes catabolism and autophagy, and suppresses anabolism and ribosome biogenesis. Significantly, KIN10/11 also target a plethora of transcription and signaling regulators to orchestrate global responses beyond metabolic regulation. In support of this view, long-term defective KIN10/11 signaling results in developmental alterations, suggesting that KIN10/11 serve as integration points of metabolic, hormonal and environmental signals to finely orchestrate plant growth and development.

**S09-05 Molecular mechanisms of cold acclimation in Arabidopsis and their integration with diurnal signalling**


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In plants, low temperatures cause massive transcriptional changes involved in the process of cold acclimation. CBF transcription factors (TFs) are the main regulators of gene expression in response to cold, but CBF-independent pathways have also been described. It has been shown that the expression of genes encoding CBFs and some cold-related genes is regulated by circadian clock, which temporally regulates many biological processes. Nevertheless, the influence of diurnal and circadian-regulated genes on cold response and on the identification of cold-responsive genes is unknown. We performed targeted expression analyses of diurnal and circadian time courses. We show that, after a short initial cold response, in diurnal conditions cold reduces the amplitude of cycles for clock components and dampens or disrupts the cycles of output genes, whilst in continuous light all cycles become arrhythmic. One consequence of the circadian control of gene expression is the gating effect; causing variable responses according to the time of day that the stress is present. Analyzing the expression of about 1900 genes encoding TFs, we show a gating effect of cold response, affecting the number and strength of expression changes for a large number of cold-induced TFs in the morning in comparison to the evening. Our data, revealing interactions between cold and diurnal regulation, will be important in the dissection of transcriptional regulatory networks controlling cold acclimation.

**S09-06 Functional identification of Arabidopsis stress regulatory genes using the controlled cDNA expression system, COS**


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Plants respond to environmental stresses by altering the expression of many genes via a complex signaling network. We developed a Controlled cDNA Overexpression System (COS) to identify genes and regulatory factors involved in stress tolerance. An estradiol-inducible cDNA expression library was tested in three genetic screens by selecting for salt tolerance, ABA insensitive germination and activation of a stress responsive ADH1-LUC reporter gene construct. Numerous
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cDNAs conferring dominant, estradiol dependent stress tolerance phenotype were identified. Screening for enhanced salt tolerance revealed that estradiol-controlled overexpression of 2-alkenal reductase (AER) cDNA confers considerably high level of salt insensitivity. Characterization of cDNA conferring insensitivity to 3 μM ABA in germination assays has identified the full-length coding region of heat shock protein HSP17.6A, suggesting its implication in ABA signal transduction. Screening for transcriptional activation of ADH1-LUC reporter gene has identified the ERF/AP-type transcription factor RAP2.12, which sustained high level ADH1-LUC bioluminescence, enhanced ADH1 transcription and increased ADH enzyme activity in the presence of estradiol.

Our data illustrate that application of inducible cDNA expression libraries such as the COS system provides an efficient tool for genetic identification and functional analysis of novel regulators of abiotic stress responses.

S09-07 Artificial selection for cellular respiration and energy use efficiency in isogenic canola populations result in epigenetically distinct populations

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Metric traits as size and weight show a normal distribution. This is even the case when the populations are isogenic. Variations in local soil quality, microclimate, sowing-depth, seed quality, ... contribute to growth differences between the individual plants of an isogenic population. Besides these physical factors, also epigenetic components could contribute to growth differences between genetically identical plants. Here we show for canola that cellular respiration and energy use efficiency contain an epigenetic component of which the state is specific for each plant in an isogenic population. Both the DNA-methylation and histone acetylation/methylation patterns show that artificial selection for higher or lower cellular respiration and energy use efficiency allow creating isogenic subpopulations that are genetically identical but epigenetically distinct. The higher respectively lower respiration and energy use efficiency states are stably inherent in seedlings and can be transmitted in reciprocal crosses. Field trials done over several years show that this epigenetic component of respiration and energy use efficiency can be used to increase seed yield in canola with up to 10%.

S09-08 Physiological and molecular characterisation of stem bending-induced diameter growth response in poplar

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Plants respond to environmental mechanical stimulation, such as wind, by modifying their growth and development. In general, plants that are grown in windy environments are shorter, stockier, and often have altered flexibility.

A biomechanical approach studying the effect of stem bending on tomato height growth revealed that the perceived mechanical variable is the strain and not the force or stresses (Coutand and Moulia 2000). Recently, a gene encoding a Cys2/His2 type zinc-fingered transcription factor was shown to be rapidly induced by stem bending in Juglans regia (Leblanc et al. 2008).

Our works aimed to study the effect of controlled stem bending on young poplar diameter growth for one part, and to understand the role of a poplar zinc finger protein (PtaZFP2) in this growth response for another part. An original experimental device was designed enabling to control the level of applied strain and to monitor radial growth continuously before, during and after bending. For checking some possible acclimation of plant to mechanical loadings, the effect of successive bending was also studied.

Results revealed that PtaZFP2 mRNAs accumulated rapidly and transiently after mechanical stimulation, and are restricted to the part of the stem where bending was applied. There is a correlation between PtaZFP2 expression level and the sum of longitudinal strains as well as to the growth response. Plants also acclimated very rapidly to mechanical loadings.

S09-09 A new strategy for engineering drought tolerance in plants via auto-regulated expression of a key enzyme of cytokinin biosynthesis

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One of the prominent morphological and physiological symptoms of plants under drought stress is the appearance of premature leaf senescence. However, the senescence program could be unnecessarily activated during drought and leads to premature death. We hypothesized the possibility of enhancing plant drought-tolerance by delaying the drought-induced leaves' senescence by cytokinins. We created transgenic tobacco plants carrying the autoregulatory system of cytokinins synthesis directed by an early senescence promoter of the senescence SARK gene. The promoter was fused to IPT, the key gene of cytokinins synthesis. The transgenic plants displayed a dramatic delay of the senescence symptoms. Most surprisingly, the tobacco transgenic plants also displayed water stress tolerance as reflected by vigorous growth after a severe drought (18 days without watering), as well as minimal yield loss when watered with only 30% of the amount of water used under controlled conditions. The transgenic plants retained photosynthetic activity, an improved water use efficiency and substantially higher water content in leaves. After rewatering, the plants recovered photosynthetic activity and active growth. Microarray analysis of stress-dependent genes showed under strong drought stress elevated expression of reactive oxygen scavenging mechanisms, indicating a stress protection mechanism as a contributor to the resistance phenotype.

PARALLEL SESSION 10: DEVELOPMENT; REPRODUCTIVE

S10-01 Integration of low-temperature and long-day flowering responses in cereals

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Physiol. Plant. 133, 2008
Temperate cereals, such as barley and wheat, perceive seasonal cues to synchronise flowering with optimal conditions in spring. Two cues that promote flowering of temperate cereals are prolonged cold (vernalization) and long-days. The requirement for vernalization overrides the effect of long-days; thus long-days do not trigger flowering until after winter. By examining interactions between genes controlling the vernalization requirement we have been able to describe how low-temperature and long-day responses are integrated in cereals. The low-temperature response is mediated by VRN1, a FRUITFUL-like MADS box gene induced by vernalization. The daylength response is controlled by FT1, a cereal orthologue of the Arabidopsis FLOWERING LOCUS T, which is induced by long-days. A third gene, VRN2, integrates low-temperature and long-day responses by maintaining low levels of FT1 expression and suppressing the long-day flowering response until plants are vernalized. Following vernalization, VRN1 represses VRN2 to allow long-day induction of FT1. According to this model, variation in vernalization requirement of cereals occurs through different mechanisms: mutations that activate the low-temperature response pathway, or mutations that allow the long-day flowering response without prior vernalization. We will present our model describing integration of flowering response pathways in cereals and compare this with the mechanisms that regulate flowering time in Arabidopsis.

**S10-02 The control of floral organ abscission by inflorescence deficient in abscission (IDA) is dependent on the receptor-like kinases HAESA and HAESA-LIKE 2**

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Small peptides may act as signaling molecules that coordinate growth and development. The IDA gene, encoding a protein of 77 amino acids (aa), is required for floral organ abscission in Arabidopsis. 35S:IDA plants have early floral abscission and ectopic abscission of other organs. IDA and IDA-LIKE (IDL) proteins, which all have an N-terminal signal sequence and a conserved 20 aa motif (EPIP) at the C-terminus, represent a novel group of putative ligands in plants. Here we present data showing that the EPIP motif of IDA can replace IDA function in vivo, both when expressed under the regulatory elements of IDA and when applied as a synthetic peptide. We also show that IDA can be processed at the C-terminus when exposed to the same cauliflower meristem extract that processes CLV3. The receptor-like kinase gene HAESA (HAE) shows overlapping floral organ expression to that of IDA. We show that a double mutant between HAE and its close homologue HAESA-LIKE 2 (HSL2) portrays the same deficiency in floral organ abscission as that of the ida mutant. We hypothesize that IDA may function as a ligand for the receptor-like kinases HAE and HSL2, in accordance with this we show that a double mutant between HAE and HSL2 is epistatic to 35S:IDA. Transcriptome profiling using RNA from AOs of hae hsl2 and ida will be performed to disclose genes that may be regulated by the IDA HAE HSL2 pathway.

**S10-03 Light-control of plant development by Arabidopsis SPA proteins**


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The four-member SPA protein family of Arabidopsis functions in concert with the E3 ubiquitin ligase COP1 to cause dark-dependent ubiquitination of transcription factors involved in light signalling. spa quadruple mutant seedlings thus exhibit strong constitutive photomorphogenesis. At the adult stage, SPA genes are essential for photoperiodic flowering; spa mutants flower early under short day, but not long day conditions. We further show that early flowering of spa1 mutants in short day is fully dependent on the floral inducer CONSTANS (CO). Consistent with the early-flowering phenotype, spa mutants show strongly enhanced FT transcript levels in short day. CO mRNA abundance, by contrast, is not altered in spa mutants. The SPA proteins interact with CO in vitro and in vivo, and, moreover, control CO protein stability. We therefore propose that SPA proteins might be involved in the dark-dependent degradation of the CO protein. Apart from controlling flowering time and seedling photomorphogenesis, SPA proteins also regulate elongation growth of adult plants. During development, the four SPA genes have overlapping but distinct functions. An analysis of SPA transcript levels suggests that differences in SPA gene expression patterns contribute to divergence in SPA1-SPA4 function. Thus, the regulation of SPA expression could be crucial in the adjustment of plant growth and development to a changing light environment.

**S10-04 The regulation of seasonal flowering and poly不可思议 in the perennial Arabis alpina**

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Flowering is an important determinant of the life cycle of a plant. Annual plants flower once and complete their life cycle within one year whereas the majority of perennials flower several times during their lifetime. To understand how flowering is regulated in perennial plants we are using Arabis alpina, a perennial relative of Arabidopsis thaliana, as a model species. Most A. alpina accessions have an obligate vernalisation requirement for flowering and already form flower initials during the cold period. The duration of the flowering season is restricted to optional conditions in the spring and perennial behaviour is maintained by axillary shoots grown after vernalisation. We have recovered a mutation in the homologue of the Arabidopsis flowering time gene FLOWERING LOCUS C (FLC) in A. alpina and shown that this gene confers obligate vernalisation requirement and regulates other traits specific to perennial plants such as seasonal flowering and polycarpic growth habit. AaFLC expression, in contrast to Arabidopsis FLC, rises after saturating vernalisation ensuring that only shoots grown before vernalisation will flower whereas shoots grown after vernalisation will stay vegetative. This unstable repression of AaFLC is correlated with different patterns of histone 3 (H3) modifications. We conclude that differences in the function and regulation of FLC between annual and perennial species are important in the evolution of life history traits.
Abstracts

S10-05 BELL genes dictate shoot apical meristem phase identity
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Floral induction is controlled by a plethora of genes acting in different pathways that either repress or promote floral transition at the shoot apical meristem (SAM). During vegetative development, floral repressors maintain the Arabidopsis SAM incompetent to respond to promoting factors. Among these, FLOWERING LOCUS C (FLC) is the most prominent. Processes underlying down-regulation of FLC in response to environmental and developmental signals have been elucidated in considerable detail. However, basal induction of FLC is less understood. Here we demonstrate that the BELL genes ARABIDOPSIS THALIANA HOMEBOX 1 (ATH1) and PENNYWISE (PNY) act redundantly as floral repressors through positive regulation of FLC expression. ath1 and pny mutants flower early in short days and display attenuated FLC levels. Moreover, both mutations partially suppress FLC-mediated late flowering of both a FRI-expressing line and that of autonomous pathway mutants. Moreover, absence of both ATH1 and PNY almost fully impairs FRI-mediated late flowering. Intriguingly, PNY, in combination with a third BEL protein, POUND-FOOLISH (PNF), has previously also been identified as a floral activator (Smith et al. 2004). To get a better understanding of the role of these three BELL proteins in the various aspects of flowering time control, we are currently investigating which pathways are affected by different combinations of these proteins.

Parthenocarpic tomato fruit-set depends on gibberellins (GA) and auxins, although possible interaction between these hormones is unknown. We showed that fruit development induced by the auxins indol-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were significantly reduced by simultaneous application of inhibitors of GA biosynthesis (Paclobutrazol and LAB 198999), and that this effect was reversed by GA3, suggesting that auxin effect was mediated by GA. Parthenocarpic fruits induced by 2,4-D had higher content of active GA1 and its precursors than unpollinated-untreated ovaries. Application experiments of radioactive-labelled GAs to unpollinated ovaries showed that 2,4-D altered in vivo GA metabolism (both biosynthesis and catabolism). Transcript levels of genes encoding copalylidiphosphate synthase (SICS), SIGA20ox1, -2 and -3, and SIGA3ox1 were higher in unpollinated ovaries treated with 2,4-D. In contrast, transcript levels of SIGA2ox2 (out of the five SIGA2ox genes known to encode this kind of GA inactivating enzymes) were lower in 2,4-D treated ovaries. Our results support the idea that auxins induce fruit-set and growth in tomato, at least partially, by enhancing GA biosynthesis (GA 20-oxidase, GA 3-oxidase and maybe CPS), and decreasing GA inactivation (GA20ox) activities, leading to higher GA1 content. The expression of diverse Aux/IAA and auxin response factors, which may be involved in this effect of auxin, was also altered in 2,4-D-induced ovaries.

S10-06 The CRABS CLAW ortholog from Eschscholzia californica, EcCRC, is involved in floral meristem determination and gynoecium development
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The Arabidopsis transcription factor CRABS CLAW (CRC) is a major determinant of carpel growth and carpel fusion, its closest relative in rice, however, has additional functions in specifying floral organ identity and floral meristem termination. We were interested in understanding the history of gene function modulation of CRC-like genes during angiosperm evolution. Here we report the identification and functional characterization of EcCRC, the Californica poppy (Eschscholzia californica) CRC ortholog. Down-regulation of EcCRC by Virus-induced gene silencing (VIGS) approaches results additional fourth floral organ whorls that develop exclusively into gynoecia resulting in a repetition of the fourth whorl. Additionally, defects in carpel polarity and fertility are apparent, and the observed phenotype is restricted to the gynoecium. Our results further show that the history of CRC-like genes during angiosperm evolution is characterized by several losses and gains of function independent of duplication processes in this gene subfamily. Moreover, our data implicate that the ancestral angiosperm CRC-like gene was involved in floral meristem termination and the promotion of carpel margin tissue development.

S10-07 Auxin-induced fruit-set in tomato is gibberellin dependent
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Physiol. Plant. 133, 2008

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Flowers are mostly bisexual in angiosperms, but it emerges in a recent evolution that plants acquire a sexual specialization, resulting in the development of unisexual flowers. Sex determination occurs in plants through the arrest of the inappropriate sexual organ in the initially bisexual flower primordia. Given that genes leading to sex determination are independent of homeotic ABC genes, most of the genetic and molecular bases for sexual differentiation remain to be elucidated. Cucurbits represent an excellent model for sex determination studies, as they provide clear and simple genes to phenotype analysis. In melon (Cucumis melo), sex determination is governed by two loci, andromonoecious (A) and gynoecious (G). The G locus is for instance responsible for the heritable appearance of gynoecy, that is the complete feminization of flowers in a whole plant. Here we present the identification of the gynoecious gene, which encodes a previously uncharacterized transcription factor in plants. Functional analysis reveal that the gynoecious gene is involved in the repression of carpel development in undifferentiated flowers, leading to unisexuality and development of male flowers. Moreover, we show that the gynoecious gene is under epigenetic regulation, which seems to be here the molecular basis for sex determination.
**PARALLEL SESSION 11: METABOLISM**

**S10-09 Regulation of flower type identity in Gerbera hybrida (Asteraceae)**
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Gerbera hybrida bears complex inflorescences consisting of different types of flowers (ray, trans and disc) that have specialized structures and functions. The specification of various flower types become established early in floral ontogeny and include differences in sex, organ fusion and flower symmetry; all present within the same genotype. This inflorescence complexity is unique for Asteraceae and not shared by the classical model species used for flower developmental research. Still, the molecular basis for flower type differentiation in Asteraceae has remained unclear. Microarray comparison of developing gerbera ray and disc flower primordia has identified a number of genes that are differentially expressed along the capitulum radius. These include many MADS box genes that encode known regulators of flower organ identity. Our data suggests, that different MADS protein complexes may contribute to the lengths of the dark period to set the rate of starch degradation. Moreover starch degradation is timed according to the subjective morning as anticipated by the circadian clock under all conditions tested.

We conclude that the circadian clock is required for the correct timing of starch turnover in A. thaliana.

**S11-02 Metabolism and metabolic regulation; the roles of β-amylase proteins in starch breakdown**
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Starch is one of the primary products of photosynthesis. Up to fifty percent of the photo-assimilated carbon is stored as starch in the chloroplast and serves as a source of energy for the plants during the night when photosynthesis is not possible. Although the pathway of starch breakdown in germinating cereal endosperm has been known for many years, relatively little was known about the pathway of starch breakdown in the leaves. Recently, it has been established that maltose is a key intermediate of the pathway in leaves, implicating the maltogenic enzyme β-amylase. We show that at least four of the nine β-amylases encoded by the Arabidopsis genome localize to the chloroplast. The analysis of different mutants and mutant combinations revealed that two β-amylases (AtBAM1 and AtBAM3) produce maltose during starch breakdown in the leaves. Additionally, we found that one isoform (AtBAM4) is not an active β-amylase but still influences starch metabolism and night-time maltose levels.

We speculate that AtBAM4 has a regulatory role, influencing other enzymes of starch degradation that act upstream of AtBAM1 and AtBAM3. This work has allowed us to complete a model for the pathway of starch breakdown in the leaves. Our future research will concentrate on the mechanisms regulating starch breakdown.

**S11-03 Trehalose metabolism and sugar signalling in plants**
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Trehalose metabolism was once thought to be uncommon in plants, until the discovery of trehalose-phosphate synthase (TPS) and trehalose-phosphatase (TPP) genes in Arabidopsis thaliana led to a complete reappraisal of its importance. Genome sequencing and mutant analyses have now shown that trehalose metabolism is not only widespread within the plant kingdom, but also that it is essential for normal growth and development. Plants with altered trehalose metabolism show marked morphological and physiological phenotypes, which are linked to changes in the level of trehalose 6-phosphate (Tre6P), the intermediate of trehalose synthesis, rather than to trehalose itself. Using an LC-MS/MS-based assay, we found that the amount of Tre6P in plant tissues is generally very low (<1 nmol·g⁻¹FW), and that it closely reflects changes in the level of sugars, particularly sucrose, leading us to propose that Tre6P acts as a signal of sugar status. The downstream targets of Tre6P signalling...
Abstracts

in plants have not yet been identified, although Tre6P has been strongly implicated in the control of photoassimilate partitioning in leaves via redox activation of ADPglucose pyrophosphorylase. We are currently testing the hypothesis that Tre6P also acts as a signal of sucrose availability in meristematic regions and developing organs, where it is integrated with other signalling pathways, e.g. auxins and cytokinins, to regulate the growth and development of the plant.

S11-04 A R2R3 MYB gene subfamily regulates aliphatic glucosinolate accumulation in Arabidopsis
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Glucosinolates are natural metabolites in the order Brassicales that defend plants against both herbivores and pathogens and can attract specialized insects (Halkier and Gershenzon 2006). We identified three R2R3 MYB transcription factors, MYB28, MYB29 and MYB76, regulating aliphatic glucosinolate biosynthesis in Arabidopsis by combining several systems biology tools (Sønderby et al. 2007). These three genes provide a unique system in which to study the evolution of MYB regulatory factors and their downstream targets. All three individual MYB genes had the capacity to increase aliphatic glucosinolates contents in leaves and seeds and induce gene expression of aliphatic biosynthetic genes within leaves when over-expressed. Leaves and seeds of single knockout mutants in MYB29 and MYB76 have reductions in only short-chained aliphatic glucosinolates whereas MYB28 mutant has reductions in both short- and long-chained. A double knockout in MYB28 and MYB29 was completely devoid of aliphatic glucosinolates suggesting a complex regulatory mechanism since the absence could not have been predicted by the chemotypes of the single knockouts. Recent results show that a double knockout in MYB28 and MYB76 did not show this epistatic effect on aliphatic glucosinolates but rather an additive effect. Results (e.g. microarray) will be presented that further unravel the differences in regulatory potential among the three genes.

Sønderby et al. (2007) PLoS ONE 19: e1322

S11-05 Mitochondrial control of cellular NAD(P)H reduction levels
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Plant mitochondria contain energy bypasses which allow respiration without inherent linkage to proton pumping and ATP production. These include alternative external NAD(P)H dehydrogenases, which oxidise cytosolic NADH or NADPH and reduce ubiquinone. In potato and arabidopsis, NDB1 is an external calcium-dependent NADPH dehydrogenase, whereas the Arabidopsis NDB2 is a calcium-stimulated NADH dehydrogenase and NDB4 is a calcium-independent NADH dehydrogenase (Geisler et al. 2007, Michalecka et al. 2004). The enzymatic system thus potentially allows fine tuning of cytosolic NADH and NADPH reduction levels. Analyses of transgenic Nicotiana sylvestris, modified to overexpress and suppress NDB1, showed that the external NADPH dehydrogenase indeed is able to specifically modify the cellular NADPH level. Comparison to the CMSII mutant, deficient in the complex I NADH dehydrogenase, also showed a lack of communication between the NADH and NADPH redox couples (Liu et al. 2008). Present work aims at determining physiological situations where changes in NADPH reduction affect cellular processes.


S11-06 Vacular malate channels in Arabidopsis
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CNR Genua, Institute of Biophysics, Italy
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In plants, malate is a central metabolite and fulfills a large number of functions. Vacular malate may reach high concentrations and fluctuate rapidly, whereas cytosolic malate is kept at a constant level allowing optimal metabolism. Recently, a vacular malate transporter (A. thaliana tonoplast dicarboxylate transporter, AtTDT) was identified that did not correspond to the well-characterized vacular malate channel. We therefore hypothesized that at least one member of the aluminum-activated malate transporter (ALMT) gene family could code for a vacular malate channel. We could show that AtALMT9 is targeted to the tonoplast and is expressed in all organs, but is cell-type specific as GU5 activity in leaves was detected nearly exclusively in mesophyll cells. Atalmt9 T-DNA insertion mutant exhibited strongly reduced vacular malate channel activity, whereas overexpression in tobacco leaves strongly enhanced the malate current densities across the mesophyll tonoplasts. Functional expression of AtALMT9 in oocytes induced anion currents, clearly distinguishable from endogenous oocyte currents. Our results demonstrate that AtALMT9 is a vacular malate channel. Actually we are investigating a second member of the ALMT gene family also localized in the tonoplast. In contrast to AtALMT9, this gene is expressed mainly in stomata and flower tissues. Patch-clamp analysis on isolated vacuoles of overexpressing plants showed a Ca2+ dependent activation of the malate channel activity.

S12-01 Impact of cationic cell-wall-peroxidase (CWPO-C) homolog on lignin in Arabidopsis thaliana
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PARALLEL SESSION 12: PEROXIDASE
2008 SATELLITE SYMPOSIUM: PLANT PEROXIDASES

S12-01 Impact of cationic cell-wall-peroxidase (CWPO-C) homolog on lignin in Arabidopsis thaliana
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Physiol. Plant. 133, 2008
A unique peroxidase isoenzyme, cationic cell-wall-peroxidase (CWPO-C), from poplar oxidizes sinapyl alcohol, ferrocyanochrome c and synthetic lignin polymers, unlike other plant peroxidases. Recently, catalytic mechanism of CWPO-C was investigated using chemical modification and homology modeling. It was suggested that Tyr residues on the protein surface, such as Tyr-177 and Tyr-74, are considered to be important for the oxidation activities of CWPO-C with a wide range of substrates, including lignin. In this study, we focused on CWPO-C homolog genes of Arabidopsis thaliana, and these T-DNA mutant lines were analyzed. Within the seven mutant lines, lignins in mutant line six and line seven were reduced by 12.6% and by 15.9% compared to wild type, respectively. Mutant line six and line seven are defective in the genes encoding peroxidase carries Tyr-177 and Tyr-74, respectively. Moreover, yield of uncondensed type monomers was increased in these mutant lines, as determined by DFRC analysis. On the other hand, other mutant lines displayed no observable differences in lignin and overall growth. These results suggest that peroxidase carries Tyr-177 and/or Tyr-74 has an impact on the amount of lignin and lignin assembly in the cell walls.

S12-03 Peroxidase and lignification in flax (Linum usitatissimum)
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In flax stem tissues, lignin represents nearly 25% of cell wall polymers in xylem whereas only very low amounts are deposited in the bast fibres. In order to gain a better understanding of the tissue heterogeneity regarding lignification in flax, we have examined two aspects related to the polymerisation step: H₂O₂ localization in the bast fibre and xylem, and cell wall peroxidases (CW PODs) activity. Indeed, many studies have shown that anionic and/or cationic CW PODs are involved in lignification. In parallel we have investigated the in vitro dehydrothermalisation of monolignol using cationic PODs.

• At flowering stage, H₂O₂ was detected in xylem cells at the bottom and the snap point of the stem whereas H₂O₂ was shown in the bast fibres at the snap point level. H₂O₂ availability might thus account for the tissue heterogeneity regarding lignification.

• Flax CW PODs occurred mostly as cationic forms in both xylem and bast fibres and were able to oxidize both coniferyl and sinapyl alcohols. The activity of xylem POD towards sinapyl alcohol was three times higher compared to fibre POD.

• In vitro polymerisation of coniferyl alcohol gave rise to β-O-4 enriched DHP when cationic horseradish POD is reduced. However a larger decline in POD allowed very low yield of DHP synthesis suggesting that POD may control lignification. The use of cationic POD enabled the recovery of DHP which had similar characteristics than DHP obtained with HRP.

S12-04 Ionically bound cell wall peroxidase activity explain the cadmium induced growth inhibition in Brassica juncea seedlings
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Research on cell wall bound peroxidases in plants has gained a considerable attention in recent years mainly due to its function in plant growth and development. The effect of cadmium on growth, lipid peroxidation, ionically bound CWP and H₂O₂ level in seedlings of Brassica juncea treated with 0–200 μM CdCl₂ were investigated. Cadmium was found to be effective in growth inhibition via reducing root length, dry weight and chlorophyll content. Treatment with CdCl₂ resulted in an increase in lipid peroxidation. A significant increase in ionically bound CWP activity in roots and leaves of seedlings were found to be directly correlated with significant increase in H₂O₂ level in same tissues when treated with 5, 50 and 100 μM of cadmium. Non redox active metals (Cd, As and Hg) were found to be more effective in ionically bound CWP induction in roots in comparison to redox active metals (Cu, Zn and Ni) is very interesting outcome of the present study. Exogenous application of sodium benzoate resulted in reduced enzyme activity in roots. So the significant increase in ionically bound CWP activity and H₂O₂ level in seedlings treated with cadmium and its reduction via antioxidant pretreatment suggests the role of ionically bound CWPs in metabolic adaptation to cadmium stress in Brassica juncea seedlings via H₂O₂ dependent cell wall peroxidase catalyzed formation of cross-linking among cell wall polymers.

S12-05 Characterisation of lignin-binding peroxidases
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The high number of peroxidase genes explains the description of numerous physiological functions and the fact that the in planta function of a single isoform has never been characterized yet. We analyzed in transgenic Arabidopsis thaliana the localization of a zucchini isoperoxidase (APRX), previously purified on his pectin function of a single isoform has never been characterized yet. We analyzed in transgenic Arabidopsis thaliana the localization of a zucchini isoperoxidase (APRX), previously purified on his pectin binding activity. We confirmed that the protein is localized near the cell wall, is mainly produced in the elongation area of the hypocotyls and respond to exogenous auxin. In addition, the ectopic expression of APRX induced changes in growth pattern and a significant reduction of endogenous indole-3-acetic acid (IAA) level. In agreement with these observations APRX showed an elevated in vitro auxin oxidase activity. We propose that APRX participates in the local negative feedback regulation of auxin level in the cell wall and consequently terminates the elongation process. To our knowledge this is the first unambiguous report of the in planta function of a specific peroxidase isoform.
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We study lignin polymerisation in Norway spruce using as a model a cell culture line that produces lignin-like precipitate into the culture medium. The polymerisation is catalysed by an extracellular peroxidase activity, possibly assisted by a low laccase-like activity. We have isolated basic peroxidases that are reversibly bound to the released suspension culture lignin (RSCL) and can be released with high salt concentration.

We analysed the specificity of this interaction using in vitro-binding assays. Culture medium and RSCL-extracted proteins were incubated with RSCL, milled wood lignin (MWL), synthetic dehydrogenation polymer (DHP), Ca²⁺-pectate or Ca²⁺-alginate, and the bound proteins were extracted with salt. While hardly any culture medium peroxidases were able to bind to the polymers, 10–50% of RSCL-extracted peroxidase activity was bound under the same conditions. Binding to pectate and alginate was similar, suggesting an unspecific interaction between charged polymers rather than specific affinity to pectin. However, peroxidase binding to DHP proved that interaction with purely phenylpropanoid-derived polymers also takes place. The greatest degree of binding was observed with RSCL, to which half of the used peroxidase activity was bound. RSCL contains some carbohydrates also, and the binding is possibly a combined affinity of peroxidases to lignin and charged carbohydrates. These interactions are being studied in more detail.

S12-06 Cross-talk between signalling pathways in plant defence: following peroxidases

L. Almagro, S. Belchi-Navarro, L. V. Gomez-Rosa, M. J. Martinez-Esteso, S. Selles, R. Bru and M. A. Pedreno

We have isolated basic peroxidases that are reversibly bound to the released suspension culture lignin (RSCL) and can be released with high salt concentration. We analysed the specificity of this interaction using in vitro-binding assays. Culture medium and RSCL-extracted proteins were incubated with RSCL, milled wood lignin (MWL), synthetic dehydrogenation polymer (DHP), Ca²⁺-pectate or Ca²⁺-alginate, and the bound proteins were extracted with salt. While hardly any culture medium peroxidases were able to bind to the polymers, 10–50% of RSCL-extracted peroxidase activity was bound under the same conditions. Binding to pectate and alginate was similar, suggesting an unspecific interaction between charged polymers rather than specific affinity to pectin. However, peroxidase binding to DHP proved that interaction with purely phenylpropanoid-derived polymers also takes place. The greatest degree of binding was observed with RSCL, to which half of the used peroxidase activity was bound. RSCL contains some carbohydrates also, and the binding is possibly a combined affinity of peroxidases to lignin and charged carbohydrates. These interactions are being studied in more detail.

S12-07 Chitin-specific peroxidases and the plant defence

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The formation of lignin in the infection zone and the expression of peroxidase genes are the most active responses of plants to fungi infection. Unfortunately, the mechanisms of plants resistance with participation of peroxidases, which play the important role in regulation of lignin level in the infection zone, are poorly studied. This assumes concentration of reactive oxygen species generators and its scavengers on infectious structures of fungi and to create the test system of chitin diagnostics on a fungi mycelium surface similar. We have revealed the property of peroxidase from different species of plants to bind both on chitin and cell wall of pathogenic fungi (Maksimov et al. 2003). The ability of peroxidase to bind on chitin without lost of enzyme activity suggests the possibility of oxidation of phenolic compounds in direct contact with fungi mycelium and directly initiate oxidative burst on a fungi mycelium surface. It is the new mechanism of peroxidase participation in the protective reactions of plants. Since in the literature there already were assumptions that polysaccharides are not simple catalysts of lignification process (Rasmussen et al. 1995) and they play a role of matrix for initiation of lignification (Liu, Kolattukudy 1997), the received results can prompt some aspects of signal transduction and start of plant protective reactions in response to elicitors action. This work was supported by RFBR 05-04-48310.

S12-08 Genetic differences among Apera spica-venti populations, revealed by peroxidase variability

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Apera spica-venti L. (silky bentgrass), an invasive weed species of winter cereals in Europe and Canada, was intensively investigated genetically by Warwick (1987) at 12 loci of nine enzyme systems, excluding peroxidases. Since peroxidases turned out to be good markers for intra- and inter-specific diversity in other grass species (Krzakowa 1996, Krzakowa & Dunaiski 2007, Krzakowa et al. 2006), in this study they were used for an analysis of genetic variability of Apera spica-venti. This grass species has become especially interesting, as it has been intensively treated with herbicides in recent years. Seeds from randomly collected panicles from 10 populations were sown in uniform greenhouse conditions, and three-week-old seedlings (30–116 individuals per population) were examined. Four electrophoretically separated loci were detected: one with anodal and three with cathodal migration. Three of them were polymorphic, so their frequencies were used for description of genetic variation in the populations investigated. Populations were spaced a few to >400 km apart. Geographic distances between populations were not reflected in their genetic similarity. The level of observed heterozygosity (Ho) in separate loci was generally lower than expected. Intra-population variation (GST = 0.32) was higher than between populations (DST = 0.17). Gene flow between populations was rather low (Nm = 7.55),
Stomatal guard cells regulate CO₂ influx into leaves for photosynthetic carbon fixation in exchange for plant water loss. Elevated CO₂ is an important signal that mediates stomatal closing. The continuing atmospheric CO₂ rise causes reduction in stomatal apertures and thus is modifying plant gas exchange and plant water use efficiency on a global scale. However, the mechanisms that mediate CO₂-induced stomatal signal transduction have remained relatively obscure. In recent research the gca2 and slac1 mutants were identified and characterized as first mutants that are strongly impaired in high (CO₂)-induced stomatal closing (Negi et al. 2008, Vahisalu et al. 2008, Young et al. 2006). Mutations in the plasma membrane protein encoding gene, SLAC1, disrupt S-type anion channels (Vahisalu et al. 2008). However, the mechanisms that directly mediate CO₂ sensing remain unknown. New results will be presented describing a gene that functions in CO₂ sensing. Previous research points to a model for how CO₂ mediates Ca²⁺ signaling, through enhancing or priming intracellular Ca²⁺ sensitivity (Young et al. 2006). This model provides a potential mechanism for encoding specificity in Ca²⁺ signaling. We have identified CDPKs that function as Ca²⁺ sensors in stomatal closing (Mori et al. 2006). New evidence will also be presented that supports this 'Ca²⁺ sensitivity priming' hypothesis.

Young JJ et al. (2006) Proc Natl Acad Sci USA 103: 7506–7511

L22 Genes gone wild: intra- and intercellular travel of DNA
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Chloroplasts (plastids) and mitochondria have evolved from free-living eubacterial ancestors through endosymbiosis. Plastid and mitochondrial genomes occur at high copy numbers, with up to thousands of genome copies being present in a single cell. In most plant species, the organelles and their genomes are believed to be inherited maternally and thus excluded from pollen transmission. In my talk, I will address three recently uncovered types of genetic leakiness organelar genomes are involved in: – the occasional paternal inheritance of organelles (paternal leakage), – the movement of organellar DNA into the nuclear genome, – the movement of organellar DNA between plants by horizontal gene transfer. Using combinations of transgenic tools and stringent selection schemes, we have reproduced these three processes in the labora-
tory. The implications for our understanding of genome integrity and evolution will be discussed.

L23 How plants survive the night
A. M. Smith
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Although plants are classed as autotrophic organisms, they face problems of carbohydrate supply on a daily basis. First, most of the cells in a plant are heterotrophic – dependent for their carbon supply on carbohydrate (in the form of sucrose) imported from the relatively small number of photosynthetic cells in the leaves. Second, plants can photosynthesise only during the day – every night all of the cells of the plant become dependent upon the mobilisation of carbohydrate (in the form of starch) synthesised and stored during the day. Mutant plants that cannot synthesise starch during the day or cannot degrade it at night have reduced growth rates. My lab is trying to understand the diurnal control of starch storage and mobilisation in leaves of the model plant Arabidopsis, using forward and reverse genetic approaches. I will present our progress in defining the pathway of starch degradation at night, which has throw up many complexities and surprises. I will discuss recent work on the control of this pathway, and hence of carbohydrate availability in the plant during the night: we have discovered that the circadian clocks play a central role in this respect. Finally I will describe unexpected findings about the ways in which non-photosynthetic cells mobilise the sucrose they receive from the leaves.

L24 Host range and signal transduction in symbiosis
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Development of root nodules in legumes in response to signals secreted from rhizobia is an example of inducible organ formation. Lipochito-oligosaccharides (Nod-factors) are the rhizobial morphogenetic signal inducing root hair deformation and cell division leading to formation of nodule primordia. An important determinant of bacterial host specificity is the structure of the Nod-factor suggesting that a plant receptor is involved in signal perception. The role of two Lotus japonicus LysM type receptor kinases, NFR1 and NFR5, in perception of Nod-factor signals will be discussed. The extracellular domains of the two transmembrane kinases carries LysM domains suggesting that they may be directly involved in binding of the Nod-factor and in deciphering the structure. Domain swaps addressing this question will be presented and the involvement of NFR1 and NFR5 receptor kinases in the earliest physiological and cellular responses will be illustrated. Mutant analysis in Lotus japonicus has identified spontaneously nodulating mutants forming empty root nodules in the absence of M. loti. This demonstrates that the complex root nodule organogenic program can be genetically deregulated. The role the identified gain of function calcium calmodulin-dependent protein kinase (CCaMK) and the cytokinin receptor (LHK1) in converting fully differentiated root cortical cells into meristematic founder cells of root nodule primordia will be discussed.
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PARALLEL SESSIONS ON FRIDAY, 22 AUGUST 2008
PARALLEL SESSION 13: SYSTEMS BIOLOGY/-OMICS

S13-01 Chloroplast proteome analysis: new insights into intracellular trafficking
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The biogenesis and functionality of plastids requires the import of several thousand nuclear-encoded proteins. The proper targeting and the selective import of cytosolic preproteins rely on a plastid protein import machinery consisting of TOC (translocon at the outer chloroplastic membrane) and TIC (translocon at the inner chloroplastic membrane) protein complexes (1). Recent data suggested furthermore, that in addition to TOC/TIC-mediated import, alternative routes exist that direct plastid proteins through the secretory pathway (2). High throughput proteomics data support this view and suggest, that intracellular protein trafficking may be more complex than previously anticipated (3–4). Therefore, we analyzed the proteome of two plastid protein import mutants, ppi1 and ppi2, lacking important components of the plastid protein import machinery. The first is defective in TOC33 and the second in TOC159. This approach aims on one hand to elucidate the contribution of the alternative import pathways and on the other to enrich plastids for those proteins that carry non canonical targeting information. We present here a comprehensive quantitative characterization of protein accumulation in these different plastid types and discuss robustness principles for the assembly of organellar proteomes as well as alternative import routes based on the identification of unusual transit peptide structures.

S13-02 Global and specific translational regulation during diurnal cycles in Arabidopsis thaliana rosette leaves
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Genome-wide transcript profiles from Arabidopsis thaliana leaves show that transcript levels undergo marked and rapid changes during diurnal cycles and after prolonged darkness. The changes in the activities of enzymes of primary metabolism are smaller, delayed, and sometimes reciprocal to the changes in the transcripts encoding them. This points to the importance of translational regulation and protein turnover. To investigate translational control during diurnal cycles in leaves of wild-type plants we compared changes in translation by polysome analysis with changes in transcripts levels and enzyme activities. In samples taken at end of the night and after 2 h light, we measured transcripts levels and translational changes of 90 genes encoding enzymes of central metabolism and the corresponding 30 enzyme activities. Polysome levels showed dramatic diurnal changes, while total ribosomes, RNA, and protein were stable. Polysomes were lowest at the end of the night, rose rapidly in the first 2 h in the light, and declined gradually during the rest of the cycle. These results reveal that protein synthesis is shaped by global changes in translational activity, with a rapid increase during the day and a marked decrease during the night, probably in relation to light and carbon status. With a few exceptions, this result was confirmed for individual enzymes. Preliminary results suggest that the daily contribution of protein synthesis is relatively minor for most enzymes.

S13-03 Molecular computer simulations to understand plant membrane channel/transporter function
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Plants regulate the permeability of their membranes to water and small, uncharged molecules by the expression of various channels and transporters. Most of the involved membrane protein families have homologs in prokaryotes and man, which serve as model systems to understand their biophysical behavior and selectivity. However, plants have different requirements and have adapted unique structural inventions to meet nutritional, energetic and environmental demands for their membrane permeability. We therefore adapted molecular computer simulations to study plant plasma membrane channels. Using a plant aquaporin tetramer structure, we confirmed that the constriction region in the monomers is the crucial determinant of their ammonia and urea conductance. The impact of the residues in the constriction region was experimentally verified using mutants of the plasma membrane aquaporin AtPIP2;1. The selectivity filter residues were exchanged to all combinations that occur in Arabidopsis NIP and TIP channels. Several, but not all AtPIP2;1 mutants with a selectivity filter of NIP homologs were capable of promoting yeast growth on ammonia or urea as sole nitrogen source. The impact of such computer simulations, together with their use on homology models of plant membrane transporters is discussed.

S13-04 A functional stem cell niche is not required in Arabidopsis root tip regeneration
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Regeneration is the process of re-establishment of cellular identities and patterns of lost structures in an adult body. It is often assumed that organ regeneration in plants depends precisely on the same stem cells supporting continuous (indeterminate) growth during post-embryonic development, so that early re-appearance of a functional stem cell niche is usually expected for regeneration. One alternative hypothesis is that regeneration differs in this respect from indeterminate growth, and that a functional niche is not required. We use the Arabidopsis root to investigate the stem cell niche role during plant organ regeneration, by integrating over time confocal imaging with global transcriptional profiling of regenerating stumps after complete whole-tip excision in various genetic and chemical backgrounds.
Our results suggest a rapid restoration of missing cell fate and function before the recovery of stem cell activity. Surprisingly, mutants deficient in stem cell niche maintenance were still able to re-establish the lost pattern and cell fates. Moreover, young leaves, lacking a stem cell niche and indeterminate growth, regenerated after partial excision, demonstrating the wide capacity to re-pattern organs without a central organizer. These results separate the function of the stem cell niche in indeterminate growth from the regeneration processes, where a combination of cell fate plasticity and stem cells-independent patterning mechanisms seems instead to play a role.

**PARALLEL SESSION 14:**
**CELL BIOLOGY**

**S14-01 Subcellular distribution of glutathione in Arabidopsis thaliana**
B. Zechmann*, F. Mauch and M. Mueller*

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The tripeptide glutathione is a major antioxidant and redox buffer with multiple roles in plant defense and metabolism. It is synthesized out of its precursors, cysteine, glutamate and glycine, in two steps which are restricted to the cytosol and plastids. Glutathione is then distributed to the other organelles. Inter- and intracellular glutathione, its precursor levels and their ratio between certain cell compartments are therefore important measurements of the plants ability to sense and fight oxidative stress and can give key information about its physiological condition. This study presents a method that allows the visualization of glutathione and its precursors in all cell compartments simultaneously in one experiment at a high level of resolution and is based on immunogold cytochemistry and computer-supported transmission electron microscopy. By applying this method on transgenic and non-transgenic Arabidopsis plants it was not only possible to demonstrate the specificity and accuracy of this method, but also to obtain a thorough knowledge about glutathione synthesis and degradation in plants. In this study, these results are summarized and compared with the subcellular distribution of glutathione and its precursors in other plant species, and the importance of compartment specific glutathione in plant cell metabolism, defense, growth and development is discussed. Acknowledgement: This work was supported by the Austrian Science Fund (P18076 and P20619).

**S14-02 Imaging the early events of TMV infection**
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Almost nothing is known of the early stages of TMV infection. To address this, we directly labelled the viral RNA of TMV by incorporation of UTP-Cy3 and injected it onto the cytoplasm of living tobacco trichome cells. The Cy3-labelled virions were infectious and the viral genome trafficked from cell-to-cell. However, neither labelled vRNA nor co-injected GFP were able to pass out of the initial injected trichome, indicating that virus movement out of trichomes is not accompanied by passive plasmodesmatal gating. Both Cy3-virions and uncoated Cy3-vRNA formed granules that became anchored to the motile cortical ER/actin network of the trichome cell within minutes of injection. Movement of vRNA granules on the actin/ER was arrested by inhibitors of the actin cytoskeleton. TMV capping was shown to be required for vRNA anchoring to the ER. Virions, or vRNA, lacking the 5'cap failed to form RNA transport granules and vRNA granules were degraded in the host-cell cytoplasm. Deleting the 3' prime UTR region from TMV virions did not affect the initial formation or anchoring of vRNA granules. We subsequently generated dual-labelled infectious TMV virions in which the vRNA was labelled with Cy3 (red) and the capsid protein was labelled with Cy2 (green). Following injection, both red and green signals were located on the same ER-bound granules, with a subsequent loss of the green signal only, indicating that in natural infections TMV virions are anchored to the ER prior to uncoating of the viral genome.

**S14-03 Plant LIM proteins – a family of actin binding proteins**
M. Dieterle*, C. Thomas, C. Hoffmann, J. Papuga, S. Tholl, F. Moreau and A. Steinmetz

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The actin cytoskeleton of plants is required for cytoplasmic organization, serves as track for intracellular transport, and functions in tip growth processes. Actin cytoskeleton dynamics and organization are regulated by actin binding proteins. We recently showed that the tobacco LIM protein NtWLIM1 associates with the actin cytoskeleton and bundles actin filaments in vitro as well as in vivo. In order to compare and understand the roles of a full set of LIM proteins in plant development we use Arabidopsis thaliana as a model organism. The Arabidopsis genome encodes six LIM proteins. The analysis of promoter-GUS lines and publically available microarray data show that three of them are mainly expressed in pollen (PLIM1-3), while the others are widely expressed in different tissues (WLIM1-3). When expressed in planta as fusion with GFP all six proteins associate with filamentous structures, suggesting an interaction with F-actin. We show that, similar to NtWLIM1, recombinant Arabidopsis LIM proteins are able to bind and bundle actin filaments in vitro with similar affinities. We isolated insertion mutants for each of the LIM genes. Since none of the single mutants was found so far to exhibit a discernible phenotype we are establishing double and triple mutants to unravel the functions of LIM proteins in plant development.

**S14-04 Pattern formation of the vascular tissues in the root of Arabidopsis**
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The plant vasculature connects all parts of the plant and allows transport of water, nutrients, and signalling molecules. Our goal of...
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research is to understand the development of vasculature and how this vital process is regulated. We have performed a genetic screen for mutants displaying mis-expression of the phloem-marker gene AtSUC2:GFP. This resulted in the identification of a set of novel mutants with patterning defects specific to the stele, distorted root vascular pattern 1-7 (dv1-7). dv1 is a novel gain-of-function allele of PHARULOISA (PHB), a member of the HD-ZIP class III family. It displays ectopic metaxylem formation in the root. short root (shr) has a similar xylem phenotype as dv1 and in addition, loss of endodermis. When both genes are knocked out, protoxylem development is rescued. This indicates that the xylem defect in shr is a result of mis-regulation of PHB and that SHR controls PHB expression.

Another mutant, dv2, resembles dv1 and shr and displays ectopic metaxylem formation and partial loss of endodermis. SHR is moving from stele into the QC and endodermis to regulate the cell division of endodermis/cortex initials and to maintain the stem cells. Interestingly, SHR does not move effectively out of the stele in dv2. It carries a mutation in one of the glycosyl transferase genes. We postulate that DVA2 is involved in cell communication in the root. Characterization of dv2 and discussion of its putative role in vascular patterning will be presented.

S14-05 Plant cell polarity and expansion is regulated by an exocyst complex
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The exocyst, an octameric tethering complex and effector of Rho and Rab GTPases, is involved in polarized secretion in yeast and animals. We provide genetic, cell biological and biochemical evidence that exocyst complex functions also in plants. In Arabidopsis thaliana, double mutants in exocyst subunits (sec5 exo70A1, or sec8 exo70A1) show a synergistic defect in etiolated hypocotyl elongation. Mutants in exocyst subunits SEC5, SEC6, SEC8 and SEC15a show similarly defective pollen germination and pollen tube growth phenotypes. Antibodies against SEC6, SEC8 and EXO70A1 demonstrate co-localization of these proteins at the apex of growing tobacco pollen tubes. SEC3, SEC5, SEC6, SEC8, SEC10, SEC15a, and EXO70 subunits co-purify in a high molecular weight fraction of 900 kDa after chromatographic fractionation of Arabidopsis cell suspension extract. Blue native electrophoresis confirmed the presence of SEC3, SEC6, SEC8, and EXO70 in high molecular weight complexes. Finally, the yeast two-hybrid system revealed interaction of Arabidopsis SEC10 with SEC15b, and SEC6 with SEC8. We conclude that the exocyst functions as a complex in plant cells, where it plays important roles in morphogenesis. Lab of V.Z. was supported by the Ministry of Education, Youth and Sports MSMT of the Czech Republic (MSMT Kontakt ME841, MSMT LC06034 “REMEROST” and MSM0021620858). The work in the lab of J.E.F. was supported by the US National Science Foundation (IBN-0420226).

S14-06 Root positive gravitropism changes on negative in the combined magnetic field
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Gravitropism is a gravity directed re-orientation of plant growth. Understanding the mechanisms of plant gravitropic reactions belongs to the one of the central problems in plant biology. Although gravitropism have been studied for many decades, a lot of questions on plant gravitropism, including the questions on Ca2+ participation in graviperception and signal transduction, remains open and requires new experimental data. We have used a new original model for the study of root gravitropism - gravistimulation in the weak combined magnetic field (CMF) with 32 Hz frequency created inside μ-metal shield, and for the first time showed the changes in the direction of a cress root gravitropic reaction in this field. A negative gravitropic reaction in the CMF occurs by a usual physiological process. Experiments in the CMF confirmed that gravitropism is plastid-based and Ca2+ ions participate in this process. Unlike control, amyloplasts-statoliths are localized along the statocyte upper longitudinal side after 1 h after gravistimulation. It is of a special interest that a root is bending to the same direction with displacing of amyloplasts: in positive gravitropism – downwards, in negative gravitropism – upwards. The obtained data are discussed in the light of the ion cyclotron resonance model: energy and direction of Ca2+ ion rotation change in the CMF with frequency adjusted to cyclotron frequency of Ca2+ ions.

PARALLEL SESSION 15: WATER, MINERALS AND TRANSPORT

S15-01 Genetics to unravell phosphate sensing and responses in Arabidopsis thaliana
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Mineral starvation is though to reduce plant growth by reducing metabolic activity, however this might be an oversimplified view. By using the Arabidopsis natural variation we have identified a major QTL (LPR1 = Low Phosphate Response1), and its paralogue LPR2, two genes that reduce the primary growth when seedlings are on a
nitrogen, which is essential for the growth of all living organisms. This process involves the conversion of ammonia (NH₃) into a form that can be used by plants. The study examines the role of aquaporins, which are proteins that facilitate the transport of water and other small molecules across biological membranes. In this case, the focus is on ammonia transport, which is crucial for the growth and development of plants. The research highlights the importance of aquaporins in maintaining the balance of ammonia across the plasma membrane, a critical process for plant health and productivity. The study also underscores the potential of using these transport mechanisms in biotechnological applications, such as in the creation of more efficient and resilient crop varieties.
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range of physiological functions. Furthermore, nitrate strongly regulates plant development, especially lateral root growth. However, molecular data concerning the nitrate signalling mechanisms are scarce. In particular, the upstream sensing systems triggering nitrate signalling are unknown. In eukaryotes, the picture emerges that transporters or transporter-like proteins play a pivotal role as sensors for nutrients. In line with this hypothesis, we have proposed that the A. thaliana nitrate transporter NRT1.1 acts as a nitrate sensor. NRT1.1 participates in root uptake of nitrate, but knock-out mutants for this transporter display alterations of lateral root growth that cannot be accounted for by a decrease in root nitrate uptake. NRT1.1 triggers a nitrate signalling pathway which promotes lateral root elongation in presence of high external nitrate concentration. Furthermore, NRT1.1-dependent nitrate signalling regulates not only root growth, but also other nitrate transporters, indicating that NRT1.1 governs both physiological and developmental responses of the plant to nitrate. Mutation of NRT1.1 results in an alteration of the expression of many genes of hormone metabolism/signalling, suggesting that hormones (especially cytokinins and auxin) act as secondary messengers in NRT1.1-dependent nitrate signalling.

S15-06 Iron bio-fortification approaches in rice by genetic engineering
J. Wirth*, B. Drosse, A. Zambrana, P. Lucca, S. Poletti, N. Yakandavala, I. Potrykus, W. Gruissem and C. Sautter
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About one third of the world population suffers from Fe malnutrition, concerning mainly women and children in developing countries, where rice is the staple food. Since rice becomes rancid during storage rice has to be polished. However, the polished rice grain lacks essential vitamins and micronutrients such as Fe and Zn. We are studying genetic engineering as an approach to improve Fe content in rice endosperm by introducing transgenes in rice, which are known to be involved in Fe metabolism. First nicotianamine synthase (NAS) producing nicotianamine, which chelates Fe and plays a role in phytosiderophore production and Fe translocation in the plant. Second ferritine, an Fe storage protein and third phytase, coding for an enzyme that degrades phytate, an Fe- and Zn-binding anti-nutritional compound. The latter are under the control of an endosperm specific promoter. Finally, we explore the Fe2+-transporter (IRT1) under the control of a root specific promoter. Two types of transgenic rice lines are currently available: One containing three transgenes (NAS, ferritine and phytase) and one containing the IRT gene. The transgenic lines have been characterized and compared to the wildtype. Endogenous rice gene expression levels were measured, phenotypic observations were made, and Fe concentrations in leaves and grains grown under different levels of Fe nutrition were determined. We will discuss results about Fe homeostasis and the consequences for genetic engineering.

S15-02 Gene clusters and metabolic diversification in plants
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Operons are clusters of unrelated genes with related functions that are a feature of prokaryotic genomes. Here we report on an operon-like gene cluster in the plant Arabidopsis thaliana that is required for the synthesis of a new and distinct group of triterpenes (the thalianol pathway) (Field and Osbourn et al. 2008. The clustered genes are co-expressed, as in bacterial operons. However, despite the resemblance to a bacterial operon, this gene cluster has been assembled from plant genes by genome reorganization rather than by horizontal gene transfer from bacteria. Furthermore, recent assembly of operon-like gene clusters for triterpene synthesis has occurred independently in divergent plant lineages (the thalianol pathway in Arabidopsis and the averacn pathway in oat), and was accompanied by the rapid expansion and functional diversification of lineage-specific gene families. Thus, selection pressure may act during the formation of certain plant metabolic pathways to drive gene clustering. Together, our findings suggest that eukaryotic genomes have remarkable plasticity and that this feature can help drive metabolic diversification and adaptive evolution.


S16-01 Fingerprinting of protein kinase function in planta using chemical-genetics and phosphoproteomics
M. Böhmer* and T. Romeis*
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S16-03 Nano-sensors for in vivo, non-invasive pH measurements
C. K. Ytting*, A. Schulz and A. T. Fuglsang
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S15-06 Iron bio-fortification approaches in rice by genetic engineering
J. Wirth*, B. Drosse, A. Zambrana, P. Lucca, S. Poletti, N. Yakandavala, I. Potrykus, W. Gruissem and C. Sautter
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Using protein based sensors for pH measurements offers several advantages: – the sensors can be targeted for expression in specific tissues, cells and organelles, which gives a self-reporting system for pH changes, – the loading of a chemical dye can be avoided, i.e. the measurements are non-invasive, – recordings of pH changes are live. The sensors we have developed consist of fusions of different variants of green fluorescent protein, GFP. Some GFP variants are more sensitive to pH changes than others with respect to emission light. The fusion of a pH-sensitive GFP to a pH insensitive variant of GFP allows us to perform ratiometric pH measurements, which are independent on differences in sensor concentration and -fluctuations inside cells. We have two different sensors with different pKa values in the physiologically relevant range. These sensors are stably expressed in Arabidopsis, in both the apoplast and the cytosol. The detection method used for in vivo measurements is confocal laser scanning microscopy and we have been able to detect changes in cytosolic pH sensor signal after external stimulation of mesophyl cells. Using this tool we are currently investigating the activity and regulation of the plasma membrane H+-ATPase, primarily in root hair and guard cells.

PARALLEL SESSION 17: PEROXIDASE 2008 SATELLITE SYMPOSIUM: PEROXIDASE STRUCTURE AND FUNCTION

S17-01 Structural requirements for lignin peroxidase activity: engineering a redox active Trp in Coprinus Cinereus peroxidase
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The degradation of lignin is known to be rate limiting in the biosphere. Clean technologies to unlock lignin and hence cellulose as renewable sources of chemicals and fuels may be of great importance in the future. Lignin peroxidase is an enzyme produced by wood degrading fungi that play a key role in the degradation of lignin. It is able to oxidise extremely stable methoxy benzenes with very high (>1.4 V) redox potentials that then play a role in the destruction of lignin. This involves a free radical mediated reaction that eventually cleaves the C-C bonds in lignin. The origin of the enzymes ability to do this seems to depend on a specialised substrate interaction site containing a unique redox active Trp residue located in a highly acidic microenvironment. The availability of a synthetic gene for Coprinus cinereus peroxidase optimised for E. coli expression, has allowed us to successfully engineer veratryl alcohol oxidation activity (lignin peroxidase activity) into a commercial peroxidase which normally lacks this ability. Only three mutations, the redox active Trp itself and two adjacent charge replacements on the surface of the enzyme were necessary to confer this activity. The engineered enzyme is kinetically competent in the oxidation of veratryl alcohol, and gives rise to a trappable Trp radical on treatment with stoichiometric peroxide. Evidence is presented that the Trp radical is specifically reduced by veratryl alcohol.

S17-02 Class III peroxidases are involved in the construction of the glycosidic epitopes of arabinogalactan proteins
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Arabinogalactan proteins (AGPs) are complex proteoglycans found at the surface of all plant cells and they have been implicated in different aspects of plant development and plant cell physiology, with much of the supporting data coming from the time- or cell-specific localization of AGPs glycosidic epitopes recognized by several MABs. Class III peroxidases (Prxs) form a large multigene family typical of plants and they catalyze the oxidation of small molecules at the expense of H2O2. Prxs have been implicated in numerous physiological processes, particularly in key processes determining the architecture and defense properties of the plant cell wall. In this work, we present data showing that Prxs seem to be involved in the construction of several of the AGPs specific glycosidic epitopes which have been implicated in plant development and plant cell physiology. In vitro, the main Prx from the leaves of Catharanthus roseus, CrPrx1, was capable to mediate intra and inter-molecular cross-linking of AGPs resulting in the formation of new Jim8 and Jim13 epitopes. Furthermore, when C. roseus leaves were infiltrated with H2O2, we observed an increase of AGPs intensity of labeling by MABs Jim8, Jim13, Mac207 and LM2, which was sensitive to Prx inhibitors. This represents strong data indicating the identity of an in vivo Prx substrate and implicates Prxs in the developmental and cell physiology functions attributed to AGPs.

S17-03 Overexpression of sweetpotato apoplastic peroxidase results in increased hydrogen peroxide production and enhances stress tolerance
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We reported 10 peroxidase (POD) cDNA clones from cell cultures of sweetpotato (Ipomoea batatas). Among them, the expression of the swpa4 gene was profoundly induced by a variety of abiotic stresses and pathogenic infections (Mol Gen Genom 269: 542–552 2003; Plant Physiol Biochem 42: 451–455 2004; Plant Physiol Biochem 45: 908–914 2007). In the present study, transgenic tobacco (Nicotiana tabacum) plants overexpressing the swpa4 gene under the control of the CaMV 35S promoter were generated in order to assess the function of swpa4. Both transient expression analysis with the swpa4-GFP fusion protein and POD activity assays in the apoplastic washing fluid revealed that the swpa4 protein is secreted into the apoplastic space. We also demonstrated that the overexpression of apoplastic swpa4 in transgenic tobacco plants showed tolerance to a variety of abiotic and biotic stresses. Moreover, swpa4 expression caused an increase in H2O2 production followed by the induction of defense-related genes, including the apoplastic acidic PR genes. These results suggest that

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the expression of swpa4 in the apoplastic space may function as a positive signal in the H₂O₂-regulated stress response signaling pathway. Transgenic sweetpotato plants with overexpression or suppression of apoplastic swpa4 POD are under development to elucidate the exact role of swpa4 in the regulation of H₂O₂ production in the apoplasts of sweetpotato under stress conditions.

S17-04 Involvement of reactive oxygen species and peroxidases in the multiple pathways leading to Arabidopsis seed germination

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Germination is controlled by temperature, light, water uptake and hormone balance. Recently, reactive oxygen species (ROS) have been shown to act as messengers during plant development, stress response and programmed cell death. We investigated the possibility for ROS being signalling molecules in the germination process. By analysing the early visible steps of germination in Arabidopsis (the testa rupture and the endosperm rupture) and by using specific ROS scavengers, we identified ROS as new factors regulating germination. We have shown that ROS are released prior to seed envelopes rupture at a specific subcellular site of production. Class III peroxidases are privileged partners of ROS, and then they could be implicated in the spatio-temporal regulation of this process. In this line, the absence of particular isoform (Arabidopsis T-DNA line) provokes a large delay of the early step of germination. Treatment with H₂O₂ can increase or reduce the testa and the endosperm rupture depending of the light quality. Exogenous H₂O₂ also modify the transcriptional level of genes necessary for germination. We discuss the connections between ROS, light, abscisic acid and gibberellic acid signalling pathways.

S17-05 Compound II revisited

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Recent mechanistic studies on the enzymatic chlorination of taurine by myeloperoxidase (MPO) (Ramos et al. 2007) have shown a different behaviour for initial rates and for rates at equilibrium, being slower at equilibrium conditions due to the accumulation of compound II (MPO-II), which is outside the chlorination cycle. Kinetic evidence and the null effect of superoxide dismutase suggest that the formed hydroperoxide/superoxide radical is not released but forms a complex with compound II (MPO-II-HO₂) (Ramos et al. 2007).

To get further insight into this observation, electronic structure calculations using the DFT-based B3LYP method have been performed to reveal the genuine structure and certain chemical features of MPO-II and, additionally, compound II of other heme peroxidases. The active site was modelled by using porphine and imidazole surrounding oxoiron(IV) probing thermodynamics and geometries of MPO-II and MPO-II-HO₂.

The structural consequences of addition of HO₂• on regular MPO-II are shown. A stable complex with hydroperoxide radical (MPO-II-HO₂) can be proposed, while superoxide anion is only released following deprotonation. The findings are discussed with respect to proposed heme-linked ionization of a distal protein residue hydrogen bonded to oxoiron(IV) (Oertling et al. 1988).

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Ramos DR et al. (2007) Arch Biochem Biophys 466: 221
Oertling WA et al. (1988) Biochemistry 27: 5395

Abstracts

P01-011 The role of nitric oxide in ozone-induced cell death in Arabidopsis thaliana

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Nitric oxide (NO) is involved together with reactive oxygen species (ROS) in the activation of various stress responses in plants. However, the biochemical mechanisms by which ROS and NO participate in these processes are still unclear. Here we have elucidated the roles and interactions of ROS and NO in the induction and regulation of ozone-induced cell death. Ozone induced a rapid accumulation of NO in plant leaves. During the later time points, NO production coincided with the formation of HR-like lesions. Experiments using ozone, the NO-donor SNP and NO-scavenger PTIO suggested that NO is not the primary signal affecting ozone-induced cell death. NO induced genes encoding enzymes of ethylene biosynthesis, which are known to be involved in early time points of lesion propagation. NO also attenuated ozone-induction of SA biosynthetic genes, known to be involved in lesion propagation. These findings suggest a dualistic role for NO in ozone-induced cell death. In order to study further the role of NO in ozone-induced cell death, mutant rcd1 (radical-induced cell death) and a knockout allele of Arabidopsis nitric oxide associated 1 (Atnoa1) were used. Here we show that Atnoa1 is ozone sensitive and that the ozone sensitive rcd1 had higher basal NO levels when compared to the wild type, and discuss the role of NO in the mutant.

P01-012 Microarray based approaches to identify new genes controlling protoxylem differentiation in Arabidopsis

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The *Arabidopsis* root vascular cylinder has a central axis of xylem cell files consisting of protoxylem at marginal positions and metaxy-lem at central positions. We have previously shown that cytokinin signalling and its inhibitor, AHP6, are major components regulat-ing protoxylem identity. We have now used Fluorescently Activated Cell Sorting (FACS) coupled with microarray analyses to identify a new set of protoxylem specific genes. We are now characteris-ing these in further detail to identify factors acting downstream of AHP6.

P01-013 Tomato seed transcriptome changes related to phytocrome control of germination

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Tomato seed germination can be inhibited by continuous irradia-tion with far-red light (FrC) and this inhibition can be relieved by a subsequent red light pulse (Rp). The information about the molecu-lar mechanisms and signalling pathways involved in germination photocontrol is scarce. Using Potato microarrays, we carried out a global transcript analysis of wild type (cv. Money Maker) and phyA mutant seeds subjected to FrC inhibitory treatment with or without a subsequent Rp. A first analysis of global changes using micro-array data showed that inhibition by FrC involves the induction of a large number of genes and the repression of a significantly smaller quantity. Correspondence Analysis showed an underlying pattern of expression dependent on the physiological treatment and incubation time. Canonical Discriminant Analysis identified different clusters of genes that could be associated with dormancy maintenance, inhibition and promotion of germination. A number of genes related to Gibberellins, ABA and other regulatory factors were differentially affected by the light treatments. We also found several genes like GIGANTEA, CSN6, ELIP and RBP that so far had been found associated to other physiological processes and might be involved in light-modulated germination as elements of phytochrome signalling.

P01-014 Biotin deficiency causes spontaneous cell death and activation of defense signaling

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Biotin is essential for normal cellular functions, growth, and develop-ment in all living organisms. In addition to its catalytic function in the transfer of CO₂, biotin is also discussed to have a critical role in regulating gene expression. The first committed enzyme in biotin synthesis is 7-keto-8-aminopelargonic acid synthase encoded by At1g04620 (BioF). We isolated a novel spontaneous cell death mutant of *Arabidopsis thaliana* showing light-dependent visible lesions in rosette leaves with a T-DNA insertion located in the pro-moter of At1g04620. Both exogenous biotin and genetic comple-mentation were able to rescue the lesion phenotype. The mutant named bio4-1 exhibited massive accumulation of H₂O₂ and constitutive upregulation of a number of genes encoding for heat shock proteins as well as marker genes for salicylic acid, jasmonic acid and reactive oxygen species signaling. Interestingly, this was not accompanied by resistance to bacterial pathogens, which could be explained by uncoupling of PR mRNA and PR protein accumula-tion. Characterization of protein profiles showed that mitochondri-al-localized biotin-containing proteins in bio4-1 were similar to those in wild type, whereas the mutant plants had a substantial reduction in chloroplastic biotinylated proteins. Furthermore, we observed a lack of a novel nuclear-localized biotinylated polypep-tide in bio4-1.

P01-015 MAPKs and their substrates

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Mitogen-activated protein kinases (MAPKs) are activated by dif-ferent biotic and abiotic stresses like salt, cold and wounding. They exhibit their actions through the phosphorylation of different substrates but, so far, few of them have been identified. An effort to identify new MAPK substrates was done using an *Arabidopsis thaliana* cell culture. Putative substrates were then isolated using a high-throughput method based on phosphopeptide isolation by immobilised metal ion affinity chromatography (Fe₃⁺-IMAC) and mass spectrometry to detect pSer/pThr/pTyr-containing peptides. By bioinformatic analysis, a number of putative MAPK substrates were selected and purified from *Escherichia coli*. Substrate speci-ficity was subsequently tested by in vitro kinase assays with active MAPKs. Confirmation of MAPK substrate specificity was obtained by mutagenesis of the phosphorylation sites. The biological function of the phosphorylation sites in these substrates is currently investi-gated by various approaches that will be discussed.

P01-016 Identification of the endosperm-specific pro-teins involved in rice grain quality improvement

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The amounts, composition and distribution of seed storage pro-teins in rice (*Oryza sativa* L.) are important factors for deter-mination of quality. To improve rice quality, we have tried to isolate proteins specifically accumulated in the endosperm of a high-quality cultivar, GOPUMBYEO or a low-quality cultivar, DOBONGBYEO by proteomic method. By two dimensional elec-trophoresis using the endosperms at 15 days or 50 days after flowering, we selected GOPUMBYEO- or DOBONGBYEO-spe-ciﬁcally accumulated proteins at each stage and identiﬁed 48 protein spots by GC-MS. Finally, we chose 11 proteins which may play an important role in quality improvement or mainte-nance through regulation of oxidative stress at grain ﬁlling or pest resistance during storage. We are now investigating their expres-sion patterns and functional roles during endosperm develop-ment. This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.
Abstracts

P01-017 Rice histone deacetylase OsHDAC1 regulates OsNAC4 that controls root development
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We had previously isolated a rice gene encoding a histone deacetylase, OsHDAC1, and observed that transgenic overexpression of the gene alters root architecture of the OsHDAC1 seedlings. To identify transcriptional repression events that occur as a result of the OsHDAC1 overexpression, global expression profiling of root genes was performed on the OsHDAC1 plants or HDAC inhibitor-treated nontransgenic plants in comparison with non-transgenic control plants. Interestingly, OsNAC4 was identified as a key component of the OsHDAC1 regulon among the selected 39 different genes. Transgenic overexpression of OsNAC4 phenocopies of the OsHDAC1 knock-out line and the OsNAC4 knockout line phenotypes of OsHDAC1 overexpressors, indicating that OsNAC4 is a key component of the OsHDAC1. To address epigenetic effects of OsHDAC1 on histone modification of the OsNAC4 promoter region, we performed chromatin immunoprecipitation assays using a group of antibodies. Our ChIP results demonstrated that OsHDAC1 negatively regulates OsNAC4 by deacetylating H4K5, H4K12, H3K14 and H3K18 and by increasing methylation of H4R3 and H3K9 in the OsNAC4 promoter region.

P01-018 Phospholipase D and phosphatidic acid mediated elicitation of silymarin in cell suspensions of Silybum marianum (L.) Gaernt
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Silymarin is an isomeric mixture of flavonolignans that accumulates in the fruits of Silybum marianum. These compounds are used for therapy of liver diseases and there is a growing interest in their anticancer and chemopreventive effects. Treatment of S. marianum suspensions with yeast elicitor (YE) improved production of silymarin and caused its release into the culture medium. Methyl jasmonate (MeJA), strongly promoted the accumulation of silymarin thus indicating that the octadecanoid pathway is presumably involved in elicitation responses. A comprehensive metabolomic profiling of S. marianum cell cultures elicited with YE or MeJA for the production of silymarin with one- and two-dimensional nuclear magnetic resonance spectroscopy showed that YE promotes the accumulation of choline thus suggesting its action on membranes and the involvement of a lipid signalling in the induction of silymarin in cultures. We report that the application of mastoparan to cultures increased the activity of the enzyme phospholipase D 15 min after treatment and, after 24 h, strongly promoted silymarin accumulation both in the biomass and in the culture medium. The addition of phosphatidic acid to control cultures also stimulated silymarin production. In the presence of 0.1% 1-butanol the elicitor effectiveness of YE or mastoparan was markedly reduced. These results indicate that S. marianum cultures utilize PLD signalling to regulate silymarin elicitation.

P01-019 Characterising the role of Arabidopsis GSK3/ Shaggy-like kinases in stress signalling
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Growth and development of plants are influenced by environmental factors. In order to face unfavourable conditions such as drought, high salinity or pathogen infections, plants have developed highly sophisticated systems in signal perception and transduction. In this respect, reversible phosphorylation plays a crucial role. Glycogen synthase kinase 3 (GSK3) is a highly conserved serine/threonine protein kinase. In plants, a gene family encodes GSK3/Shaggy-like kinases (GSKs) that can be grouped on the basis of their sequence homology. Genetic and biochemical approaches have indicated that GSKs are involved in various processes such as hormone signalling, development and stress response. We are interested in studying the role of the Arabidopsis GSK3/Shaggy-like kinases AtK4, AtK5 and AtK6 in stress signalling. These kinases were found to be expressed in all Arabidopsis organs using Real Time semi-quantitative RT-PCR analysis. Furthermore, their temporal and spatial expression throughout development was analysed in a histochemical assay. In order to study the involvement of AtK4, AtK5 and AtK6 in stress signalling, plants with altered levels of these kinases were generated. We are characterising these mutant lines biochemically and physiologically, and will test them for tolerance towards a variety of stresses. Furthermore, to analyse the in vivo kinase activity of AtK4, AtK5 and AtK6, specific antibodies have been designed.

P01-020 Function of auxin-binding protein 1 for Physcomitrella patens development
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Two auxin receptors have been identified so far in higher plants and are conserved in Physcomitrella. This includes the F-box protein Transport Inhibitor Response 1 (TIR1) and the membrane bound Auxin Binding Protein 1 (ABP1). The focus of our research is on ABP1 which is essential to the life of higher plants. ABP1 is involved in cell division and cell expansion however its precise mechanisms of action remain to be elucidated. In Physcomitrella the gene corresponding to ABP1 was identified. The corresponding predicted protein displays all the conserved domains. However the leader peptide and the first cysteine were missing. The accurate splicing pattern of ABP1 mRNA was determined by 5’ RACE. Expression analysis showed that ABP1 is expressed constitutively in filaments and leafy shoots and is not affected by auxin treatment. Precise expression pattern was investigated by fusion to reporter genes. The role of ABP1 during moss development will be assessed by construction of an inducible knock-out. Transgenics are currently under selection and will also be presented.

P01-021 DNA and arginine methylation in Arabidopsis: the relationship between AtMBD7 and AtPRMT11
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DNA methylation in vertebrate cells marks out functionally specialized regions of the genome and is strongly associated with...
transcriptional repression. Highly conserved DNA-binding proteins (containing methyl-CpG binding domain, MBG) has been identified and share high specificity in recognizing methylated DNA. In Arabidopsis thaliana several members of the MBG family have been characterized, showing similarities to animal MBG. Only few of them can recognize methylated DNA and their physiological and functional significance is still unclear. To study the role of AtMBD7, the only one bearing three MBG domains, we identified AtPRMT11, an arginine methyltransferase, among the protein partners. Our experiments confirmed that these two proteins interact in vitro and can be isolated together in the same complex. Analyses revealed that AtPRMT11 methylates arginines in an asymmetrical fashion and is active both on histones and cellular proteins. These data reveal for the first time in plants the direct interaction between one member of the MBG family and an arginine methyltransferase, analogously to animals where MBG2 is able to interact with and is modulated by the PRMT5 enzyme (Tan and Nakielny 2006). Moreover we found that AtPRMT11 can methylate one of the DNA binding domains in AtMBD7 thus suggesting that AtMBD7 might act as a bridge between two global epigenetic mechanisms of gene regulation in plants. Data describing these particular interaction will be presented and discussed.

P01-022 Differential aphid-induced gene expression in partly resistant and susceptible barley lines

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The bird cherry-oat aphid (Rhopalosiphum padi L.) is an important pest on cereals transferring plant viruses and causing plant growth reduction at high infestation levels. Resistance to R. padi in barley (Hordeum vulgare L.) probably involves several genes and is manifested as reduced aphid growth. In an attempt to identify candidate sequences for resistance-related genes, we performed a microarray analysis of gene expression after 2 days of aphid infestation in two susceptible barley lines and two genotypes with partial resistance. The analysis revealed large differences in gene induction between the four lines, indicating substantial variation in response even between closely related barley genotypes. Genes induced were in functional categories similar to those found with other cereal aphid/plant combinations: defense, primary metabolism, signaling, oxidative stress and secondary metabolism. Twenty-eight genes were induced in all lines. Few genes were down-regulated and none of them in all lines. There were differences and overlaps in aphid-induced gene regulation between resistant and susceptible lines as well as differences in constitutive gene expression between the two types of lines. Candidate sequences for both induced and constitutive resistance factors have been identified.

P01-023 Cytokinin signaling in Arabidopsis upon R. fascians infection

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Abstracts

P01-024 RICES: A novel data mining tool to find cis-elements in rice gene promoter regions

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We have developed a novel data mining tool named ‘RICES’, which searches for cis-element candidates in the upstream, downstream, or coding regions of differentially regulated genes of Oryza sativa subsp. japonica. RICES is designed so that any biological researchers can use it easily. Thus it is implemented as a web-based application software and publicly opened at http://hpc.iri.cgiar.org/tool/nias/ces. RICES first accepts a certain gene set defined by users themselves. RICES then makes a preliminary cis-element candidate list by one of three ways. One of them is motif searching based on the supposition that if cis-elements playing important roles in the regulation of a given gene set, they will be statistically overrepresented and conserved. RICES evaluates the likelihood scores of the listed candidate motifs by association rule analysis, to pick up overrepresented motifs. The preliminary cis-element candidate list can be also made from previously known cis-element motif list prepared in RICES system. Furthermore, users can define their own preliminary candidate motifs, using regular expression with which users can express ambiguous sequences. These features enable users to perform further investigation, such as co-existence or positional relationship of multiple cis-elements. Here we describe detailed feature, usage and recent upgrade of RICES, as well as some example works to which RICES applied.
Abstracts

P01-025  Pectin methylesterase is a sentinel of plant transcriptome  
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Experimental Botany AS CR, Czech Republic  
Physiol. Plant. 133, 2008

P01-026  The role of brassinosteroids in transduction of light signals  
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P01-027  Elucidation of auxin signaling for regulation of PIN endocytosis  
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The signaling molecule auxin has multiple effects on different aspects of plant growth and development. At the cellular level auxin is known to modulate gene expression by regulated degradation of transcriptional repressors from Aux/IAA family. In addition, auxin regulates sub cellular protein trafficking and thus activity of plasma membrane proteins including PIN auxin efflux carriers. By inhibiting PIN endocytosis, auxin increases levels of PINs at the plasma membrane and concomitantly promotes its own efflux from cells. This data imply a previously undescribed mode of plant hormone action: by modulating PIN protein trafficking, auxin regulates PIN abundance and activity at the cell surface, providing a mechanism for the feedback regulation of auxin transport. This auxin effect on endocytosis requires activity of the Calossin-like protein BIG but further molecular mechanism remains elusive. Here we present new data regarding the molecular mechanism underlying auxin effect on endocytosis. We conducted forward genetic screen and identified three loci showing auxin-insensitive PIN internalization. The molecular analysis of these mutants provides molecular insights into the auxin effect on vesicle trafficking, in particular reveals a connection to clathrin-dependent vesicle formation.

P01-028  Adenine/adenosine deaminase interaction with cytokinins  
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Recombinant adenine deaminases (EC 3.5.4.2) from yeast were obtained from E. coli under IPTG-inducible promoter and shown to hydrolyze cytokinins using UV spectral assay. The enzymes AAH1 from Saccharomyces cerevisiae and SPBC1198.02 from Schizosaccharomyces pombe are able to cleave cytokinins to hypoxanthine and an amine derived from the N6 side-chain, the latter enzyme showing a notable protein sequence homology to CHASE domain of the cytokinin receptor CRE1/WOL/AHK4. The reaction products were identified by HPLC coupled to UV and Q-TOF detectors. A closest Arabidopsis thaliana homologue to these enzymes encoded by the gene At4g04880 and annotated as a putative adenosine deaminase (EC 3.5.4.4) was prepared by the same method. The recombinant protein shows low activity with adenosine, AMP and ATP, but does not hydrolyze cytokinins at all. The protein may perform different role than metabolism of purine compounds,
Abstracts

P01-029 Pectin methylesterase is a stress-related factor of plant transcriptome stability
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Pectin methylesterase (PME) is an ubiquitous enzyme, which is involved in the cell wall (CW) growth and development. Recently it was revealed that PME participates in gene silencing, plant virus reproduction and influences the expression of transgenes. In this work we studied the role of PME in antiviral resistance. It has been shown that tobacco plants carrying additional PME gene or even T-DNA from ‘empty’ binary vector display higher level of PME enzymatic activity and are able to suppress reproduction of tobacco mosaic virus (TMV) including cell-to-cell and long-distance virus movement in plant. We proved that PME activity is increased in stably transformed plants despite of the insert used. For example, transgenic tobacco and Nicotiana benthamiana plants expressing TMV movement protein gene or GFP both demonstrate heightened PME activity. The tomato plants with several supplementary polygalacturonase genes have increased level of PME activity as well. Moreover, induction of light-sensitive pbsO gene was accompanied by reinforcement of PME gene transcription. So we can conclude that either introduction of foreign insert into plant genome or excessive transcription of its own genes resulted in high level of PME expression, which leads to return to status quo of cell transcriptome.

P01-030 An Arabidopsis Golgi-localized membrane protein and a chloroplastic carbonic anhydrase may interact in the secretory pathway
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The Arabidopsis MPS (Membrane Protein induced by Salt) is a conditionally expressed Golgi-localized membrane protein of unknown function, which seems to regulate chloroplastic proteins. Indeed, split-ubiquitin data showed that the MPS interacts with immature form of several chloroplastic proteins, including a carbonic anhydrase, AtCAH1. Interestingly, AtuCAH1 belongs to a restricted number of characterized proteins that have been shown to be targeted to the chloroplast through the secretory pathway in plant. To elucidate a possible role of the MPS in the transport of defined chloroplastic proteins, we are reconstituting in vitro the transport of AtuCAH1 in the presence or absence of MPS. First, a histidine-tagged MPS was expressed in yeast, solubilized and purified on Ni-NTA resin. Then, the interaction between the MPS-HIS6 and AtuCAH1 from crude leaf extract was verified by pull-down assay. The efficient uptake of in vitro transcribed/translated AtuCAH1 in ER-derived dog pancreas microsomes was confirmed by proteinase K and its glycosylation by endoglycosidase H. Using generated specific antibodies, we found that in vitro synthesized or AtuCAH1 in leaves extract can be mainly detected as a dimer which could represent the structurally matured and/or active form of AtuCAH1. According to these data, MPS can be a partner protein of AtuCAH1 in vivo and may be involved in the AtuCAH1 ER-Golgi transit en route to the chloroplast.

P01-031 Role of a novel protein phosphatase in phytochrome-mediated light signaling pathway
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In plants, light signal transduction is finely regulated by interactions between specific signaling proteins, as well as by protein modifications such as phosphorylation and ubiquitination. The identification of novel phytochrome-interacting proteins and the precise signaling mechanisms that they mediate is still ongoing. In our current study, the newly identified putative phytochrome-associated protein, PAPP2C (phytochrome-associated protein phosphatase type 2C), was found to be interacted in the nucleus with phytochrome A (phyA) and B (phyB), both in vitro and in vivo. Moreover, the phosphatase activity of PAPP2C and its association with phytochromes were enhanced by red light, indicating that it plays a role in mediating phytochrome signaling. In particular, PAPP2C specifically binds to the N-terminal PHY domain of the phytochromes. We thus speculate that this interaction reflects a unique regulatory function of this phosphatase toward established phytochrome-associated proteins. In addition, it was found that PAPP2C effectively dephosphorylates phytochromes and indirectly mediates the dephosphorylation of phytochrome interacting factor 3 (PIF3) in vitro. Taken together, we suggest that PAPP2C functions as a regulator of PIF3 by dephosphorylating phytochromes in the nucleus.

P01-032 Tobacco mosaic virus enhances the non-cell-autonomous spread of RNA silencing
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RNA silencing is a fundamental mechanism that, among other important tasks, controls the accumulation of viruses through the degradation of their RNA intermediates. Since viruses encode suppressors of RNA silencing it is assumed that RNA silencing has evolved as an antiviral defense response. Thus, the idea of an arms race between the virus and the host, which the virus has to win for a successful infection, is now widely accepted. Our results question this concept of an arms race by showing that a virus-encoded protein, the movement protein (MP) of Tobacco mosaic virus (TMV), supports the intercellular trafficking of the non-cell-autonomous...
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silencing signal. A virus mutant with defects in the suppressor is shown to be more prone for silencing with MP than without MP indicating that MP supports antiviral silencing during infection. Previous studies have demonstrated that the expression of silencing suppressors leads to viral overaccumulation and the death of the plant. Therefore, we suggest that the ability of MP to support the spread of signal may contribute to the control of virus propagation in the infected host.

P01-033 Boron deficiency down-regulates the expression of several cell wall-related genes in Arabidopsis roots
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The cell wall is a basic structure in plant growth and development, as well as in the response to environmental stresses. There is direct evidence for the role of boron (B) in cross-linking with apiose residues of cell wall rhamnogalacturonan II (RG-II) to form dimeric RG-III what allows the pectin assembly in the cell wall. Thus, nowadays there is general agreement in which the primary function of B is its structural role in the cell wall. The aim of this work was to investigate the effects of B deficiency on the expression of cell wall-related genes in Arabidopsis roots, and how the expression of some of these genes could be regulated by short-term B deficiency. Plants were grown hydroponically in a nutrient solution supplemented with 2 μM B and then transferred to a B-free medium for 6 and 24 h. A transcriptome analysis was carried out and we identified several cell wall-related genes whose expressions were down-regulated by B deficiency. The genes belonged to arabinogalactan protein, xyloglucan endotransglycosylase/hydrolase, polygalacturonase, pectate lyase, pectin methyltransferase, expansin, and cellulose synthase-like gene families (Camacho-Cristóbal et al. 2008). Besides the essential role for B in the cell wall structure, these results seem to indicate that B could regulate expression of genes involved in the biosynthesis and modification of cell wall.

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P01-034 The MKKKC5 - MKK2 connection - joint players in plant stress response?
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In plants MAPK (mitogen activated protein kinase) signalling networks are major modules in biotic and abiotic stress responses. In Arabidopsis thaliana 20 MAPK’s, 10 MAPKK’s (MAPK kinases) and 60 putative MAPKKK’s (MAPKK kinases) interact in different combinations, depending on the stress the plant is facing. In this way MAPK pathways offer a large capacity to regulate the reactions of plant cells to changing environmental conditions. The MKK2 pathway was shown to be involved in plant resistance to cold,salt (Teige et al. 2004) and biotic stresses (Brader et al. 2007). A yeast-two-hybrid screen with MKK2 led to the discovery of a new player in MKK2-mediated signalling, named M KK KC5. In vitro co-immunoprecipitation confirmed the interaction between MKK2 and this putative MAPKKK. To address the function of M KK KC5 in the MKK2 signalling pathway, we analyzed mkkkc5 mutant plants under different stress conditions. As observed for mkk2 mutants, germination of mkkkc5 seedlings was inhibited on high salt concentrations. However, in contrast to mkk2 mutants, mkkkc5 plants showed increased sensitivity to the necrotrophic fungus Alternaria brassicicola, an effect also seen in MKK2 overexpressors. We are currently evaluating transcriptome data from Alternaria infected mkkkc5 plants and MKK2-overexpressors. To obtain additional information on the function of M KK KC5 in these stress responses, we are currently also analysing M KK KC5 overexpressing lines and M KK KC5 promoter::GUS lines.

P01-035 Network interactions in jasmonate induced resistance priming
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Jasmonates are fatty acid derived signalling molecules synthesised by plants in response to a plethora of biotic and abiotic stresses. They are key components of signalling networks involved in inducing and priming plants’ natural defences. Foliar spraying of jasmonates has been shown to prime plants, through systemic resistance mechanisms, so they are better able to respond to attack. However, there are known and potential interactions of jasmonate signalling with a number of other networks. The opportunity for jasmonate based pest management approaches requires consideration of these interactions to ensure pest control and productivity are optimised. A novel seed treatment approach to jasmonate induced pest resistance priming is being investigated with potential to integrate the procedure into pest management strategies particularly where biological controls are employed. Biological approaches may be hindered by agronomics as time to reach effective control may fall beyond the economic damage threshold of the crop. At this point chemical controls over and above the intended management plan must be applied. Priming can offer a ‘buffer’ allowing plants’ own defences to assist biological controls by reducing speed of pest colonisation. The work in progress will be presented in consideration of how interactions with other stress and developmental networks might impact on application of the technology.

P01-036 Metroxylon sago: current progress on molecular biology of starch synthesizing enzymes
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Starch biosynthesis pathway involves several interactions of starch synthesis enzymes that play specific function in synthesizing starch molecule subunits. Moreover, the presence of isoforms in each enzyme contributes complexity into the pathway. In sago palm Physiol. Plant. 133, 2008
(Metroxylon sagu), to date there are eight different starch synthesis enzymes that have been published in NCBI database. The database consists of partial sequences encoding the enzyme ADP-glucose pyrophosphorylase large subunit (apglp1 and apglp19) and ADP-glucose pyrophosphorylase small subunit (apglp11). In addition, there are also partial sequence of starch branching enzyme 1 and 2 (SBE 1 and 2) which is 1329 bp and 1383 bp respectively, as well as partial sequence of pullulanase-type starch debranching enzyme with the size of 714 bp and also 642 bp partial sequence of soluble starch synthase (SSI). Current work involves amplification of 5′ region of granule bound starch synthase (GBSS) and 5′- and 3′-end of soluble starch synthase 1 in order to obtain the full length open reading frame (ORF) encoding these enzymes. Through RT-RACE PCR methods, extension of 300 bp cDNA regions of 5′ end and 3′ end of SSI sequence was successfully obtained. However, amplification of 5′ region of the GBSS gene is still in progress. In addition, further endeavour in molecular work for identification and amplification of the entire coding region involving starch biosynthesis enzymes in sago palm is also being carried out.

P01-037 RCD1 and SRO1, the Arabidopsis WWE domain-containing proteins, modulate plant environmental responses and development in combinatorial manner


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The RCD1 (for Radical-induced Cell Death1) protein is known to be essential for many plant responses, mainly related to abiotic stress and programmed cell death. The biochemical function of the protein is unknown, but it has a trypartite domain structure formed by a WWE domain implicated in protein-protein interactions, a catalytic core of ADP-ribose transferases and a thus-far uncharacterized C-terminal domain, which bears homology to TAF4 proteins (components of TFIIID). RCD1 is a member of a small plant-specific gene family and, together with its closest homolog SRO1 (Similar to Arabidopsis homologues and the location of introns is conserved. In contrast to RCD1, the lack of proper SRO1 function causes very mild phenotypical changes. However, when both genes are dysfunctional, the result is a hardly viable plant, whose phenotype exceeds that of rcd1 mutant by far. We present data from promoter activity analysis, protein localization, protein-protein interactions together with stress and developmental phenotypes of rcd1, sro1 and their double mutant to demonstrate that the combinatorial function of these proteins is necessary for many aspects of plant life and that RCD1 and SRO1 are likely to take their effect through their interactions with transcription factors.

P01-038 EARLY BIRD1 acts in red light signalling and has a role in the circadian clock


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P01-039 Identifying factors involved in the regulation of gene expression in mesophyll cells of a model C₄ plant

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The C₄ photosynthetic pathway concentrates CO₂ around Rubisco and reduces the rate of photorespiration. The initial fixation of carbon, the decarboxylation reaction and the regeneration of the substrate are spatially separated in two specialised cell types, mesophyll (M) and bundle sheath (BS), and to achieve this the expression of the key genes is cell-specific. We are interested in the evolution of the regulation of cell-specific gene expression and we are studying Cleome gynandra (Brown et al., 2005, TIPS 10: 215–221; Marshall et al. 2007, Plant J 51: 886–896), the closest known C₄ relative of Arabidopsis (C₃). Taking a comparative approach allows us to identify which components have been altered during the evolution of C₄ photosynthesis in Cleome gynandra. In this study we present data on the evolution of genes that are expressed specifically in M cells: phosphoenolpyruvate carboxylase kinase, a pyruvate carrier, a carbonic anhydrase and pyruvate,orthophosphate dikinase. All of these genes from Cleome gynandra are over 75% identical to their Arabidopsis homologues and the location of introns is conserved. We will present data on cell-specificity of these genes using RT-PCR on cell-specific cDNA from laser capture microdissection. In addition, we report on the importance of their 5′ and 3′ UTRs for cell-specific expression.
Chitin and its fragments as a typical fungal MAMP trigger various defense responses in a wide range of plant species. We recently isolated CEBiP, chitin elicitor binding protein, from rice cells and showed that CEBiP plays an important role as a cell surface receptor for chitin elicitor signaling (Kaku et al. 2006). However, CEBiP lacks intracellular domains, indicating it may require other partner protein(s) for elicitor signaling. To survey such a partner protein, we used reverse genetic approach and identified CERK1 (Chitin Elicitor Receptor Kinase 1), an essential component for chitin elicitor signaling in Arabidopsis (Miya et al. 2007). OsLysM-RLK9 in rice genome showed the highest homology with CERK1. Knock-down transformants of OsLysM-RLK9 were established to analyze the function of this molecule. Several lines of these transformants significantly reduced the expression of OsLysM-RLK9 but not of CEBiP (Miya et al. 2007). These lines also showed almost no ROS generation as well as phytoalexin biosynthesis in response to chitin elicitor. Interestingly, Yeast two-hybrid analysis showed the positive interaction between CEBiP and OsLysM-RLK9. These results indicated that OsLysM-RLK9 may collaborate with CEBiP, and plays an essential role for chitin elicitor signaling in rice.

Kaku et al. (2006) Proc Natl Acad Sci USA 103: 11086
Miya et al. (2007) Proc Natl Acad Sci USA 104: 19613

**Microarray analysis reveals several defence genes induced by volatile emissions from undamaged barley in other barley cultivars**

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Plant-plant communication mediated by volatiles has received increasing attention in recent years. The role of volatile organic compounds released from herbivore-infested plants in priming of defences in neighbouring plants has been demonstrated in several species. We have earlier established that plant-plant communication can occur via volatile interaction also by unwounded plants. The effects have been manifested as reduced aphid acceptance and increased attraction of aphid natural enemies to responding plants in certain barley (Hordeum vulgare) cultivar combinations of inducing and responding genotypes. In order to study this phenomenon at molecular level, microarray analysis of gene expression was carried out in responding barley genotypes. Gene expression was analysed in plants that had been exposed to volatiles from an inducing genotype and compared with plants that had been exposed to volatiles from their own genotype. A number of genes were found as up-regulated in the treated plants compared to the control, although the induction was weak. A large part of the annotated genes are classified as defence-related. The volatile-induced regulation was confirmed for a number of sequences in independent experiments, using real-time PCR. In future we plan to relate the volatile-induced gene expression to the earlier established effects on aphids and their natural enemies.

**Analysis of microRNA expression and their target transcripts in Eucalyptus**

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MicroRNAs (miRNAs) are small, noncoding RNAs that can play important roles in eukaryotes by mRNA degradation, and translation repression. To investigate whether miRNAs regulate gene expression in woody plants, we analyzed small RNAs expressed at flowering stage of Eucalyptus. Small RNAs isolated from flower buds were separated by polyacrylamide gel electrophoresis. Twenty-two bases of the small RNAs were sequenced by MPSS method, and about 120,000 sequences were determined. Next the sequences that have homology with rRNA, tRNA, snRNA, scRNA were removed from the 120,000 sequences, and then 30,000 genome sequences including the small RNAs were taken. Finally we identified about 500 miRNA candidates by prediction of secondary structures of the 30,000 sequences. Some miRNA candidates have sequence conservation in Arabidopsis or Populus, but many new miRNA candidates were found in Eucalyptus. We are searching the miRNA target transcripts against the EST data, and identified some miRNA target genes so far. One of the target genes has homology with Arabidopsis SPL2 gene, and this gene was expressed in all organs in Eucalyptus. Another target gene has sequence conservation with MYB genes. 5’- RLM-RACE analysis demonstrated that these two genes were cleaved in the middle of their target sites. These results indicated that miRNAs regulate gene expression of the transcription factors in Eucalyptus.

**Calmodulin-binding transcription factor, OsCBT, functions both in plant defense signaling and tiller development in rice**

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Previously we isolated OsCBT gene encoding a calmodulin (CaM)- binding transcription factor from the rice expression library screening. To understand the biological role of OsCBT, we have generated transgenic plants constitutively expressing OsCBT under the control of CaMV 35S promoter and also isolated an oscbt-1 loss-of-function mutant from the screening of rice T-DNA insertion mutant pool. Since we have isolated OsCBT from the expression library screening constructed from fungal elicitor treated rice callus, we tested whether
OsCBT possibly participate in defense signaling against pathogen attack. Whereas wild-type rice and 35S:OsCBT transgenic plants show susceptibility to rice blast fungus, Magnaporthe grisea, as well as bacterial leaf blight pathogen, Oscht-1 exhibits strong resistance both to fungal and bacterial pathogens, suggesting OsCBT functions as a negative regulator in defense signaling. In addition, oscht-1 shows interesting phenotype in tiller development. Anatomical analysis showed that the outgrowth of tiller buds were stimulated in oscht-1 compared to wild-type rice. Oscht-1 plants showed ectopic tiller development at upper internodes where tiller outgrowth is normally suppressed in wild-type plants. Here we intend to discuss the possible role of OsCBT in defense signaling and regulation of tiller development and also the possible interaction between these two signaling pathways in rice.

**P01-044** The blue light receptor CRY1 mediates plant responses to high irradiances

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Exposure to high irradiances results in dramatic changes in nuclear gene expression in plants. However, little is known about the mechanisms by which changes in irradiance are sensed and how the information is transduced to the nucleus. Here we describe a novel function of CRY1 in mediating plant responses to high irradiances that is essential to the induction of photoprotective mechanisms. The cry1 and hy5 mutants show specific mis-regulation of ELIP1/2 and we show that the induction of ELIP1/2 expression is mediated via CRY1 in a blue light intensity-dependent manner. Furthermore, we showed that 77 of the HL responsive genes are regulated via CRY1, and 26 of those genes were also HY5 dependent. As a consequence of the mis-regulation of these genes the cry1 mutant displayed a high irradiance-sensitive phenotype with significant photoinactivation of PSII. This indicates that high irradiance can be sensed in a chloroplast-independent manner by a cytosolic/nucleic component.

**P01-045** Interaction between Scots pine (*Pinus sylvestris* L.) and *Methylobacterium* endophytes

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During the past years several plant associated microbial endophytes have been reported to promote plant vitality, growth and defense in various species. Endophytes can provide capacity for plants to live in a broader range of environments and endure harsh conditions, such as drought. We have previously identified distinctive plant-endophyte interaction between *Methylobacterium extorquens* sp. and Scots pine (*Pinus sylvestris* L.). *Methylobacterium* spp. are the most dominant endophytic species in the cells of apical meristem of Scots pine. These methylotrophic endophytes can be found within the meristematic tissues throughout the year. However, after cell differentiation endophytes can no longer be detected from fully developed tissues. Overall the endophytic methylotrobacterias have positive effect on early growth and development of Scots pine, meanwhile its biological significance is yet to be solved. Our preliminary results indicate *Methylobacterium* infected pine seedlings have increased lateral root formation, root length and biomass when compared to uninfected ones. To characterize the interaction of these species we are using microarray methods to study the gene expression in both species during the interplay. Methylophic photosymbionts are potential tools in the biotechnology to increase crop productivity and biomass.

**P01-046** The WRKY superfamily of transcription factors play an important role in plant defense and stress responses

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Plants are exposed to several biotic stresses in their environment. This has led to the evolution of complex strategies allowing rapid modulation of cellular functions and mounting an active defense response. Induced defenses play a major role in plant disease resistance and are regulated by a network of interconnected signal transduction pathways with the plant hormones ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) as crucial mediators. These specific hormone-mediated signaling cascades trigger distinct sets of defense-related genes leading to enhanced resistance to particular pathogens. In Arabidopsis, among a number of families of transcription factors, WRKY belongs to a class of predominantly plant specific TFs often involved in biotic stress signaling. We are studying on WRKY group III which consist of 13 members and my research focused on the contribution of WRKY54 and WRKY70 on gene expression and defense response in Arabidopsis. We will elucidate the cross-talk between these WRKYS and identity proteins with which they interact. In order to investigate the WRKY70 or WRKY54 regulon, oligonucleotide-microarrays have been used to study the target gene expression in the wrky70 and wrky54 single as well as in the wrky70/wrky54 double mutants. Moreover, we have constructed conditional overexpressors for WRKY54 and WRKY70 and will use these for identification of WRKYS.

**P01-047** Regulation of ABA in birch, *Betula pubescens* and its drought sensitive genotype *B. pubescens* f. *hibernifolia* during water stress

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A plant stress hormone abscisic acid (ABA) is involved in regulation of plant’s responses in various abiotic stresses such as drought and cold as weil as seed dormancy. These aspects are less studied in trees, which might respond differently than annual plants to various environmental stresses due to their large size and long lifespan. Our aim was first to characterize whether inability of drought sensitive birch to increase its ABA level under stress conditions was correlated with expression level of the ABA biosynthesis genes. We made a dehydration experiment measuring water content and water potential of the two genotypes of birch and related these parameters with the level of ABA. The drought sensitive genotype dried much faster and was unable to increase its ABA level during the slow drying as the more tolerant genotype. We will relate this inability to
accumulate ABA level to the expression of the known ABA biosynthesis genes as well as genes related to ABA signalling and ABA response genes by quantitative real time RT-PCR. Defect in expression of some of these genes might reveal how to find the reason for this drought sensitivity. In addition we will study the relation of ABA with other plant growth regulators, as our results suggest that different plant growth regulators might interact in drought stress in trees.

**P01-048** Silencing of the *Rps10* gene can be initiated in different growth stages in *Arabidopsis thaliana* generating spectrum of phenotypes

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Our work is the first report describing the effects of downregulation of *Rps10* gene in plants. The RNAi strategy was applied to silence the *Rps10* gene encoding mitochondrial ribosomal protein S10. Transgenic plants grown under short day conditions presented spectrum of phenotypes classified into four categories P1–P4 depending on phenotype severity. We have shown that the observed phenotypes result from two reasons: state of zygosity (most severe P1 phenotype observed in homozygotes), and the time of the onset of silencing (initiation of *Rps10* gene silencing at different developmental stages causing P2–P4 phenotypes in hemizygotes). Irrespective of the phenotype category silencing in hemizygous plants causes decrease of the *Rps10* transcript level to 20–30% of that observed in control plants, but in homozygotes it drops below 10% leading to lethality. Induction of silencing in P1 and P2 plants occurs in early vegetative phase causing severe alterations in the whole rosettes. In P3 and P4 plants silencing is initiated within long period lasting from the late vegetative phase until the early reproductive one resulting in abnormal development of the upper part of rosettes (P3) and inflorescence (P4 and P5). We have also shown that the switch from short to long day conditions results in elimination of P3 and P4 phenotypes but not P1 and P2. This suggests that long day conditions disable *Rps10* silencing initiation in later growth stages.

**P01-049** Aluminum modulates microRNA expression in rice roots

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MicroRNAs are small 21 long RNA molecules with regulatory roles in development and in response to stresses. Expression of plant microRNAs have been specifically associated with responses to abiotic stresses caused by cold, light and metal ions. In acid soils, with increased aluminum solubility, this metal can severely affect plant growth. At the present, there is no work on miRNAs in response to aluminum stress in plants. Modulation of miRNA expression may constitute a key element to explain the mechanisms implicated in aluminum toxicity and tolerance. The objective of this work was to characterize the expression of at least one miRNA member from each miRNA families in rice roots under high concentration of aluminum. A total of 46 miRNAs out of 62 predicted families were effectively detected by quantitative PCR. Among these, 13 were down regulated and six were up regulated in plants after 8 h of aluminum treatment. Analysis of their putative targets suggest that these 19 rice miRNAs are involved in the regulation of super imposed and independent metabolic pathways in response to aluminum. (Work supported by CNPq).

**P01-050** Activation of the TAS3-derived tasiRNA pathway in the root system of *Arabidopsis thaliana*

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Plant and animals use small RNAs (microRNAs and siRNAs) as guide for post-transcriptional and epigenetic regulation. In plants, miRNAs and trans-acting siRNA (tasiRNA) result from different biogenesis pathways but both interact with target transcripts to direct their cleavage. Four ta-siRNA gene families (TAS1–4) are known in Arabidopsis thaliana. TAS gene transcripts are cleaved by miRNAs; the cleavage products are copied into dsRNA by RDR6, and diced into tasiRNAs by DCL4. Biogenesis of TAS3-derived tasiRNAs involves miR390 and they target mRNAs encoding AUXIN RESPONSE FACTORS ARF3/ETTIN and ARF4. Specific degradation of ARF3 in the leaves by TAS3-derived tasiRNA is critical for leaf development and phase transition. In a screen for large non-protein coding RNAs in Arabidopsis using a dedicated micro-array, we identified that TAS1a gene is also expressed in root tissues, in particular at the secondary root branching points. Using reporter constructs for TAS3a and miR390 loci, as well as analysis of the accumulation of their derived RNAs, we characterized the expression pattern of the TAS3 pathway during root development. We present evidence that this pathway might be linked to the architecture of the root system.

**P01-051** Roles of CTD phosphatases (CPLs) in the attenuation of wound-induced transcription of jasmonic acid-biosynthetic genes in *Arabidopsis*

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Trienoic fatty acids (TAs) are essential for stress signaling as precursors of the phytohormone jasmonic acid (JA). *Arabidopsis FAD7* encodes a plastidial ω-3 fatty acid desaturase, which catalyzes the TA production. In coordination with other JA-biosynthetic genes, expression of *FAD7* is locally induced by wounding. This provides a feedforward mechanism for rapid and sustainable JA accumulation. To identify regulatory components involved in this mechanism, a transgenic line of *Arabidopsis carrying the FAD7* promoter (*FAD7 pro*) fused to the firefly luciferase gene (*LUC*) was constructed. Reciprocal crossing experiments using this transformant revealed that the *FAD7* induction depends largely on JA biosynthesis and the SCLS-mediated signaling mechanism, while JA alone is insufficient for its maximal induction. Full induction required synergistic interactions between JA-dependent and independent wound signaling pathways. A genetic screen for aberrant *FAD7 pro:LUC* expression yielded a mutant showing enhanced wound induced *LUC* bioluminescence. The mutation was associated with the *cpl1* (*CTD phosphatase-like 1*) locus and

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P01-053 Osmotic stress signal perception and transduction in *Populus*

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Response of poplar to water deficit stress is one of the major concerns for poplar culture. This stress is perceived by the plant cell as a change in osmotic pressure. The osmosensing pathway has been studied in *Arabidopsis thaliana* and it was shown that a system related to the bacterial two-component system is involved in this process. In order to understand the molecular basis of this signal perception and transduction in *Populus*, we identified a cDNA encoding a Histidine-asparte Kinase, and 4 cDNA encoding Histidine-containing Phosphotransfer proteins, HPI-4. The HK1 protein sequence deduced from the cDNA, shows similar structures to the ATHK1 osmosensor of *A. thaliana* and to the SLN1 osmosensor of *Saccharomyces cerevisiae*. The 4 HPTs are characterized by histidine-aspartate kinase domains. We have shown that HK1 expression is upregulated during an osmotic stress in hydroponic culture and that this protein is able to interact specifically with HPT2 in the yeast two-hybrid system. All these results suggest the existence of a multi-step phosphorelay pathway involved in osmotic stress sensing in *Populus*. Furthermore, transgenic poplar callus expressing a fusion protein HP2-GFP were produced in order to determine the cellular localization of HP2. We showed a cytoplasmic and nuclear localization in control condition. This approach will help us to define the molecular targets of this protein.

P01-054 The *Arabidopsis NRT1.1* transporter acts as a nitrate sensor and is crucial for nitrate signalling governing root colonization of nitrate-rich patches

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Nitrate is both the main N source for nutrition of higher plants, and a signal molecule regulating their metabolism and development. The roots sense the NO3- concentration in the soil, and trigger signalling pathways allowing plant adaptation to changes in its external availability. Localized proliferation of lateral roots in NO3- rich patches is a striking example of the nutrient-induced plasticity of root development. Using an in vitro split root system, we show that preferential LR growth in NO3- rich patches is predominantly governed by NO3- concentration in NO3- poor patches. Mutants of the *NRT1.1* nitrate transporter but not *NRT1.2*, another nitrate transporter, display a strongly decreased LR proliferation response. This results from both increase of LR elongation in NO3- poor patches and reduced elongation in NO3- rich patches. This phenotype that is not due to lower specific NO3- uptake activity in the mutants has been correlated to modification of auxin accumulation in LR and differential regulation of meristematic activity. These results show that *NRT1.1* promotes localized root proliferation independently of any nutritional effect. We concluded that *NRT1.1*, which is localized at the forefront of soil exploration by the roots, is a key component of the NO3- sensing system that enables the plant to detect local nitrate concentration and exploit NO3- rich soil patches. However, the sensing/signalling mechanism is largely unknown and several hypothesis will be discussed.

P01-055 Global gene regulation by oxidized lipids (phytoprostanes) in *Arabidopsis*

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Oxylipins play an important role in signalling in plants, especially related to plant stress responses and innate immunity. The best characterized oxylipins are jasmonic acid (JA) and its precursor 12-oxo-phytodienoic acid (OPDA). In addition a number of biologically active oxylipins are formed non-enzymatically, including several classes of phytoprostanes. Microarray analyses revealed that the cyclopentenone OPDA and structurally related phytoprostanes induce the expression of genes related to detoxification, stress responses and secondary metabolism, that is different from the genes induced by the cyclopentenone JA through the COI1 (CORONATINE INSENSITIVE 1) pathway. More than 40% of the
promoters of induced genes contain binding sites for TGA transcription factors. Microarray analysis showed that 60% of all phytoprostanes- and 30% of all OPDA-regulated genes depend on the TGA transcription factors TGA2, TGA5 and TGA6. Besides being potent signals, cyclotides and other lipid peroxidation products are reactive molecules that can covalently bind to and damage proteins. To this end, we show that at least two of the induced detoxification enzymes efficiently metabolize cyclotides in vitro. These reactive metabolites accumulated in *Pseudomonas* infected *Arabidopsis* plants.

**P01-056 Three SnRK2 protein kinases have important roles in seed maturation and germination of *Arabidopsis* through phosphorylation of ABI5**

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Abscisic acid (ABA) is an important phytohormone regulating various plant processes including seed maturation and germination. A bZIP transcription factor ABI5 is involved in ABA signaling during seed maturation and germination. Amino-acid substitution of putative target sites for Ser/Thr protein kinases of ABI5 resulted in suppression of ABA-dependent transactivation. The ABI5 polypeptide was phosphorylated by three redundant ABA-activated SNF1-RELATED PROTEIN KINASE2 (SnRK2) protein kinases, SRK2D, SRK2E, and SRK2I. These SnRK2 genes were expressed differentially during seed maturation and germination. The srk2d srk2e srk2i mutant showed stronger in ABA insensitive phenotypes than srk2d srk2i double mutant, but not srk2d or srk2i single mutants. Changes in seed dormancy consistent with ABA insensitivity were observed. Viviparous phenotype was also observed in the triple mutant under high humidity conditions. The srk2d srk2e srk2i mutant had a greatly reduced level of a 42-kD kinase activity capable of phosphorylating peptide from ABI5. Microarray experiments indicate that expression of more than 1000 genes was reduced in seeds of the mutant, but not srk2d or srk2i or srk2e srk2i single mutants. These results demonstrate that SRK2D, SRK2E, and SRK2I have important roles in ABA signaling during seed maturation and germination of *Arabidopsis* through phosphorylation of ABI5.

**P01-057 Plants and environment: signals and response networks induced by compatible virus infection**

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Plants continuously encounter various environmental stresses of abiotic and biotic nature. To ensure survival, plants have evolved response mechanisms like adaptation, defence, and tolerance. These responses are mostly of dynamic and transient nature as they need to be of low cost for the plant’s growth and development. One of the responses to various environmental stimuli including virus infection was recently shown to affect the frequency of homologous recombination events. With respect to infection with the tobramovirus Tobacco mosaic virus (TMV) and Oilseed rape mosaic virus (ORMV) increased homologous recombination frequency depends on the inactivity of the N resistance gene in tobacco, thus implying that compatibility is an important prerequisite for this specific response. To better understand response networks to compatible virus infection, we here investigate the interaction between ORMV and *Arabidopsis*. Our aim is to explore whether we can resolve responses specific for the virus and to characterise their functional implications. Our approach combines the application of metabolite, transcriptome, and small RNA profiling techniques. We hope that with this comprehensive analysis of a compatible plant-virus interaction we will gain detailed knowledge about the interplay of signals and responses to compatible virus infection.


**P01-058 Searching for responses to bacterial signals in *Arabidopsis***

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Many plant-associated bacteria utilize a mechanism of cell-cell communication referred to as quorum sensing to regulate functions involved in interactions with plants. In gram-negative bacteria quorum sensing signalling is mediated by N-acyl-homoserine lactones (AHLs). In recent years, several studies have indicated that, in addition to bacteria, plants have the ability to recognize and respond to bacterial AHLs. We are using AHLs from the soft rot pathogen *Erwinia carotovora* ssp. *carotovora* to further investigate this aspect of plant-microbe interactions in *Arabidopsis thaliana*. DNA-microarrays are used to screen for genes responding to AHLs. Also, expression of genes known to function in defense is studied with quantitative real-time RT-PCR to determine if *Erwinia* AHLs activate defense responses in *Arabidopsis*. So far, preliminary results have not revealed extensive responses to AHLs. Further studies will be conducted and the results will be discussed.

**P01-059 Interaction between plant phospholipase D and actin**

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Phospholipase D (PLD) hydrolyses membrane lipids to yield phosphatic acid (PA) and free-head group. This enzyme and its product (PA) play an important role in many cellular processes. They are involved in vesicular trafficking, cytoskeleton rearrangement, remodelling and degradation of membranes, cell proliferation,
hormone action and signal transduction. Despite of usefulness of plant PLD, mechanisms of activity regulation at molecular level are less understood. Calcium ions, phosphoinositides, heterotrimeric G-proteins and local changes in physical state of membrane are important regulatory factors. Experiments in vitro with recombinant PLD indicate that interaction of PLD with actin cytoskeleton is another regulatory mechanism. In our work we investigate interaction between PLDs from tobacco (Nicotiana tabacum L.) and actin. Based on pull down assay with fragments of tobacco PLDs we suggest that interaction PLD-actin is isofrom specific. This hypothesis is also supported by in vitro measuring activity of different PLDs after treatment with actin. PLD binds to the actin via conserved actin-binding region (ABR). On the basis of sequence analysis of eukaryotic ABR we speculate that actin-specific binding is probably mediated by a few amino acids rather than global conformational change of the whole region. This work was supported by grant of Czech Science Foundation, grant no 522/05/0340 and Ministry of education of CR grants no. LC06034 and 6046137305.

P01-060 Functional characterization of one Arabidopsis TETRASPANIN using the split-ubiquitin system to screen for interaction partners
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TETRASPANINS were first described in animals and are common to all multicellular organisms. They are broadly expressed membrane glycoproteins with four conserved transmembrane domains, a small cytoplasmatic loop and an extracellular loop. This loop contains highly conserved amino acid residues, essential for the interaction with a wide range of functionally diverse partners. The TETRASPANINS act primarily as adapter proteins, helping the establishment of a supramolecular network. Most significantly, they often associate with others TETRASPANINS forming a TETRASPANIN web in specific membrane microdomains. These act as organizers of membrane-signalling complexes. In animals, the TETRASPANINS are associated with various diseases (cancer, immune disorders and many infectious diseases). To date only two TETRASPANINS, from a family composed of at least 24, have been partially characterized in Arabidopsis. These studies neither report a detailed functional characterization, nor investigate the dynamics of TETRASPANIN complexes, their regulation in response to diverse stimuli and their role in signalling. Here, we present the results of an interaction screening for one of Arabidopsis TETRASPANINS, using the Split-Ubiquitin system. Several putative interaction partners were identified and framed in TETRASPANIN context. The data supports a complex model for TETRASPANIN function in signalling and membrane organization. Oliveira is supported by FCT (SFRH/BD/19005/2004).

P01-061 Importance of phosphorylation of small peptide induced from upstream open reading frame sequence of SAMDC gene on its own transcription
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S-adenosylmethionine decarboxylase (SAMDC), a key enzyme for polyamines biosynthesis, was tightly regulated for their homeostatic levels. 5'-leader sequence of carnation SAMDC(CSDC9) mRNA contains two overlapping tiny and small uORFs. To explore the role of tiny and small uORFs of SAMDC gene in controlling transcription and/or translation, we used a reporter GUS gene driven with the 35S promoter for making transgenic tobacco plants. When we measured GUS transcripts and GUS activity in various transgenic tobacco plants, translated protein of GUS was remarkably diminished, which implied that uORF protein was already reported as a translational inhibitor. Also, GUS transcripts was fully prevented in some lines of transgenic plants, where point-mutations(SÆA) were introduced to four putative phosphorylation sites of small uORF protein. In transgenic plants with point mutated-protein of all four putative phosphorylation sites, transcripts levels was resulted in almost similar level with in those with wild-type protein. However, the putative phosphorylation of ser10 and ser28 in uORF peptide, respectively, was significantly inhibited GUS transcripts. These results suggested that phosphorylated protein of ser10 or ser28 might act as a transcriptional inhibitor, which resulted in subsequent translational decrease in reporter gene expression. Point mutated-peptide of ser54 functioned as a transcriptional inhibitor, but that protein did not show any effect on translational inhibition.

P01-062 Functional characterization of NbNCP1 encoding a nucleus- and chloroplast-targeted protein using virus-induced gene silencing
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Virus-induced gene silencing (VIGS) is a widely used tool for functional genomics in plants. In this study, we characterized cellular function of NbNCP1 (Nicotiana benthamiana Nucleus- and Chloroplast-targeted Protein1) using VIGS. NbNCP1 encodes a protein of 325 amino acids, which contains both the chloroplast transit peptide and the nuclear localization signal. Indeed the NbNCP1: GFP fusion protein was dual-targeted to the nucleus and to distinct regions in the chloroplasts. The NbNCP1 transcript level was higher in young tissues such as young leaves and flower buds than in mature tissues. VIGS of NbNCP1 resulted in pleiotrophic defects in plant development, including leaf yellowing and abnormal leaf development. At the cell level, degeneration of the chloroplasts and the nucleus, and abnormal morphology of mitochondria were observed. Dual localization of the NbNCP1 protein in the nucleus and the chloroplasts indicates that it may play a role in the coordination of nuclear and chloroplast physiology in response to developmental and environmental signals.

P01-063 Rapid growth effects of Arabidopsis thaliana due to bacterial volatiles
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Bacteria are well known to release secondary products (e.g. toxins, antibiotics) which possess antagonistic potential to other organisms. Most of the known toxins and antibiotic compounds
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Reversible conjugation with amino acids is used by plants to adjust endogenous auxin levels as required for concerted growth and development. One of the auxin-amidohydrolases included in this regulatory mechanism, BrILL2, was cloned from Chinese cabbage (Brassica rapa L.) and tested for hydrolytic activity towards conjugates of indole-3-acetic acid. We further characterized the enzyme including substrates such as alanine (Ala) conjugates of indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA), both of which are endogenous auxins. Enzymatic activity of purified BrILL2 was tested in the presence of diethiothreitol and Mn++. Progress of the cleavage reaction was monitored by HPLC using absorbance at 284 nm. IPA-ala was hydrolyzed at the highest rate, followed by IBA-ala and IAA-ala, in this order. The 3D structure of BrILL2 was modeled by the program Modeller9v2 using the X-ray structure of IAA-amino acid hydrolase from Arabidopsis thaliana (PDB_id 1XMB) and the Mn++ binding site was determined. Ligands: IBA-Ala, IAA-Ala and IPA-Asp were docked into the protein using the AutoDock3.05 program. The most populated binding site was selected as the most probable one. The obtain complexes were neutralized by adding Na+ ions, the systems was solvated in the 8 Å thick water layer and energy minimized using the program AMBER9. To learn more about the complex stability and the substrate binding site we accomplished a series of molecular dynamics simulations.

Potato is one of the most economically important crops. Potato viruses are major menace for the potato production. Genetically engineered expression of double-stranded RNA (dsRNA) derived from virus sequences has been proposed as an efficient system to confer protection against virus diseases through RNA interference (RNAi). RNAi is a homology-dependent RNA degradation system designed to act as a nature defense barrier against virus infection. In this study we applied RNA interference to engineer transgenic potato plants that are resistant to potato virus Y, potato virus X and potato leaf-roll virus. RT-qPCR was used to clone three sequences separately from coat protein gene of PVY, coat protein gene of PVX and replicase gene of PLRV. The three sequences were connected and replicase gene of PLRV. The fused gene was inserted into the vector pHELLS-GATE which facilitates the rapid, efficient and simple production of hpRNA constructs, then introduced in potato plants by agrobacterium tumefacions mediated transformation. PCR and Southern Blot were used for the detection of transgenic potato plants. Northern Blot was used for transcript analyses and siRNA detection. The presence of the viruses was evaluated in plants by DAS-ELISA and Real-Time PCR. This research is helpful to develop plants resistant to multiple viruses using RNA.

P01-066 Tilling screening of an EMS mutant collection of Pismum sativum and identification of met1 lines affected in global CG methylation

J. Schmidt*, M. Dalmais*, J. Burstin and A. Bendahmane*

*INRA/URGV, France

The systematic characterisation of gene functions in species recalcitrant to Agrobacterium-based transformation, like Pismum sativum, remains a challenge. To develop a high throughput forward and reverse genetics tool in pea, we have constructed a reference P. sativum EMS-mutant population and developed a Tilling platform and associated database, UTILLdb (Dalmais et al. 2008, Genome Biology 9: R43). The Met1 gene is implicated in epigenetic control of transcription through maintenance of DNA methylation. We are interested in isolating Psmet1 mutant lines to study the role of DNA methylation in plant development and stress responses, both for fundamental and applied purposes. Starting from the sequence of gene AtMet1 of A. thaliana, we identified and tilled homologous sequences in P. sativum. We identified 96 Psmet1 mutant lines, 51 of them with amino-acid change. Their methylation level was compared by Methylation-Sensitive Amplified Polymorphism. Methylation of Cyclops transposons was also investigated by Sequence-Specific Amplified Polymorphism. Seven lines showing a decrease of their methylation level have been retained for further analysis. They also are being crossed to WT plants in order to generate epi-Recombinant Inbred Lines. Such lines will allow to identify epi-QTL involved in stress responses, thus linking methylation of a given region to a specific QTL; besides, interesting epimutations for crop improvement shall eventually be fixed.

P01-065 Characterization of the Brassica rapa amido-hydrolase, BrILL2

B. Savic*, J. Ludwig-Müller, S. Tomic, V. Magnus and B. Salopek-Sondi

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are liquid or solid at room temperature. But bacteria also emit a wealth of volatiles, comprising monoterpenes, sesquiterpenes, aromatics, fatty acid derivatives etc. In co-cultivation systems the volatiles of many rhizobacteria and phytopathogenic bacteria effect the growth of various fungi and A. thaliana negatively. Strains/isolates of Pseudomonas, Senetrophomonas and Serratia revealed strong reductions of the fresh weight of A. thaliana, while Burkholderia cepacia and Staphylococcus epidermidis promoted the growth of the plant. To monitor the effects of the bacterial volatiles, morphology changes, H2O2 production and activation of stress inducible promoters were registered during the development of A. thaliana. The plants react very fast to the bacteria application; within one day stress promoters are activated.

P01-064 RNA interference mediated resistance against potato virus Y, potato virus X and potato leaf-roll virus

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Peel of potato, containing a large number of amino acids, is one of the most economically important crops. Potato viruses are major menace for the potato production. Genetically engineered expression of double-stranded RNA (dsRNA) derived from virus sequences has been proposed as an efficient system to confer protection against virus diseases through RNA interference (RNAi). RNAi is a homology-dependent RNA degradation system designed to act as a nature defense barrier against virus infection. In this study, we applied RNA interference to engineer transgenic potato plants that are resistant to potato virus Y, potato virus X, and potato leaf-roll virus. RT-qPCR was used to clone three sequences separately from coat protein gene of PVY, coat protein gene of PVX, and replicase gene of PLRV. The three sequences were connected and replicase gene of PLRV. The fused gene was inserted into the vector pHLLS-GATE which facilitates the rapid, efficient and simple production of hpRNA constructs, then introduced in potato plants by agrobacterium tumefaciens mediated transformation. PCR and Southern Blot were used for the detection of transgenic potato plants. Northern Blot was used for transcript analyses and siRNA detection. The presence of the viruses was evaluated in plants by DAS-ELISA and Real-Time PCR. This research is helpful to develop plants resistant to multiple viruses using RNA.

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P01-066 Tilling screening of an EMS mutant collection of Pismum sativum and identification of met1 lines affected in global CG methylation

J. Schmidt*, M. Dalmais*, J. Burstin and A. Bendahmane*

*INRA/URGV, France

The systematic characterisation of gene functions in species recalcitrant to Agrobacterium-based transformation, like Pismum sativum, remains a challenge. To develop a high throughput forward and reverse genetics tool in pea, we have constructed a reference P. sativum EMS-mutant population and developed a Tilling platform and associated database, UTILLdb (Dalmais et al. 2008, Genome Biology 9: R43). The Met1 gene is implicated in epigenetic control of transcription through maintenance of DNA methylation. We are interested in isolating Psmet1 mutant lines to study the role of DNA methylation in plant development and stress responses, both for fundamental and applied purposes. Starting from the sequence of gene AtMet1 of A. thaliana, we identified and tilled homologous sequences in P. sativum. We identified 96 Psmet1 mutant lines, 51 of them with amino-acid change. Their methylation level was compared by Methylation-Sensitive Amplified Polymorphism. Methylation of Cyclops transposons was also investigated by Sequence-Specific Amplified Polymorphism. Seven lines showing a decrease of their methylation level have been retained for further analysis. They also are being crossed to WT plants in order to generate epi-Recombinant Inbred Lines. Such lines will allow to identify epi-QTL involved in stress responses, thus linking methylation of a given region to a specific QTL; besides, interesting epimutations for crop improvement shall eventually be fixed.

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P01-067  Effects of types of nitrogen nutrition on growth and GUS-activity in Arabidopsis thaliana plants transformed with pARR5:GUS construct
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The aim of the work was to elucidate the effects of nitrogen nutrition on the expression of the GUS gene under the control of the cytokinin-dependent ARR5 gene promoter in transgenic A. thaliana as dependent on plant growth and development. The system enables a evaluation of the content of physiologically active cytokinins from the GUS expression. The plants were grown on 0.5 MS salts and 20 mM nitrate or ammonium. Expression of the GUS gene was quantified by staining with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide and image processing. In nitrate-fed plants active cytokinins accumulated in leaves’ blade in the period of intensive growth mainly in the regions of vascular system differentiation. In the early period of growth there was apical-basal gradient of GUS-expression. Maximal intensity of GUS activity was observed in base of blade and hydathodes. The basal-apical gradient of GUS-expression in blade was observed when upper leaves formed. GUS-activity decreased remaining in the basal regions of petioles. In the course of plant development cytokinin activity shifts to upper adult leaves. In ammonium-fed plants, GUS activity was detected mainly in cotyledons, being often more intensive than in the nitrate-fed plants. Coincidence of GUS-staining with vascular systems during early cotyledon’s development was less pronounced. Leaf growth was suppressed, and this coincided with the reduced content of active cytokinins in them.

P01-068  Arabidopsis PIP5K4 regulates pollen tube growth and polarity through modulation of endocytosis and membrane secretion
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Pollen tube growth involves activation of complex signaling networks, rapid synthesis and release of specific molecules. Phosphatidylinositides are emerging as novel second messengers in plant cells, and our group has reported their importance in pollen tube growth. We investigated the function of an Arabidopsis pollen-expressed gene encoding phosphatidylinositol-4,5-monophosphate 5-kinase 4 (PIP5K4) using a reverse genetics approach. Homozygous mutant plants revealed that both pollen germination and tube growth were significantly impaired requiring more time to achieve fertilization, when compared to wild-type pollen tubes. The T-DNA insertion in PIP5K4 is associated with a partial transmission defect, such that the mutant allele is transmitted to the progeny at a reduced frequency. PIP5Ks produce PtdIns(4,5)P2 and have been involved in vesicle trafficking and cytoskeletal rearrangements. Analysis of secretory events using FM dyes showed that pip5k4 cells incorporate less dye suggesting a reduction in endocytosis and membrane recycling. Imaging of elongating tobacco pollen tubes transiently transformed with PIP5K4-GFP fusion construct revealed that the protein localizes to the plasma membrane with a higher concentration in the flanks of the apical region. Over-expression of the fusion protein led to a relocalization of the signal extending to the apex and to abnormal phenotypes. Our results showed that PIP5K4 play a role in the regulation of pollen tube growth.

P01-069  Ethylene and ABA may share signalling component upon A. thaliana plants response to UV-B
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Mechanism governing a wide range of UV-B (280–320 nm)-induced responses in plant cells is largely unknown. One of the possibilities is that UV-B activates distinct signalling routes dependent on ethylene and ABA. To verify a hypothesis that in UV-B-irradiated plant cells ethylene and ABA signalling pathways may share signalling component(s), ethylene-insensitive Arabidopsis mutants etr1 and ctr1 were used. No visible morphological changes were observed in plants irradiated with UV-B of low or intermediate levels although significant changes in ethylene and ABA contents were revealed. Since apoptotic DNA laddering was not apparent, the growth perturbations observed in all genotypes may be accounted rather for UV-B radiation specific response then for apoptosis. Therefore the survival of UV-B-treated plants was partly due to switching on a UV-B-specific signalling. We identified MAPks for which UV-B is likely to be a primary signal. These MAPks differ from ethylene-activated 47-kD MAPK. Biochemically UV-B-induced MAPks are similar to AtMPK3/6. UV-B-induced elevation in ABA content may switch on ABA-dependent MAPK module. But it is premature to make the final conclusions since we could not to identify reliably ABA-induced MAPK even when in vitro cultivated cells of A. thaliana (wild-type, etr1 and ctr1) were used. We propose a model depicting the signalling events occurring in response to UV-B irradiation.

P01-070  MAP kinase substrates in Arabidopsis
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MAP kinases are highly conserved across species, but their substrates are not. By an earlier search based on residues surrounding the phosphorylation site, several putative AtMPK3/6 substrates were identified. In vitro kinase assays confirmed that most of these proteins were in vitro substrates of AtMPK3 and AtMPK6. These substrates were from diverse protein families with the only apparent commonality being this phosphorylation motif. These proteins included a transcription factor and small basic proteins with no biochemical role yet defined. Mutation of the predicted phosphorylation sites in the candidate proteins abolished the MAPK phosphorylation. In vivo phosphorylation of these new candidates has been shown by mass spectrometry and ProQ diamond staining for four of these proteins. Similar to the MAP kinases, these substrates may be involved in development or stress related processes. Two of the substrates were phosphorylated after flagellin treatment, but two
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candidates do not appear to be phosphorylated during biotic stress. One candidate that does not seem to be stress activated exhibited clear developmental effects in true leaves if overexpressed in planta, whereas plants expressing an unphosphorylatable version of this protein showed clustering of stomata in cotyledons providing a link to loss-of-function studies of AMPKα3/6. These plants also have a defect in the anthocyanin pathway and currently we are investigating a link to sugar metabolism/signaling.

P01-071 Do members of the fantastic four (FAF) protein family modulate meristem size via trehalose-6-phosphate synthesis?


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**Max Planck Institute for Developmental Biology, Germany

The plant specific FANTASTIC FOUR (FAF) gene family is extensively regulated throughout Arabidopsis development. All four genes are strongly expressed in the vasculature, but individual FAFs are present in different domains that overlap only partially, FAF2 and FAF4, which are expressed in the centre of the meristem, repress WUS expression in the organising centre, and are themselves under repression by CLV3. Constitutive expression of any of the FAFs results in shoot meristems that appear significantly smaller than wildtype. Additionally, overexpression or knock-down leads to defects in vein patterning. From microarray analysis on plants constitutively expressing individual FAFs, we identified genes encoding enzymes involved in sugar metabolism, especially trehalose biosynthesis. Interestingly, we found that Trehalose-6-Phosphate Synthase 1 (TPS1) interacts with all FAF proteins in yeast two-hybrid screens. TPS1 transcript is present in the vasculature and in the peripheral zone of the vegetative meristem, and trehalose-6-phosphate (Tre6P) has been implicated in sugar-signalling pathways in plants. Using liquid chromatography coupled to triple quadrupole mass-spectrometry (LC-MS/Q3) and fluorimetric assays we found increased levels of both Tre6P and trehalose in shoot apices of FAF overexpressors. We are currently investigating how the FAFs affect trehalose metabolism at the shoot apex.

P01-072 HD-Zip class I genes as components of ABA and drought signalling system in Arabidopsis thaliana

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The ability of coping with environmental changes is vital for the conservation of any life form and, even more for plants in particular, as they are sessile organisms. Lack of water is one of the most critical conditions since it reduces growth and ultimately survival. Such perception elicits a signal cascade that eventually activates genes involved in the adaptation process, such as the homeodomain leucine zipper (HD-Zip) class I genes, plant-specific TFs upregulated under ABA treatment, drought and osmotic stress. Some of these genes suppress stem growth in close relation to drought; others are linked to light signalling providing the plant with a system to integrate information on both external stimulus. Despite these genes were originated by genome duplication events, they all have been selected in the evolution. So, although similar regulatory functions apply for the whole gene class, each HD-Zip gene itself contains a special trait that allows the plant a tighter control. However, little is known about each specific gene function and its regulation. Both the target gene repertoire, and the nature of the dimers involved in DNA binding is still unknown. ChIP and BiFC are used to focus on DNA- and protein-protein interactions, showing HD-Zip genes cross-regulation, as well as a putative feedback regulation among these and the ABA-related ab1 and ab2 genes, as some of the regulatory connections involved in the adaptive plant response to changing environmental conditions.

P01-073 Genome-wide expression analysis shows significant differences in root and shoot induced jasmonic acid responses in a feral Brassica species

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The plant specific FANTASTIC FOUR (FAF) gene family is extensively regulated throughout Arabidopsis development. All four genes are strongly expressed in the vasculature, but individual FAFs are present in different domains that overlap only partially, FAF2 and FAF4, which are expressed in the centre of the meristem, repress WUS expression in the organising centre, and are themselves under repression by CLV3. Constitutive expression of any of the FAFs results in shoot meristems that appear significantly smaller than wildtype. Additionally, overexpression or knock-down leads to defects in vein patterning. From microarray analysis on plants constitutively expressing individual FAFs, we identified genes encoding enzymes involved in sugar metabolism, especially trehalose biosynthesis. Interestingly, we found that Trehalose-6-Phosphate Synthase 1 (TPS1) interacts with all FAF proteins in yeast two-hybrid screens. TPS1 transcript is present in the vasculature and in the peripheral zone of the vegetative meristem, and trehalose-6-phosphate (Tre6P) has been implicated in sugar-signalling pathways in plants. Using liquid chromatography coupled to triple quadrupole mass-spectrometry (LC-MS/Q3) and fluorimetric assays we found increased levels of both Tre6P and trehalose in shoot apices of FAF overexpressors. We are currently investigating how the FAFs affect trehalose metabolism at the shoot apex.

P01-074 Diversity and redundancy within the Arabidopsis CLAVATA2 family


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The CLV2 gene in Arabidopsis encodes a Leucine-rich repeat receptor-like protein (RLP) that is hypothesized to dimerize into a CLV1/CLV2 receptor complex and is involved in restricting the number of stem cells in the shoot apical meristem. RLPS typically consist of an extracellular LRR-domain, a transmembrane domain and a short
cytoplasmic tail that lacks any apparent signalling domain. Our previous genome-wide functional analysis of 57 Arabidopsis RLP genes revealed that mutant phenotypes were only observed for a few genes including the two reported genes CLV2 and TMM, despite that a wide range of developmental stages and treatments were tested. To gain further insights into the biological role of these receptor like proteins, we identified eight RLPs which closely resemble CLV2 based on sequence similarity and domain structure. Out of these eight RLPs, only RLP2 and RLP12 were able to functionally rescue the clv2 mutant when expressed under the control of the CLV2 promoter, implicating a functional redundancy among these receptors. Double mutant combinations were created and GUS reporter fusion genes were constructed within this subfamily to further characterize the function and expression pattern of these receptors. In order to determine the functional domains of CLV2, several deletion constructs of CLV2 and domain-swaps between CLV2 and RLP38 were created and tested for their ability to complement the clv2 mutant and the results will be presented.

P01-075 Global changes in gene expression during embryonic development in Picea abies and Pinus sylvestris
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The characterization, expression patterns and functions of genes regulating embryoid development in gymnosperms is interesting from an evolutionary point of view. It is also of use when developing efficient protocols for mass propagation via somatic embryogenesis. We have compared global changes in gene expression during embryoid development in Picea abies and Pinus sylvestris in order to elucidate how specific developmental processes are regulated which differ between the two species. This was done using a microarray with more than 12,000 spotted cDNA clones from Pinus taeda. The embryogenic tissue samples for RNA profiling were proliferating embryogenic cultures in the presence of plant growth regulators (PGRs), early somatic embryos 1 and 2 weeks after withdrawal of PGRs and late somatic embryos after 1 and 2 weeks on maturation medium for P. abies and P. sylvestris, respectively. Significance Analysis of Microarray (SAM) testing identified 849 genes from P. abies and 767 genes from P. sylvestris that were deemed as significantly differentially expressed. These sets of genes have been assigned Gene Ontology (GO) terms based on sequence homology to translated Arabidopsis proteins. Over and under representations of these GO terms have been identified. Furthermore, candidates for genes regulating embryoid development will be presented.

P01-076 Subcellular localization and functional characterization of overexpressed plant acetylcholinesterase (AChE) gene in rice plant
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We previously proposed that the acetylcholine (ACh), ACh receptor (AChR) and acetylcholinesterase (AChE) system (ACh-mediated system) in plants may play a signal transduction in the same manner as the animal system. Recently, we purified and cloned AChE genes from maize and sorghum seedlings. In this study, to understand function of ACh-mediated system in plants, we generated transgenic rice plants overexpressed maize AChE gene and rice AChE homologous gene. The transgenic plants exhibited extremely high AChE activity compared with the wild-type. Thus, rice AChE homologous gene might be rice AChE gene. Further, we investigated the subcellular localization of maize AChE in transgenic rice plants using expression of green fluorescent protein (GFP) fusions. The maize AChE protein was localized in extracellular spaces in transgenic plants. Furthermore, these plants were analyzed for gravitropic response. The transgenic rice seedlings showed a drastic gravitropic response, indicating that the plant AChEs play important roles in the gravitropic response. It means that the ACh-mediated system can act as a candidate for potential-gating regulator. [This research was supported by Ground-based Research Program for Space Utilization promoted by Japan Space Forum.]

P01-077 Phosphorylation-mediated regulation of a rice ABRE binding factor activity
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OREB1 is a rice ABA-inducible group A bZIP transcription factor. We previously reported that OREB1 is phosphorylated by a SnRK2 kinase in vitro at multiple sites of functionally different domains. In the present study, we have investigated phosphorylation-mediated regulation of OREB1 activity by using site-directed mutagenesis of phospho amino acid residues. The N-terminus 28 amino acids region is essential for transactivation function of OREB1. Two highly conserved phosphorylation modules (P1 and P2) are located nearby the transactivation domain, and our data implicate that P1 and P2 may have differential regulatory functions on transactivation activity of OREB1. Mutation of C-terminus phosphorylation module abolished the protein-protein interaction between OREB1 and 14-3-3 protein. Mutation of basic domain of OREB1 resulted in a complete loss of transactivation function, possibly through impaired DNA binding activity. On the other hand, those mutations did not significantly affect nuclear localization of OREB1 protein. Our present data suggest that the presence of multiple phosphorylation modules could provide efficient and diverse way of fine control of OREB1 activity in the complex network of ABA and stress signaling. This work was supported by NIAB and On-site Cooperative Agriculture Research Project, RDA, Republic of Korea (Y.I.S.).

P01-078 De-masking the role of ELF3 in the circadian clock
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P01-079 Using the important pot plant poinsettia as a model for studying abscission
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Organ abscission is an important event in plant development since the abscission of flowers will reduce ornamental value and abscission of seeds, fruits or berries may reduce yield in many agricultural and horticultural crops. Bearing this in mind, surprisingly little research is performed to investigate this event in a plant’s life cycle. Our research group has investigated floral abscission using poinsettia as a model plant. We have developed a reliable and precise method for induction of abscission. Using this method, we have studied abscission from molecular and physiological angles during the 7 day duration from induction to abscission. We obtained 127 sequences from a differential display, where some of these have been investigated further through quantitative real-time PCR, RACE and homology alignments in databases. Furthermore, we have performed RNA in situ hybridisation for determination of gene expression in time and space in sections of poinsettia flowers. Using monoclonal antibodies for carbohydrate epitopes and FT-IR analysis of the abscission zone, we have found very interesting evidence of the breakdown of the cell walls during the abscission process. Finally, we have studied the hormonal balance in the buds and the effect of applying exogenous hormones on the abscission process. This has lead us to a hypothesis on auxin gradient and the ability to use this together with ethylene to shift the abscission zone to a different location in the bud.

P01-080 Polyamine derived apoplastic ROS orchestrate abiotic stress responses, either induction of stress-responsive genes or PCD
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Polyamine (PA) oxidases (PAOs) are ROS-generating enzymes mostly localized in the apoplast. In this work, we examined the contribution of PAO to ROS generation during salinity, and whether these ROS are sufficient to initiate transduction signals. We developed transgenic plants over- (S-pao) or down-regulating (A-pao) the maize pao. These plants were supplied with salt, and using ROS detection techniques we were able to detect H2O2 in the apoplast. As expected, S-pao plants accumulated significantly higher ROS levels, while the opposite was true for A-pao plants compared with WT. These ROS could promote PCD in a dose-responsive manner, with S-pao plants showed higher PCD, in contrast to A-pao. Also, we followed the transcription patterns of two stress-responsive genes, namely adc (arginine decarboxylase) and samdc (S-adenosyl-L-methionine decarboxylase) after the exogenous supply of Spermidine (Spd) and Spermine (Spm). These two mRNAs were accumulated only in A-pao and WT plants, while S-pao plants failed to respond. Furthermore, we showed that salinity induces the secretion of PAs and mostly of Spd to the apoplast, were they are oxidized by PAO, and the size of ROS titers determines whether stress-responsive genes will be expressed or the PCD syndrome will be induced. These results are in accordance with an emerging novel role for the apoplastic compartment and for PAs in the stress tolerance/sensitivity molecular responses. 1Funded by EPEAKII-Pythagoras and COST858.

P01-081 Characterisation of genes involved in photoperiodic control of growth cessation and bud formation in Norway spruce
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In trees of the temperate zone shoot elongation is under photoperiodic control. In night lengths shorter than a critical length growth cessation, bud set and dormancy are induced. It is well established that the phytochrome system is a light length sensor in trees. In photoperiodic control of flowering in Arabidopsis thaliana the phytochrome system acts together with the blue light receptors cryptochrome. However, information on the role of cryptochromes in woody species, particularly gymnosperms, is scarce. To improve our understanding of photoperiodic control of dormancy-related processes in gymnosperms we have characterised phytochrome and cryptochrome genes as well as genes thought to act downstream of these light receptors in Norway spruce (Picea abies). These genes include PHYO, PHYN (PHYA-like) and PHYP (PHYB-like), two different cryptochrome genes, two CONSTANS-like genes (PaCOL1 and 2), a TFL1-like gene as well as a MADS-box gene with homology to SOC1 in Arabidopsis. Transcript levels...
of PHYN, PaCRY1, PaCRY2, PaCOL1, PaCOL2, PaTFL1 as well as the PmMADS box gene were all affected by photoperiod and light quality.

P01-082 Effects of temperature-light interactions on flowering
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In Arabidopsis small changes in ambient temperature can have relatively large effects on flowering time. In the wild type (WT) of Arabidopsis flowering is induced more rapidly in 22 or 23 than 16°C (Blazquez et al. 2003, Halliday and Whitelam 2003, Halliday et al. 2003). In autonomous-pathway mutants (ica, tve and fha) this is not the case, they flower at the same time in both these temperatures (Blazquez et al. 2003). This suggests that the autonomous pathway also acts as a thermosensory pathway. Blazquez et al. (2003) found that effects of temperature on flowering time in the WT are not correlated with changes in the expression of FLC, but with altered expression of FT. In our studies we observed that the time to flowering of the autonomous pathway mutants was affected by diurnal temperature variations. The mutants flowered earlier under low day and high night temperature than under the opposite temperature regime or low or high constant temperature, where they flowered at the same time, regardless of the temperature.

P02-011 Going deeper into the composition of the chloroplast protein import apparatus in Arabidopsis
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The assembly of the photosynthetic apparatus requires the import of approximately 2000 different nuclear encoded proteins in plant chloroplasts. Nuclear encoded proteins are synthesized in the cytosol as precursors and must be imported into the nascent chloroplast. The chloroplast is enclosed by an envelope consisting of two membranes. Both contain translocons to facilitate the import of precursor proteins. These are termed the Toc- and Tic-complexes (Translocon at the outer/inner chloroplast membrane). The Toc-complex consists of three major components forming a stable complex, Toc159 and Toc34 are surface exposed, GTP-binding integral membrane proteins. The available evidence indicates that the two proteins act in concert to recognize the chloroplast targeting peptide. Toc75, the third component, forms a hydrophilic channel through which precursors are translocated across the outer membrane. The work in our lab is aimed at the elucidation of Toc-GTPase function using a combination of in vivo and in vitro methods. Here we described the used of the TapTag (Tandem Affinity Purification) approach to identify new members of the Toc complex, and get new information about the complex structure. To increase the chance of identifying the entire set of Toc-interacting proteins, different Toc-proteins are being used as bait. Here we present preliminary results for the Toc159 bait protein, thought to act as the primary receptor for transit sequence.

P02-012 Characterization of Tic62: a possible link between chloroplast preprotein import and photosynthesis?
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The vast majority of chloroplast proteins is translated in the cytosol and posttranslationally imported into the organelle. The preprotein translocon at the inner envelope of chloroplasts (Tic complex) facilitates the import of these preproteins. It contains seven distinct subunits as identified so far. For each of those, specific functions have been proposed based on structural prediction or experimental evidence. Three out of the seven Tic subunits possess modules which could act in a redox-regulation of the import process: Tic55 (containing a Rieske-Fe-S center), as well as the two dehydrogenases Tic32 and Tic62. Early on, Tic62 had been proposed as a putative redox sensor based e.g. on its redox activity and association with the FNR. Up to date however, the functional implication of this close cooperation remains enigmatic. Therefore, we set out to investigate the role of Tic62 and its photosynthetic partner protein in vivo in more detail. Here we present the characterization of tic62 knockout plants in the model organism Arabidopsis thaliana. Knockouts do not show a strong visible phenotype but have a drastically reduced amount of membrane-bound FNR. This, together with data from other assays like PAM measurements, coexpression and Affymetrix analyses as well as enzymatic assays strengthens the close relationship of Tic62 and FNR and indicates a connection of Tic62 to further photosynthetic and metabolic components on the expression and protein level.
also evidence for a substantial disturbance of the mitochondrial redox homeostasis with increases in the oxidation state of glutathione in seedlings and an upregulation of the whole cellular antioxidant defences in older plants. Reduced MnSOD levels had biochemical consequences that were rather specific to the mitochondrion with an inhibition of specific mitochondrial TCA cycle enzymes (aconitase and NAD-isocitrate dehydrogenase) and a consequent decrease in mitochondrial TCA cycle flux. However, total respiratory CO₂ release actually increased which may indicate an over-compensatory diversion of carbon into the cytosol to bypass inhibited steps via cytosolic isoforms of aconitase and ICDH. Increases in the activity of extra-mitochondrial antioxidant defences in older plants suggest that the altered mitochondrial redox and metabolic status is sensed by the nucleus. Overall, the results demonstrate that reduced MnSOD affects mitochondrial redox balance and plant growth.

P02-014 Function and evolution of plastid metabolite transporters
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Plastids are organelles of plant cells that can be traced back to a cyanobacterial ancestor. Plastids of higher plants are surrounded by two membranes, the outer and inner envelope membranes. Solute transport across these membranes is catalyzed by porins and by specific metabolite transporters is key to connecting plastid metabolism with that of other cellular compartments. Thus, it has been suggested that the insertion of different transporter proteins into the envelope membranes was one of the first steps for the establishment of the primary endosymbiosis, allowing the ancestor of the Plantae to profit from cyanobacterial carbon fixation. It became clear during the last years that the plastid envelope transporters have multiple evolutionary origins. Only a few transport proteins can be traced back to the cyanobacterial ancestor of plastids while most of the envelope transporters are derived from the host cell. One group of these proteins, the phosphate translocators, are derived from nucleotide sugar transporters of the ER and Golgi membranes of the host cell. Another group of plastid proteins belong to the large mitochondrial carrier family (MCF). In contrast, the ADP/ATP transporter of the plastid envelopes belong to a family that is restricted to plants and obligate intracellular parasites like Chlamydia or Rickettsia. So, these proteins might have been introduced by horizontal gene transfer.

P02-015 Prediction of dual protein targeting to plant organelles
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P02-016 Plant mitochondrial rhomboid, AtRBL12, has a different substrate specificity from its yeast counterpart
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Regulated intramembrane proteolysis (RIP) is a new mechanism where substrates undergo proteolysis within their membrane-spanning domain. One of proteases family involved in RIP is rhomboids – polytopic serine proteases. Rhomboids were shown to play distinct roles in plasma membrane, Golgi apparatus and mitochondria. The first identified mitochondrial rhomboid was Pcp1 from Saccharomyces cerevisiae. The main goal of this work was to examine if Pcp1 possess any homologue(s) in plant mitochondria. Among 13 rhomboids in Arabidopsis thaliana, we selected five predicted as mitochondrially targeted. Among these proteins we identified one mitochondrial rhomboid, RBL12 (At1g18600) via the GFP transient assay. Despite a high sequence homology between RBL12 and yeast cells with RBL12 was not processed in vivo. RBL12 does not recognize the yeast substrates, cytochrome c peroxidase (Ccp1) or dynamin-like GTPase (Mgm1). We also did not observe processing of Ccp1 or Mgm1 when incubated with Arabidopsis mitochondrial extracts. Our results imply that plant mitochondrial rhomboids function in a specific manner and thus differ from their yeast and mammal counterparts. In light of these findings we decided to examine plant-specific function of RBL12 using a proteomic approach. A comparison of mitochondrial proteome of Arabidopsis wild-type and rbl12 plants will be presented.
P02-017 Chloroplastic NADPH thioredoxin reductase mediates photoperiod-dependent development of leaves in Arabidopsis

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Thioredoxins are small proteins which catalyze disulphide-dithiol interchange in their target proteins thus being involved in the regulatory redox networks in cellular compartments. Plant plastids and cyanobacteria have unique components of the thioredoxin systems: ferredoxin-dependent thioredoxin reductase (FTR) and a recently found plastidial NADPH-thioredoxin reductase (NTRC) with an extra thioredoxin-folding domain in the C-terminus to the reductase sequence. To reveal the physiological significance of NTRC, we have characterized SALK T-DNA insertion mutant lines of the NTRC gene in Arabidopsis thaliana. Besides retarded growth, ntrc plants show severe developmental (small cell size, few chloroplasts, delayed flowering and senescence) and metabolic (low carbon assimilation rate, hormone and amino acid imbalance) disorders when grown under short-day conditions, which indicates NTRC’s non-redundant function relative to FTR. The mutant phenotype was less severe in plants grown under long-day or continuous light. Transcriptome profiling of ntrc and wild type plants revealed distinct repression of two genes related to the photoperiodic growth. Furthermore, the growth in short days induced a unique combination of differentially expressed genes in the ntrc leaves related to profound mutant phenotype of ntrc plants: up-regulation of five photosynthetic and two chlorophyll biosynthesis genes, seven genes encoding heat-shock proteins and four genes encoding chloroplast proteases.

P02-018 The Novel factors involved in intracellular transport of seed storage proteins in Arabidopsis thaliana


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In higher plants, seed storage proteins are synthesized on the ER as precursors and then are transported to protein storage vacuoles, where they are processed into mature forms. We isolated two Arabidopsis thaliana mutants, mag1 and mag2, that accumulated the precursors of two major storage proteins, 2S albumin and 12S globulin, in dry seeds. mag1 seed cells mis-sorted storage proteins out of the cells. Our findings suggest that MAG1 is a component of the exit of the ER-localized t-SNAREs, AtSec20 and AtUfe1. Our findings suggest that MAG2 functions in the exit of storage protein precursors from the ER in plants. In order to clarify mechanism of MAG2-dependent transport, we also identified factors that form a complex with MAG2. Here, we report characterization of MAG2-complex components (MACCs). Our results suggest that MAG2 complex plays a significant role in the transport of storage proteins in Arabidopsis thaliana (Li et al. 2006).


P02-019 Functional gene transfer from the chloroplast to the nucleus in tobacco

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DNA transfer from the organelles (mitochondria and plastids) to the nucleus has been a major driving force of eukaryotic nuclear genome evolution. The vast majority of DNA transfer to the nucleus is non-functional. However, in a small subset of transfer events the gene is activated and functional gene transfer established. While significant steps have been made in elucidating the frequency of organelle to nucleus DNA transfer, less is understood about gene activation upon arrival in this new environment. The work presented here has enabled experimental observation of these evolutionary events and molecular analysis of the steps leading to activation. We had previously generated a number of independent tobacco lines in which a segment of the chloroplast genome had been transferred to the nucleus. Twelve of these lines were screened for nuclear activation of a chloroplast transgene (aadA). From the ~1.5 billion cells screened three plants were generated that showed spectinomycin resistance due to activation of aadA through acquisition of a nuclear promoter. In at least one case this resistance was mitotically unstable. These results suggest that functional gene transfer involves a dynamic interplay between transfer, rearrangement, function and loss of nuclear organelle DNA sequences.

P02-020 Multi-facetted controls in chloroplast gene regulation: the impact of multiple sigma transcription factors and their master regulator

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Chloroplasts contain two RNA polymerases, each with important roles in plastid gene expression: the (nucleus-encoded) single-subunit NEP and the multi-subunit PEP with a plastid-encoded bacterial-type core and (nucleus-encoded) regulatory proteins. The latter include multiple sigma factors for gene-, developmentally-stage-, and environmentally-regulated initiation of transcription (Loschelder et al. 2006, Schweer et al. 2006). Furthermore, a PEP-associated Ser/Thr protein kinase named PTK (plastid transcription kinase) is responsible for phosphorylation of sigma factors and other
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PEP constituents (Baena-González et al. 2001). Here we address the questions of, (1), redundant vs specialized functions of individual members of the sigma factor family from Arabidopsis and, (2), the control mechanism(s) for sigma phosphorylation by PTK. The experimental strategies include both bacterially expressed recombinant proteins and their specifically altered derivatives as well as Arabidopsis knockout lines and re-formation with sigma and/or PTK variants. This way it has e.g. become possible to gain information on phosphorylation sites and their impact on transcription, to map critical determinants for sigma and kinase functions, and to elucidate the mechanism of SH-group redox control of PTK.


P02-021 SRP-dependent LHCII transport to the thylakoid membrane of chloroplasts

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The nuclear encoded light-harvesting chlorophyll-binding proteins (LHCs) are the most abundant integral membrane proteins in chloroplast thylakoids and the question of how LHCs are routed to and inserted into the membrane has been the subject of research for several years. It has been shown that a chloroplast signal recognition particle (cSRP) and a SRP receptor homologue (cpFtsY) are required for the transport of LHCs from the chloroplast envelope to the thylakoid where the integral membrane protein Alb3 is essential for stable insertion of LHCs. CpSRP consists of an evolutionarily conserved 54-kD subunit (cpSRP54) and a unique 43-kD subunit (cpSRP43) (Schünemann D. 2007). In this contribution the molecular details of the SRP-dependent transport of LHCs to the thylakoid membrane are explained and novel data elucidating the docking mechanism of the transit complex to the Alb3 insertase are presented. These data focus on the molecular details of a direct interaction between cpSRP43 and Alb3.


P02-022 Redox-regulation of protein import into chloroplasts

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The vast majority of chloroplast proteins is translated in the cytosol and posttranslationally imported into the organelle. The preprotein translocon at the inner envelope of chloroplasts (Tic complex) facilitates the import of these preproteins. Seven distinct subunits have been identified so far. For each of those, specific functions have been proposed based on structural prediction or experimental evidence. Three of those subunits possess modules which could act as redox-active regulatory components in the import process: Tic55 (containing a Rieske-Fe-S center), as well as the two dehydrogenases Tic32 and Tic62. To date however, the mode of redox-regulation of the import process remains enigmatic. To investigate how the chloroplast redox state influences translocon behaviour and composition, we are studying the redox-regulatory components of the translocon in more detail. By the design of our experimental approaches we try to investigate the redox control using a variety of biochemical methods. Our results suggest that the composition of the Tic complex and the localization of certain redox-regulatory components depend on the NADP+/NADPH ratio in the chloroplast stroma. This ratio also influences the interactions of one of the redox-active proteins, Tic62, with the translocon and with the flavoenzyme ferredoxin-NADP+ oxidoreductase. Furthermore, a high NADP+ concentration seems to enhance the import of certain preproteins into the chloroplast.

P02-023 Mitochondria of common bean contain two classes of sublimons differing in copy number and maintained by various mechanisms

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Heteroplasmy – the presence of more than one type of mitochondrial genome – is a common state in plants. Usually one mitotype is prevalent, while the alternative one is present in a lower proportion. Substoichiometric mitochondrial DNA molecules (sublimons) are products of recombinations occurring in the prevalent mitotype. However, some sublimons are not accompanied by parental sequences that would serve as recombination substrates. Thus, it is not clear how sublimons are maintained and if recombination plays any role to sustain their population. We applied the real-time PCR to analyze sublimons of common bean. We found that sublimons were much more abundant (107–109) relative to the pre-
Oilseed rape (*Brassica napus*ssp *oleifera* L.) is the third most important oil crop in the world. Its total acreage is expanding very fast especially in areas with moderate climatic conditions. In Turkey, rapeseed is planted on an area of 5390 ha on which production results in 12 615 tons with in the overall local edible oil production, in spite of its being a new crop. This research was carried out to determine yield and yield components of some summer rapeseed (*Brassica napus* ssp *oleifera* L.) varieties in Ankara/Turkey conditions. The experiments were established in randomized complete blocks design with eight varieties in four replications. The research was planned for three growing seasons from 2004–2006. As a material Gladiator, Heros, Sary, Licosmos, Jura, Jumbo. Tiger and Lambda summer rapeseed varieties are used in the experiment. Observations and evaluations were made for seed yield/ha, oil content %, oil yield/ha, 1000 seed weight, plant height, branch number, capsule number per plant, seed number per capsule and percent seed moisture in harvest. Three-years results of this research showed statistically significant (0.01%) seed yield differences among eight varieties. Plant height 115.0–135.3 cm, capsule number per plant 119–129, seed number per capsule 20–25 and seed yield per hectare 2223–2747 kg were found among eight summer rapeseed varieties.

**P03-012 The control of autumn senescence in aspen (Populus tremula)**

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Bud set and autumnal senescence are highly regulated processes in *Populus tremula* which shows strong variation with latitude of origin. The environmental cue for initiation of bud set is photoperiod, where northern populations set bud at a longer day length (earlier in the season) than southern populations. Initiation of autumn senescence in aspen is also under tight photoperiodic control. We want to understand the regulation of autumn senescence — and its relation to bud set — and have studied onset and progression of senescence in many different genotypes grown under different conditions, and our data support a mechanistic model where bud set and autumnal senescence are triggered by two independent photoperiodic perception events, but that the tree is not competent to respond to the autumn senescence trigger unless sufficient time have passed since bud set, the tree has to be ‘ripe to senesce’. Timing of autumnal senescence is likely to represent a tradeoff between the carbon and nitrogen balance of the tree, since too early senescence would result in loss of carbon fixation, while too late senescence may result in loss of a large fraction of the nitrogen of the tree, that will still be present in the leaves at the time when leaf cells are killed by frost damage and the leaves are subsequently shed. To get more information about this, we have also made a metabolite study during the progression of autumn senescence.

**P03-013 Delayed greening in Theobroma cacao L.**


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Delayed greening is a specific phenomenon found in many tropical species tolerant of shade environments. Young leaves of *Theobroma cacao*, a shade-adapted evergreen tree, are thin and transparent, have low chlorophyll contents and remain in a vertical position until they nearly reach the final size. Coordination of leaf growth and photosynthetic development was investigated during the delayed greening as well as greening phase in leaves of *T. cacao*. Relative growth rate (RGR) and epidermal cell size were measured to study leaf growth while photosynthetic development was examined by analysing chlorophyll content, chlorophyll a fluorescence and leaf gas exchange. Rapid chlorophyll accumulation started concomitantly with epidermal cell expansion and decreasing RGR. The maximal photosystem II efficiency, capacity of thermal energy dissipation and net CO₂ assimilation all increased in parallel with the chlorophyll accumulation. Based on these observations, leaf development in *T. cacao* can be characterised by three distinct stages: (1) early exponential growth stage with high RGR, active cell division, low chlorophyll content and low photosynthetic activity, (2) late exponential growth stage with low RGR, pronounced cell expansion, chlorophyll accumulation and development of photosynthetic apparatus, and (3) mature stage with slowly continuing chlorophyll accumulation in fully expanded leaves. Developmental processes in delayed greening leaves will be discussed.

**P03-014 Seasonal changes in ultrastructure and pigment composition of the leaves of high alpine Loiseleuria procumbens (Ericaceae)**

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*Loiseleuria*, a procumbent growing dwarf-shrub, colonizes small to large areas at the alpine and nival zone. The shrubs develop, beneath green, also yellow and red coloured vital leaves. The red colour is caused by anthocyanins in the vacuole of the upper palisade parenchyma cells and disappears in summer from July to August. Yellow leaves can be found on unprotected growing branches, which are exposed to strong sunlight and drought. It has been investigated how the extreme alpine environmental conditions are reflected in the ultrastructure and the content of plastid pigments of the leaves, and how the green leaves differ from the red and yellow ones. Therefore leaves were fixed for electron microscopy and extracted photosynthetic pigments were analyzed with HPLC. They show differences in the ultrastructure between the different coloured leaves and between the seasons. Red and green leaves contain a greater number of organelles than the yellow ones. There are major differences between the cells in winter and during the vegetation period from May to October. In winter the organelles accumulate at the basal part of the cell. Starch is absent, the number and size of plastoglobuli increases. The organisation of thylakoids changes completely, they are stretched evenly along the whole plastid. The content of carotenoids and chlorophylls correlates positively with the temperature in the population. α-tocopherol has its greatest occurrence in the yellow, less in red leaves.

**P03-015 The study of biodiversity and different morphological characters of rocket (Eruca sativa)**

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Loiseleuria, a procumbent growing dwarf-shrub, colonizes small to large areas at the alpine and nival zone. The shrubs develop, beneath green, also yellow and red coloured vital leaves. The red colour is caused by anthocyanins in the vacuole of the upper palisade parenchyma cells and disappears in summer from July to August. Yellow leaves can be found on unprotected growing branches, which are exposed to strong sunlight and drought. It has been investigated how the extreme alpine environmental conditions are reflected in the ultrastructure and the content of plastid pigments of the leaves, and how the green leaves differ from the red and yellow ones. Therefore leaves were fixed for electron microscopy and extracted photosynthetic pigments were analyzed with HPLC. They show differences in the ultrastructure between the different coloured leaves and between the seasons. Red and green leaves contain a greater number of organelles than the yellow ones. There are major differences between the cells in winter and during the vegetation period from May to October. In winter the organelles accumulate at the basal part of the cell. Starch is absent, the number and size of plastoglobuli increases. The organisation of thylakoids changes completely, they are stretched evenly along the whole plastid. The content of carotenoids and chlorophylls correlates positively with the temperature in the population. α-tocopherol has its greatest occurrence in the yellow, less in red leaves.
Rocket (*Eruca sativa*) is a member of the Brassicaceae family and is a minor crop worldwide. In the last few decades, plant breeders have come to realize the importance of genetic diversity. In addition to breeding for better cultivars of *E. sativa*, research on this species may also lead to improved yield, quality, resistant and environmental benefits of its related crops, since *E. sativa* can be considered a genetic resource for all Brassicaceae crops. In this investigation, 1000 rocket seeds were cultured in 20 flower-pots. The different morphological characters were studied in different growth steps, from seedling growth to seed production. The leaves, stems and fruits morphology, flowers and seeds color and size, trichum present and others were variation. The different characters have different frequency. In this analysis were determined 29 lines of rocket with 12 characters associated with growth and production. There was wide variability as regards the characters of the silica. Similarly, there was wide genetic variability for seed production per plant and related characters.

**P03-016** The chemical composition of essential oils from some Romanian spontaneous species of Lamiaceae and their taxonomic significance

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The essential oils of 10 Romanian spontaneous species of Lamiaceae family were compared in order to determine the main and the specific compounds of these aromatic plants and their taxonomic significance. There were 116 compounds identified by GC/MS in the hydrodistillates of the whole aerial parts of *Acinos alpinus* L., *Calamintha einseleana* F.W.Schultz, *Montha piperita* L., *Marrubium peregrinum* L., *Marrubium vulgare* L., *Nepeta musimii* L., *Phlomis pungens* L., *Phlomis tuberosa* L., *Salvia nutans* L. and *Melissa officinalis* L. All species contain, with a single exception (melissa), and with a great variability α-pinene, β-pinene, linalool, β-caryophyllene, γ-germacrene D, α-cadinol. *M. officinalis* is an exception of this *Lamiaceae* rule, because of its volatiles: α-, β-citronellol, α-, β-citral, methyl citronelol. The other studied species contain particular volatiles as a species specificity: *Acinos alpinus*-germacrene D (29.33%), carvacrol, β-caryophyllene, *C. einseleana*-piperitone (29.05%), eucarvone, *M. piperita*-piperitone oxide (61.93%), eucalyptol, *M. peregrinum*-germacrene D (40.02%), germacrene B, *M. vulgare*-γ-elemene (35.34%), β-caryophyllene, *N. musimii*-germacrene D (26.71%), cis β-ocimene, nepetalactone, *P. pungens*-germacrene D (79.34%), *P. tuberosa*-germacrene D (42.45%), β-caryophyllene, *S. nutans*-germacrene D (66.56%), β-caryophyllene. A taxonomical dendrogram based on the principal component was designed and discussed.

**P03-017** Polyomorphhic histo-anatomical adaptations of halophytes under different natural stress factors

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Atriplex prostrata Boucher ex DC., *A. tatarica* L., *Halimione veruciera* M. (Bieb.) Aellen, *Petrosinum oppositifolia* Pall. (Litiv.), *P. triandra* (Pall.), *Salicornia europaea* L. (*Chenopodiaceae* family), *Spergularia media* (L.) C. Presl. (*Chenopodiaceae* family), *Juncus gerardi* Loisel. (*Juncaceae* family), *Aster tripolium* ssp. *pannonicus* (Jacq.) Soo and *Artemisia santonicum* L. (*Asteraceae* family) have been histo-anatomically studied. Multiple environmental factors such as the salinity and the hypoxic, anoxic and xerophytic conditions are often convergent and involved in defining various stress types. The hypoxic/anoxic stress and the salt stress which includes the ionic and the dehydration stress are some of the multiple stress types. Under these circumstances the halophyte species develop histo-anatomical features that represent a response to the corresponding environmental factor. The halophytes harvested from wet or flooded saline soils (*A. tripolium* ssp. *pannonicus*, *J. gerardi*, *S. media*) present aerenchyma, a common adaptive feature of halophyte species that grow in these soils. The species harvested from dry saline soils present xerophytic features: water storage tissues that induce succulence (*P. oppositifolia*, *P. triandra*, *A. santonicum*) and sunk stomata (*L. saligna*, *A. prostrata*). Some species developed other adaptive strategies. *S. europaea* developed tracheids and the phenomenon of succulence while *H. verruciera* and *A. tatarica* presents salt hairs.

**P03-018** Essential oil compositions of natural and culture materials of Salvia tomentosa Mill

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Essential oils obtained by hydrodistillation from natural and culture of Salvia tomentosa Mill. were analyzed by GC-MS. Plant material and cuttings of *S. tomentosa* were collected from the locality: A4, between Kastamonu and Tosya on the 6th km in Turkey. Cuttings were used in order to establish the plantation on the experimental field of our department in Ankara. The aerial parts collected from natural and culture plants were dried in shadow. In natural material, 10 compounds representing 94.64% of oil were identified while in culture material, 17 compounds representing 99.41% were determined. The major components were δ-pinene (37.56%) and camphor (19.5%) and camphor (19.15%) in natural material when camphor (30.15%) and δ-pinene (19.44%) were identified as main compounds in culture material. The other minor compounds were isoborneol, borneol and camphene in natural material and δ-pinene, camphene and borneol in culture material.

**P03-019** Variations in nutritional value among berry fruits produced in Hungary

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Fruits of four berry species (strawberry, raspberry, red and black currant) were compared in their element contents and redox parameters involving total phenol content (TPC), ferric reducing ability (FRAP), DPPH and total radical scavenger activity (TRSA) (the latter measured in a photochemiluminescence assay). Berry cultivars contained high amounts from most of the detected elements with the black currant ‘Otelo’ showing outstanding values. Black currant was characterized by the greatest antioxidant capacity in all assays. The results supplied by the FRAP, TPC and TRSA assays were closely correlated; while TRSA and DPPH varied independently. Our study provides valuable information on the antioxidant value of several berries grown in Hungary and highlights that genotype has a crucial influence on the element content and antioxidant power of berry fruits. This makes selection possible for obtaining cultivars with special nutritional purposes or helps to assign parental lines in functional breeding programs. This work was funded by the Hungarian National Scientific Research Fund (OTKA T046622).

P03-020 Dynamics of stomatal response under changed ambient [CO2]

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Transpiration of vascular terrestrial plants is governed by several environmental factors such as light, temperature, CO2 concentration [CO2] and air humidity. These abiotic factors can strongly vary in space and time which forces the plants to use an efficient stomatal control in order to achieve an optimal water balance and CO2 supply. Stomata can react rapidly to sudden changes of environmental stimuli. Dynamic of the stomatal response is best documented for changing light intensities, far less is, however, known on the response under changing [CO2]. We hypothesized that the dynamic of the stomatal response to sudden increase of [CO2] will depend on the inherent characteristics of the plants, i.e. type of stomata, type of photosynthetic carbon metabolism and that plants growing at more variable gaseous conditions (variable [CO2] at sites with natural CO2 enrichment – molettes) could be capable of faster stomatal action. In the series of the experiments we studied stomatal response of several grassland species and some agricultural plants to increase of ambient [CO2], from 350 to 700 μmol mol⁻¹, strictly controlling other factors that could influence stomatal action. Measurements revealed species specific differences. Some of the C4 grasses responded much faster to CO2 increase than other species tested. When comparing the populations of the same species growing at different CO2 environments (stable, variable), however, no difference in response was found.

P03-021 Genetic variability of clonal invasive water weed, Elodea canadensis

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Canadian water weed (Elodea canadensis) is a submerged aquatic angiosperm native to North America, from where it has spread to other continents. This invasive species causes problems in many of its new environments by changing the balance of ecosystems and making the recreational use of lakes more difficult. E. canadensis was first brought to Europe in the beginning of the 19th century and to Finland, to the Botanical Garden of University of Helsinki, in 1884. At present, it is common in the whole of Southern and Central Finland. There are only female plants in Europe, thus reproduction is only vegetative. We have recently developed 11 microsatellite markers for E. canadensis by using genomic screening with ISSR (inter simple sequence repeat) primers. With these presumably neutral markers, the basic population genetic characteristics of Finnish populations of the species are being analyzed. Since the species has spread to Finland very recently and originates from a few, clonally reproducing founder individuals, the Finnish populations are expected to contain only small amounts of genetic variation. Here we present preliminary results of the population genetic analysis. Our future aim is to identify stress-responsive genes and to compare the level, structure and distribution of genetic variation in study populations based on both neutral and adaptive markers.

P03-022 Ecophysiological characteristics of new promising producer of essential oils Artemisia lerchiana Web. ex Stechm

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Artemisia lerchiana Web. is wide-spread in the South-East of Russia. A. lerchiana is a codominant species in the phytocoenoses on chestnut soils and dominant on solonetzes. The aim of the work was to study its potential as source of essential oils in the natural and cultivated conditions. Under natural conditions, the experiments were carried out during 2004–2007 at different sites of Volgograd region differing in climatic and soil conditions. Pot experiments were carried out in the phytotron to determine the capacity for accumulation of photosynthetic pigments and maintainance of carbohydrate metabolism and water relation under increasing doses of NaCl. Essential oils were prepared by hydrodistillation or using CH2Cl2. Their individual components were analysed by chromato-mass-spectrometry. The total content of essential oils in plants comprised 1,1–1,5% of d. w. The major components were camphor, borneol, 1,8- cineole and camphene. More than 60 minor components were identified: sesquiterpenic lactones, alkanoles, sitosterols, germakrene D. In plants growing at chalk and chestnut soils, the ratios of components varied markedly. This work was supported by Department of Biological Sciences of RAS (Program ‘Fundamental bases of management of biological resources’).

P03-023 Establishing DNA barcodes for plants

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It has been shown that biological species can be identified using a short DNA sequence from a standardized position in the genome - a DNA barcode. Our main focus is to provide DNA barcodes as a new taxonomical key for plant taxa occurring in Finland. There are many areas of practical importance, especially those related...
Abstracts

to knowledge of biodiversity, verification of herbal medicines, foodstuffs or controlled species, monitoring of harmful or invasive species and ecological surveys. Additionally, DNA barcoding contributes to phylogenetic knowledge and is effective in resolving the taxonomy of poorly known groups. In our project, we are generating barcoding information of the matK gene and the trnH-psbA spacer, which both represent the chloroplast genome. We have developed new primer pairs, which work across a wide range of plant taxa. We are testing, whether these two barcoding regions provide enough taxonomic resolution or whether additional genomic regions should be included. Additionally, we are exploring how to solve taxonomic questions in difficult plant groups. A case study concerns the genus Salix. The species of this genus are very cross-fertile and numerous hybrids occur. Thus, the accurate identification of Salix is not always easy to accomplish. Finally, we will introduce a combined barcode database and e-learning project aiming to provide state-of-the-art learning techniques and means for the identification of plants and fungi.

P03-024 The quantitative assessment of ecological aftereffects
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The system approach to biological studies attracts attention of scientists. Considerable progress has been recently made in developmental, quantitative and reproductive biology. Many aspects of developmental biology require further investigation into the genotype-environment system. There are but few data concerning the details of the influence exerted by environmental factors underlying the genotype realization in a preceding generation on formation of quantitative characteristics in plants of a hereditary generation. This research summarizes the results of studying the basic mechanisms of the effects exerted by environmental factors participating in formation of the phenotype of maternal generation plants on the growth and development of offspring generation plants. Consideration has been given to the details of the influence on the individual phenotype produced by limiting environmental factors such as air temperature, soil humidity and intensity of mineral nutrition of plants. Using certain representatives of the division Magnoliophyta, the data are presented, revealing the mechanisms of influence the pre-vegetative environment has on the physiological quality of diaspores, disseminules (seeds, tubers) and quantitative characteristics of plants. The role of pre-vegetative limiting factors in the formation of adaptive response of a genotype to the environment is discussed. The problems of ecological heterogeneity of seeds form the subject matter of the investigation.

P03-025 Structure and metabolism of perennial cereals rhizomes in the late Autumn
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Rhizomes are an important component in the source-sink system of perennial plants. The reed canary-grass rhizomes amount to 50% of the total plant weight. The weight ratio of aboveground to underground organs decreased from 2.5 to 1.0 in the period of time beginning with July till October. The reduction of the rhizomes cross-section area and the relative volume of the central cylinder alongside with increasing of the cortex parenchyma volume were revealed in autumn. The decreasing of the thickness and layers of the endodermal cell walls was found. Apparently the cell walls suberin and polysaccharides were hydrolized up to sugars which can be cryoprotectors. The oligosaccharides content considerably increased after the first frosts. The cytokinins content was about 1000 μg g⁻¹ DW that was 34 times higher compared to that in summer period. Cytokinins regulate the rhizomes morphogenesis and synthesis of cryoprotectors substances. The rhizomes growth rate decreased greatly and the optimum temperatures for the rhizome growth shifted to low positive temperatures. The results are discussed in connection with the adaptation of the rhizomes metabolic processes at low temperatures.

P03-026 Shortday responses of growth and carbon allocation in leaves of Lombardy poplar
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Autumnal growth cessation induced by daylength shortening is a prerequisite for winter acclimation in deciduous trees in the northern hemisphere. Since leaf growth is affected by intrinsic (day-night) rhythms in plants, like many other processes including carbohydrate metabolism, we investigated how daylength interacts with the diel growth activity and carbon allocation in leaves of Lombardy poplar (Populus nigra var. italica Koehne). Under longday (16 h/8 h day/night), leaf growth rate reached the maximum at the end of the day (14–16 h after light-on) and the minimum at the end of the night. Under shortday (9 h/15 h), growth quickly slowed down and the maximum shifted to 7–9 h after light-on, i.e. to the end of the day under the shortday regime. This maximum was less pronounced compared with the longday maximum, and there was still a small peak in growth rate at the time point of the longday maximum. The shortday treatment resulted in decreased carbohydrate contents in growing leaves, especially at 2 h and 12 h after light-on. A pulse-chase experiment with 14C, indicated dramatically reduced export of 14C-labeled assimilates from lamina to midvein in growing leaves in the morning under shortday. The contribution of the growth activity programmed at 14–16 h after light-on under both daylength conditions and adaptive carbohydrate allocation in shaping the diel growth pattern in poplar leaves will be discussed.

P03-027 Seasonal variations of total phenol content, antioxidant and radical-scavenging activities of three halophytic species
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P03-027 Seasonal variations of total phenol content, antioxidant and radical-scavenging activities of three halophytic species
Eryngium maritimum L. (sea holly, Apiaceae), Crithmum maritimum L. (sea fennel, Apiaceae) and Cakile maritima Scop. (sea rocket,
Brassicaceae) are three halophytic species commonly found along Atlantic sand hills. These plants have found many applications in folk medicine. Studies on antioxidant properties of these halophytes mainly deal with sea fennel essential oil, and radical scavenging activity was recently studied in sea rocket leaves. However, little is known about seasonal variations of total phenol content, antioxidant and radical scavenging activities of these species. Aerial parts of halophytes were collected along the shoreline sandy area at ‘Pointe du Toulinguet’ (Brittany, France). Seasonal samplings were made over one year and a half. Total phenol content was determined with Folin-Ciocalteu reagent. Antiradical scavenging activity was evaluated on DPPH radical and total antioxidant capacity was assessed with phosphomolybdenum reagent. The three halophytic species presented different levels of total phenols and radical scavenging activity. However, they exhibited similar total antioxidant capacity. C. maritima and C. maritimum accumulated phenolic compounds in summer, along with a strong antiradical activity. E. maritimum differed from the two other plants, exhibiting both a low level of phenolics and radical-scavenging activity.

P03-028  Characterisation of S-alk(en)yl-L-cysteine sulfoxide lyases from Allium sativum genetic resources
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Garlic (Allium sativum L.) have been used as a vegetable and a type of alternative medicine for thousands years. Sulphuric amino acids (cystein-sulphoxides) are stored in vacuoles and they are converted to allicin and its analogues, via the enzyme alliinase [S-alk(en)yl-L-cysteine sulfoxide lyase (EC 4.4.1.4)] when the garlic clove tissue is crushed. For PCR amplification three primer pairs were designed to cover whole gene sequence. Amplicons from four different genotypes representing basic morphological types were cloned and sequenced. We found that the length of the gene including 5 exons and 4 introns may differ among cultivars. After comparison of sequences from different cultivars single nucleotide polymorphisms (SNPs) and single sequence repeats were identified. Based on identified differences a system to detect SNPs was designed using SNaPShot approach and ABI platform. The four genotypes of alliinase gene were cloned using TOPO TA Cloning® (Invitrogen). Twenty-one SNaPshot markers were tested. The reaction conditions for each of the primer were optimised. Then mixture of five to six SNaPshot primers were used in multiplex reactions. The assay was applied to analyse the set of 135 garlic varieties. The SNaPShot Multiplex procedure confirmed 10 SNPs. Results and discussion will be presented. Supported by projects 1G58084 of the Ministry of Agriculture of the Czech Republic, 1PO5OC055 of the Czech Ministry of Education, Youth and Sports and COST924.

P03-029  Pigment apparatus of the Northern plants
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Pigments complex in the leaves of 129 plant species inhabiting in three sites (1–65°22′ N, 60°46′ E–2–62°45′ N, 55°49′ E and 3–61°38′ N, 50°43′ E) on the European North-East of Russia was studied during July, 2004–2007. Content and ratio of chlorophylls and carotenoids were varied greatly in depending on plant species, life forms and geographical groups. Pigments concentration decreased in the row: herbs > shrubs and dwarf shrubs > club-mosses. Plants growing in the middle taiga (3) had higher photosynthetic pigments concentration than those growing in Sub-Polar Ural Mountains (1). Arctic and alpine species had lowest value of chlorophylls to carotenoids ratio (2.0–3.5) as compared to hypoarctic (northern taiga) and boreal species (3.8–4.9). It means that carotenoids content ratio increased on ~30–40% in leaves of plants towards the North. Role of carotenoids in the tolerance of photosynthetic apparatus and protection against photodioxidative damage in the northern plants is discussed.

P03-030  Development and optimisation of RT-qPCR assay to characterise β-amylase expression in developing barley grains
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The β-amylase is one of factor affecting the barley malting quality. The protein is synthesised in developing grain and activated during seed germination. Previously we identified differences in β-amylase DNA sequences among Czech spring malting cultivars that resulted in an effort to elucidate whether there are differences in gene expression pattern as well. We will present development of the detection assays that allow us to monitor β-amylase expression in developing barley grain. RNAs were isolated from grains 5–25 days after pollination. Different isolation protocols were tested. RNAs were transcribed into cDNA and further quantified by specific primer sets allowing quantification of 10 different housekeeping genes and β-amylase sequence using SybrGreen chemistry. Several parameters were tested including specificity, reproducibility and repeatability of the assay. Presence of inhibitors and enhancers of PCR reaction was tested using dilution series and spiking the reaction with recombinant plasmid containing soy lectin gene. Internationally validated TaqMan based method was used for quantification. Substantial sources of uncertainty of the assay was identified and was assigned to reverse transcription. We identified the most reliable house-keeping genes and suggest reliable protocol for quantification of gene expression in developing barley grains. Expression of β-amylase grain has been described.

P03-031  The capture of algae by the aquatic utricularia: a vegetarian carnivorous plant?
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Carnivorous plants of the genus Utricularia possess elaborated suction traps that catch and utilise a wide range of small organisms. In aquatic species, research focused so far on animals like Protozoa, Crustacea or insect larvae. However, in many traps algae can often be found as well. The prey composition in traps of Utricularia

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minor, *U. brevit, U. australis* and *U. intermedia* of eight sites in Austria was analysed. Trapped algae were determined, quantified and compared with the water chemistry of the habitat. In 46% of the traps, only algae but no animals were found. 43 genera of algae were trapped, forming up to 80% of the total prey. Unicellular and filamentous species of Desmidiaceae and Zygnemataceae were most abundant. The percentage of algae increased highly significantly with decreasing conductivity of the water \((r_S = \ – 0.417; P \ = \ 0.000)\). In the extremely soft waters of ombrotrophic bogs, algae were the most abundant prey. Differences in prey composition between various species of *Utricularia* were insignificant, as well as between different geographical regions. More than 90% of the trapped algae were dead and apparently digested. Two interpretations are discussed: algae supplement animal prey in extremely oligotrophic habitats or the capture of algae is rather unprofitable or even causes stress, thus limiting the distribution of *Utricularia* in some habitats. Furthermore, the capture of immobile algae questions our understanding of the trapping mechanisms of *Utricularia*.

**P03-032 Carnivory in Lathraea squamaria and Salvia glutinosa?**

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Besides the well established carnivorous plants, a large number of species has been suspected to trap and utilise animals. The subterraneous leaves of *Lathraea squamaria* (Orobanchaceae) contain lacunae with glands, similar to the traps of Geniaceae. *Salvia glutinosa* (Lamiaceae) has sticky infl orescences with glandular hairs trapping insects. To test for carnivory in these species, the morphology of the potential traps was examined, as well as their capturing efficiency and the ability to digest and absorb nutrients. Samples of the soil were analysed for N and P to clarify the need for prey derived nutrients. Lacunas of *L. squamaria* did not contain any animals except for a few earthworms. Callimella and Ciliates were not trapped under controlled conditions. Neither the glands nor the lacuna fluid showed any digestive activity. No capability to absorb nutrients was detected. *S. glutinosa* showed high capture rates (31 ± 23 victims/inflowescence, including large prey like Dermaptera). No digestive enzymes were produced, but some hemipatens (mainly Dicyphus pallidus) were nourishing on the prey. However, neither the glands on the flowers nor on the leaves showed any absorbing capacity. In the habitat of both species, the soil was rich in nutrients. According to these results carnivory can be denied in both species. The lacunas of *L. squamaria* seem to excrete water instead of trapping animals while the sticky glands of *S. glutinosa* serve rather for defence than trapping prey.

**P03-034 Natural distribution and accumulation of alkamide in Acmella radicans**

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Alkamides comprise over 200 related compounds. There are many studies revealing much useful information about the structure, chemistry and biological activity of this novel class of compounds. However, there is a lack of information about the natural distribution and their synthesis. Here we report the synthesis of the alkamide during the growth of the plant *Acmella radicans*. The *A. radicans* seed were surface-sterilized and then put them in soil. The seed began germination 1 week after placement in the soil, with 100% of germination. The alkamide content was evaluated every week during 1 year. We found six alkamides in this plant: Affinon biosynthesis initiated, just before seedling and, during the third week appeared the rest of the alkamides. The profiles of these six alkamides in *A. radicans* vary with the time and developmental stage. Affinon and other alkamides have been found to alter the architecture of the root system and to regulate cell division and differentiation process in *A. thaliana*. Our results suggest a possible like-plant hormones role of the alkamides in this plant since changes in alkamides concentration varies with plant age.

**P03-035 Variations in nutritional value among different apricot genotypes**

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More than 40 commercial apricot cultivars were compared in their fruit colour, total phenol (TPC) and vitamin C contents, ferric reducing ability (FRAP) as well as DPPH and total radical scavenging capacity (the latter measured with a chemiluminescence method). The FRAP and TPC assays revealed a great diversity in the antioxidant power
of apricot fruits. Variability in the carotenoid contents was assessed by a 1.4-fold difference in the hue angle. The closest correlation occurred between the FRAP and DPPH-radical scavenging capacity. The variations in antioxidant capacity of different genotypes could be attributed mainly to the phenolics but vitamin C also had a considerable contribution to it. Our results demonstrate the great genetic diversity of apricot. A breeding program has been initiated to enhance the functional properties of apricot fruits through exploiting the large variability within the tested germplasm. An advanced selection was identified and named as ‘Preventa’ as this produced outstanding values in all antioxidant assays. This work was funded by the Anyos Jedlik programme NKFP06A2-BCETKA06.

P03-036 Variation of microbial load and chemical composition of fruits of some Hippophae rhamnoides L. Romanian varieties, depending on storage conditions
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The estimation of microorganisms present on Hippophae rhamnoides L. fresh, dehydrated and frozen fruits points out the quantitative modifications in microbial communities depending on fruit provenance and their storage conditions. The analyses were performed on seabuckthorn fruits from plants originating in different Romanian geographic areas (Danube Delta and Bacau District). The size of microbial communities, ranging between 63 × 100–198 × 100 colony forming units g⁻¹ plant matter, displayed a variation depending on concrete conditions of the respective origin areas and their pollution degree. The microbial load – generally constituted by species resistant at low temperatures – of 3 months frozen fruits is significantly diminished (4.0 × 10⁻⁵–50.0 × 10³ CFU g⁻¹ plant matter). The fruits dried at 400°C and stored in paper bags generally have an average value of CFU/g plant matter 2–10 times greater than the microbial load of frozen fruits. The following biochemical parameters were analysed: total proteins, total lipids, reducing monosaccharides, reducing disaccharides, soluble reducing polysaccharides. The results evidenced different behaviours of these biochemical indicators, depending on location of plants from which the biological material (fruits) was harvested and on applied treatment in view of fruit storage.

P03-037 A common polymorphism affecting biomass segregates both in the global and in local populations of Arabidopsis thaliana

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Early-onset leaf necrosis is a character often found in incompatible F1 hybrids, and is proposed to be related to hyperactivation of pathogen response pathways. A similar, but less severe phenotype, late-onset necrosis, can be observed in natural populations of Arabidopsis thaliana. Using a combination of genome-wide association studies and QTL mapping, we identified a common hyper-active allele of ACD6 (ACCELERATED CELL DEATH 6) as causal for late-onset necrosis, and for an associated reduction in biomass of about 30%. ACD6 has been implicated in biotic stress response before. The severe reduction in biomass coupled with the hyperactive allele being reasonably common in the global as well as in local populations of A. thaliana suggest a fitness trade-off between biomass production and stress resistance. However, we do not observe broad-spectrum disease resistance, suggesting either pathogen-specific effects or improved resistance to other stresses. We also identified a third, highly divergent allele family that enjoys a similarly wide distribution, and whose origin could pre-date the separation of A. thaliana from other members of the genus.

P03-038 Cytogenetic traits in two Romanian varieties of Hippophaë rhamnoides L.

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For Hippophaë rhamnoides L. dioecious species controversy exists even on the diploid chromosome number and on its basic number. This study contains data on the number and principal morphological characteristics of mitotic chromosomes of two Romanian varieties – Letea 8 and Sulina 16. Although for both varieties the diploid chromosome number is 2n = 24, their karyotypes display some differences. The karyomorphological data show that their complements have chromosomes of relatively small size (1.46–4.07 µm, in Letea 8; 1.13–2.90 µm, in Sulina 16) and the length of haploid complements is 26.98 µm for Letea 8 and 22.18 µm for Sulina 16. The karyotype is symmetric, with only two types of chromosomes, metacentric and submetacentric. According to Levan et al. (1964) nomenclature, we established that their haploid complements have 8m + 4sm chromosome formula, for Letea 8 female karyotype, respectively 8m + 3sm + XY, for Sulina 16 male karyotype X chromosome has small size ~ 1.44 µm, with arm ratio = 1.60 and centromeric index = 38.19, while Y chromosome is 2.38 µm in length, its centromier having a more median placement, r = 1.15 and i = 46.22. In literature the data relative to the morphology and size of seabuckthorn heterosomes are few. Therefore, our considerations on Sulina 16 heterosomes are based on the fact that generally in dioecious plants (Silene latifolia, Canabis sativa) Y chromosome is bigger than X chromosome. No secondary constrictions were found.

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POSTER PRESENTATIONS, TOPIC 05: STRESS AND ACCLIMATION; BIOTIC

P05-011 Metabolic alteration in orthostichous leaves of aspen in response to mechanical damage
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The evolution of chemical defense systems is indicative for the ‘ecological success’ of trees. In aspen, phenolic glycosides are the most important defense compounds against herbivory. Both primary and secondary metabolites influence herbivore performance and considerable variations in the allocation of these compounds depend on the vascular architecture. The metabolic alteration on orthostichous leaves to mechanical damage among 12 clones of *Populus tremula* from different origin was analyzed in this study. Damaged and intact orthostichous leaves in distal and proximal position from the main stem, relative to the damaged leaf, were harvested for metabolomic analyses. By applying multivariate projection methods such as OPS-DA on the GC-MS data, it was shown that orthostichous leaves after damage, showed clear separation between proximal and non-proximal (damaged and distal) leaves. Shikimic acid, phenylalanine and phenethylamine (volatile) were distinctive for non-proximal leaves. In contrast, the loading showed strong separation of proximal leaves in sugars (sucrose, trehalose, maltose and manitol). The results showed strong induction of systemic chemical defense in leaves with vascular connections: (1) Volatile biosynthesis in non-proximal leaves; and (2) Increased levels of sugars to proximal leaves supporting that carbon transport and partitioning may be changed towards storage. Our results offer a first overview of metabolic alterations within-plant defense signaling.

P05-012 Gall midge-induced leafy galls on *Salix*: changes of photosynthetic performance and oxidative enzymes
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Gall midge *Rhachtsophaga rosaria* L. induces neoplastic formations on vegetative buds of *Salix*. Changes of chlorophyll content, chlorophyll a fluorescence, ascorbate peroxidase, polyphenol oxidase and peroxidase activity were studied in order to understand physiological changes caused by the gall-former. Chlorophyll content (R = 0.93), maximum photosynthetic efficiency of photosystem II (Fv/Fm; R = 0.83) as well as photosynthetic performance (R = 0.89) decreased in gall leaves from outer part towards the centre of the gall. Non-photochemical quenching first decreased in parallel with decrease in Fv/Fm but slightly increased again near the centre of the gall. Both peroxidase and polyphenol oxidase activity increased in gall leaves. In contrast ascorbate peroxidase activity decreased with the distance from the outer part of the gall. It is concluded that the activity of the gall former affects both photosynthesis- and defense-related characteristics in galled leaf tissues.

P05-013 The multidisciplinary monitoring of biotic and abiotic stress on strawberry and soybean plants during the treatments with fungal extracts
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Plant diseases caused by *Botrytis* and *Phytophthora genera* are difficult to control chemically, thus the induced resistance by treatments with fungal extracts is a new management strategy of biotic stress control. Extracts from selected pathogen and antagonistic fungal isolates were applied on leaves and in soil in order to elicite plant immune response. Experimental variants of strawberry and soybean cultivars were organized in greenhouse with different biotic and abiotic stress degrees in order to elucidate the relationship between treatments and the dynamics of some biochemical and physiological plant parameters. The leaf gas exchange parameters, during the vegetation period, will be discussed in correlation with the contents of amino acids, oligosaccharides, oligopeptides, fatty acids or peroxidase activity. The plant response towards the induced stress will be managed to improve the resistance of these species and to minimize the infection in strawberry and soybean fields.

P05-014 WRKY III transcription factor family in plant stress signalling
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In their natural environment plants are exposed to biotic or abiotic stresses and respond to such unfavorable conditions by activating effective defence and adaptation systems. On a molecular level, the perception of a stimulus by the plant is mediated by a signal transduction that leads to reprogramming of the plant transcriptome for the activation of physiological, metabolic and morphological changes resulting in adaptation of the plant. Distinct types of pathogens or stresses might trigger different responses in the challenged plant. So, signal transduction in plant stress responses does not act as an isolated linear signal pathway, but is integrated in a complex signalling transduction network, including phytohormones (ethylene, jasmonate, salicylic acid) and transcription factors. In this context, our project is to elucidate the part of each WRKY III transcription factors in the regulation of plant stress signalling in *Arabidopsis*. To this aim, expression patterns of the 13 *Arabidopsis* WRKY III transcription factors are studied in response to different pathogens and hormonal treatments. Moreover, a reverse genetic approach is used to check stress tolerance of T-DNA mutants and microRNA silenced lines affected in WRKY expression. Finally, the WRKY III interaction network is studied by yeast-two-hybrid
and co-immunoprecipitation to find some regulation or crosstalk between different stress signalling pathways.

**P05-015** Multiple CDPK-mediated calcium signaling in plant innate immunity

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Innate immunity is based on the recognition of microbe elicitors by plant receptors that activate downstream signaling cascades to modulate gene expression and promote plant resistance. Signal transduction pathways are driven by second messengers among which calcium is the most widespread in plants. Changes in intracellular calcium levels can be sensed and transduced by several calcium sensors including calcium-dependent protein kinases (CDPKs). To investigate the role of CDPKs in plant innate immunity, we carried out a functional genomic screen using cell-based transient expression assays with flg22-responsive gene markers and a complete set of constitutively active CDPK constructs. By co-expressing a flg22 marker gene and active CDPKs in a transient assay, we have identified a subset of CDPKs that are able to mimic flg22 signaling. The kinase activity is required as a mutation in the ATP binding site of these CDPKs abolishes flg22 marker gene responses. Using both loss-of-function and gain-of-function genetic approaches, we further identified several CDPK target genes in multiple flg22 signaling pathways. Our studies have revealed complex interactions between CDPK and MAPK cascades to regulate flg22-induced gene expression. The new findings have provided compelling evidence that CDPKs play multiple essential roles in plant innate immunity. This research was supported by a Marie Curie International fellowship within the 6th ECFP to M.B. and the NSF (MCB 0446109) grant to J.S.

**P05-016** Signalling pathways involved in interaction *Brassica napus* – *Leptosphaeria maculans*

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*L. maculans* is a hemibiotrophic fungal pathogen causing a stem cancer of oilseed rape (*B. napus*). Despite importance of this disease, signalling pathways participating in defence response against this serious pathogen remain still unclear. After a short necrotrophic period following inoculation, *L. maculans* switches into biotrophic mode colonizing the whole plant without formation any visible symptoms, and turns back to a necrotrophic lifestyle at the end of the season. In our experiments we focused on the activation of salicylic acid (SA)-dependent and SA-independent pathways monitoring the expression of marker genes by means of RT-qPCR. The primers were designed on ESTs with high homology to *Arabidopsis* genes and their specificity to particular signaling pathways was verified by treatment with chemical inducers sodium salicylate, methyl jasmonate, ethephone, abscisic acid and chitosan. The expression of salicylic acid (SA) responsive gene PR-1 strongly increased on the fourth day after inoculation, whereas no changes in expression of jasmonic acid responsive gene AOS were detected during the 10 days after inoculation. The results indicate the importance of SA-regulated signalling pathway, which is typical for biotrophic interaction, in defense response of oilseed rape to *L. maculans* even during a necrotrophic phase of infection. Supported by the Czech Grant Agency (no. 522/08/1581).

**P05-017** Involvement of reactive oxygen species (ROS) in the defense response of *Capsicum annuum* to *Phytophthora capsici*

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Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and superoxide (O₂⁻), were analyzed as part of the oxidative burst in the interaction between the oomycete *Phytophthora capsici* and cell suspensions of two pepper varieties showing different degrees of sensitivity to the pathogen: The resistant Serrano Criollo de Morelos (SCM) and the susceptible California Wonder (CW). The addition of filtrate from *P. capsici* to pepper suspensions produced both H₂O₂ and O₂⁻ in both varieties but with substantial differences concentrations. After elicitation, superoxide formation was instantaneous and its concentration did not change with elicitation time, although it was higher in the SCM cells than in CW cells. The use of SOD inhibitors confirmed that O₂⁻ is quickly converted to H₂O₂, which showed significant differences between varieties and also with elicitation time. The addition of catalase decreased the H₂O₂ concentration by 23% which suggests that H₂O₂ is produced by a ROS other than superoxide. The addition of diphenyleneiodonium, inhibited the O₂⁻ synthesis, so that NADPH oxidase could be a source of ROS. The addition of salicyl hydroxamic acid, an inhibitor of peroxidase, did not influence O₂⁻ synthesis and so cannot be considered a source of O₂⁻. However, we do not exclude a role for peroxidase in the generation of H₂O₂, which is subsequently involved in tissue lignifications. This work has been partly supported by the Project BFU2004-4707-C02-01 from CICYT, Spain.

**P05-018** Class III peroxidases and plant defence in *Arabidopsis thaliana*

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Class III peroxidases (Pxs) form a large multigene family typical of plants and they have been implicated in resistance to biotic and abiotic stress, although the mechanisms of action remain largely uncharacterized or hypothetical. In order to clarify the role of Pxs during plant defense, we investigated the behaviour of Pxs...
Catalase (H₂O₂ oxidoreductase) is one of the central enzymes involved in scavenging the high level of reactive oxygen species (ROS) by degradation of hydrogen peroxide to oxygen and water. The adverse effect of H₂O₂ increases, resulting in the up-regulated expression of catalase. Catalase stringently regulated H₂O₂ and was involved in eliminating ROS. In rice, three catalase genes, CatA, CatB and CatC have been reported. Rice catalase cDNA clones were isolated from rice (Oyza sativa). To analyze the function of a catalase cDNA, we generated transgenic rice overexpressing CatA, CatB and CatC. These cDNAs were overexpressed under control of a CaMV 35S promoter. Genomic Southern analysis of transgenic plants showed increased and reduced induction of PR1, respectively. Therefore, we suggest that a CaM binding receptor-like protein kinase (CBRLK1) plays a negative role in a plant disease resistance signaling pathway.

P05-019 Overexpressing of catalase gene increases tolerance to drought stress in transgenic rice

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Catalase, a peroxidase belonging to the heme oxygenase family of enzymes, catalyzes the degradation of H₂O₂. The adverse effect of H₂O₂ increases, resulting in the up-regulated expression of catalase. Catalase stringently regulated H₂O₂ and was involved in eliminating ROS. In rice, three catalase genes, CatA, CatB and CatC, have been reported. Rice catalase cDNA clones were isolated from rice (Oyza sativa). To analyze the function of a catalase cDNA, we generated transgenic rice overexpressing CatA, CatB and CatC. These cDNAs were overexpressed under control of a CaMV 35S promoter. Genomic Southern analysis of transgenic plants showed increased and reduced induction of PR1, respectively. Therefore, we suggest that a CaM binding receptor-like protein kinase (CBRLK1) plays a negative role in a plant disease resistance signaling pathway.

P05-020 A CaM binding receptor-like protein kinase (CBRLK1) functions as a negative regulator in plant defense response

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In plant Ca₂⁺/calmodulin-regulated protein phosphorylation plays a pivotal role in amplifying and diversifying the action of Ca₂⁺. By screening of an Arabidopsis cDNA expression library using HRP-conjugated calmodulin we identified a calmodulin binding receptor-like protein kinase (CBRLK1). CBRLK1 has the conserved amino acids characteristic of the S-domain family. Using domain mapping, we identified a Ca₂⁺-dependent CaM binding domain in the C-terminus of CBRLK1. The specific binding of CaM to CaM binding domain was confirmed by a gel mobility shift assay, split ubiquitin assay, and a competition assay using a CaM-dependent enzyme. The CBRLK1 kinase domain was capable of autophosphorylation and substrates phosphorylation. Phosphoamino acid analysis of autophosphorylated CBRLK1 kinase domain indicated that serine and threonine residues were phosphorylated. The CBRLK1 transcript was strongly induced by a bacterial pathogen and salicylic acid (SA). Interestingly, the CBRLK1 mutant displayed enhanced resistance to a virulent strain of the bacterial pathogen whereas CBRLK1 transgenic plants showed reduced resistance. The enhanced and reduced resistance in mutant and transgenic plants was associated with increased and reduced induction of PR1, respectively. Therefore, we suggest that a CaM binding receptor-like protein kinase (CBRLK1) plays a negative role in a plant disease resistance signaling pathway.

P05-021 The glutathione peroxidases in Chlamydomonas reinhardtii: sequence analysis and biochemical characterization

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The glutathione peroxidases (GPXs) belong to the ubiquitous non-heme thiol/selenol peroxidase family. Theses enzymes were initially shown to catalyze the glutathione dependent reduction of hydrogen peroxide and diverse alkyl hydroperoxides to water or the corresponding alcohol via a thiol/disulfide exchange mechanism. In mammals, most GPXs contain a highly reactive selenocysteine in their active site while yeast and land plants are devoid of selenium and contain a cysteine instead. The plant non-selenium NS-GPXs have been shown to use thioredoxins (TRX), rather than glutathione (GSH) as reducing substrate. In Chlamydomonas reinhardtii, the presence of one NS-GPX, GPXH/GPX5 and two seleno-enzymes, GPX1 and GPX2, has been reported so far. The now available Chlamydomonas genome sequence offered the opportunity to complete our knowledge on GPXs in this organism. Beside the three known isoforms, two additional NS-GPXs could be identified. Phylogenetic analysis, putative subcellular localization and expression level based on EST data revealed similarities with orthologs in plants and animals. Furthermore, biochemical analyses of two of the Chlamydomonas NS-GPXs indicate a clear TRX-dependency of the NS-GPXH/GPX5 similar to plant GPXs. GPX3 does not show high activity with either TRX or GSH as reducing substrate. The function and putative role of the different NS- and SelenoGPX isoforms in green algae will be discussed.

P05-022 The effect of cadmium stress on ascorbate metabolism in Physcomitrella patens

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Cd has a negative effect on development, damages the photosynthetic apparatus and causes oxidative stress. Ascorbate (ASC), a H₂O₂ scavenging enzyme. Catalase stringently regulated H₂O₂ and was involved in eliminating ROS. In plant Ca²⁺/calmodulin-regulated protein phosphorylation plays a pivotal role in amplifying and diversifying the action of Ca²⁺. By screening of an Arabidopsis cDNA expression library using HRP-conjugated calmodulin we identified a calmodulin binding receptor-like protein kinase (CBRLK1). CBRLK1 has the conserved amino acids characteristic of the S-domain family. Using domain mapping, we identified a Ca²⁺-dependent CaM binding domain in the C-terminus of CBRLK1. The specific binding of CaM to CaM binding domain was confirmed by a gel mobility shift assay, split ubiquitin assay, and a competition assay using a CaM-dependent enzyme. The CBRLK1 kinase domain was capable of autophosphorylation and substrates phosphorylation. Phosphoamino acid analysis of autophosphorylated CBRLK1 kinase domain indicated that serine and threonine residues were phosphorylated. The CBRLK1 transcript was strongly induced by a bacterial pathogen and salicylic acid (SA). Interestingly, the CBRLK1 mutant displayed enhanced resistance to a virulent strain of the bacterial pathogen whereas CBRLK1 transgenic plants showed reduced resistance. The enhanced and reduced resistance in mutant and transgenic plants was associated with increased and reduced induction of PR1, respectively. Therefore, we suggest that a CaM binding receptor-like protein kinase (CBRLK1) plays a negative role in a plant disease resistance signaling pathway.
scavenger, is a member of the antioxidant defence system. The response to Cd was studied in P. patens by applying a range of physiologically relevant CdSO4 concentrations (1, 5 & 10 μM) during 1 day, 5 days and 3 weeks. Accumulation of biomass decreased in samples for 3 weeks in a concentration-dependent way. Both chlorophyll-a and -b content decreased in mosses treated for 5 days or 3 weeks. As quantified by HPLC, after 1 day of treatment total and reduced ASC levels increased (5 μM) and total ASC levels increased (10 μM). After 5 days of treatment, total ASC levels were only increased for 5 μM Cd. Long-term Cd stress resulted in increased ASC levels in mosses treated with 1 μM Cd, but in decreased total ASC levels in 5 μM Cd treated mosses and decreased ASC levels in 10 μM Cd treated mosses. Redox status decreased after 1 day of Cd stress (1, 5 & 10 μM) but increased after 3 weeks of Cd stress (5 & 10 μM). The applied cadmium evoked stress in P. patens, and the moss reacts by adjusting ASC levels. To determine if the increased ASC level is due to changes in synthesis or turnover, transcript level of key genes involved in these processes will be monitored by Real time PCR: L-galactono-1,4-lactone dehydrogenase, (performs the last step in ASC synthesis) and ASC peroxidase (reduces H2O2) in mosses treated with 5 μM CdSO4 (1 day) and 1 μM CdSO4 (3 days & 3 weeks).

P05-023 Functional characterization of the Ustilago maydis/maize interaction by virus-induced gene silencing and overexpression

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The basidiomycetous fungus Ustilago maydis is a biotrophic pathogen parasitizing on maize. During the early infection stages U. maydis is recognized and elicits massive plant defense reactions. With establishment of the biotrophic interaction U. maydis initial responses are attenuated. For a functional characterization of plant effectors involved in this biotrophic relationship, systemic overexpression and silencing of candidate maize gene is required. Since stable transformation of maize is a laborious procedure, we use virus based systems to facilitate a faster analysis of candidate genes. For virus induced gene silencing (VIGS) a Brome mosaic virus (BMV)-vector system (Ding et al. 2006) was established in combination with U. maydis infection. We identified two maize cultivars (Early Golden Bantam and Va35) which are susceptible to U. maydis and tolerant to substantial levels of BMV as well. U. maydis was inoculated after BMV infection, when systemic spread of viral symptoms was visible. Since BMV does not interfere with systemic overexpression, the system allows rapid analysis of the role of maize genes in this interaction. Furthermore we are testing alternative systems for virus induced overexpression (VIOE) in association with U. maydis infection. We present recent progress in both VIGS and VIOE approaches to identify maize genes that are required to establish the biotrophic interaction with U. maydis.

P05-024 Identification of two grapevine genes regulated upon Botrytis cinerea infection

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By differential display and suppression subtractive hybridization we identified several grapevine genes that are regulated in leaves infected by Botrytis cinerea. Among them, two genes BIG 24.1 and BIG 102 were strongly up-regulated in this interaction. BIG 24.1 is coding for a F-box protein. No function has been found in databases for BIG 102. Expression pattern analysis by real-time quantitative RT-PCR revealed that the two genes are also up-regulated by biotic and abiotic stresses including infection with Pseudomonas syringae pv pisi and UV-C exposure. Exogenous treatments with salicylic acid (SA) and methyl jasmonate (JA) induced BIG 24.1 and BIG 102 expression. BIG 24.1 and BIG 102 homologs were identified in Arabidopsis thaliana. Their expression pattern was analysed in response to B. cinerea infection, after challenge with Pseudomonas syringae DC3000 ± AvrRPM1 or after UV-C exposure in wild type plants and in plant affected in SA or JA signalling pathways. Both genes were up-regulated in response to all the biotic and abiotic stresses tested and At 102 expression was mostly affected in mutants impaired in SA signalling. Functional analysis of At 102 and At 24.1 mutants of A. thaliana toward sensitivity to various pathogens and during plant development is currently under investigation. Our first results showed a clear increase in sensitivity to B. cinerea in the At 102 mutant compared to wild type plants.

P05-025 A pleiotropic drug resistance transporter from Nicotiana plumbaginifolia is involved in plant-pathogen interactions

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The ATP-binding cassette transporter family contains several subfamilies among which the pleiotropic drug resistance (PDR) subfamily is specific to fungi and plants. We previously showed that NpPDR1 is expressed in Nicotiana plumbaginifolia leaf trichomes and root. NpPDR1 silencing by RNA interference resulted in increased susceptibility of the plants to Botrytis cinerea. We tested the possible involvement of NpPDR1 in the response to three other fungi characterized by different life styles: Fusarium oxysporum, Rhizoctonia solani and Phytophthora parasitica. Infection of N. plumbaginifolia leaves strongly increased expression of NpPDR1 in leaf tissues as shown by immunodetection using anti-NpPDR1 antibodies and by following the β-glucuronidase activity of a transgenic plant expressing the gusA reporter gene under the control of the NpPDR1 transcription promoter. Using pathogenicity tests, N. plumbaginifolia NpPDR1-silenced plants were found to be susceptible to these fungi while wild-type plants were little affected. These data demonstrate that NpPDR1 is involved in the plant defense against different pathogens, possibly by exporting antifungal molecules. We therefore sought to identify the NpPDR1 substrates by a comparative GC-MS analysis of the leaf surface metabolites from wild-type and NpPDR1-silenced plants. Reduced concentration of sucrose esters and diterpenes was found in the silenced lines, indicating that PDR1 transporter has a broad range of antifungal substrates.

P05-026 The impact of primary metabolism on pathogen defence in tobacco leaves – compatible versus incompatible interaction

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Timely recognition of pathogens as well as rapid and effective induction of defence responses make a key difference between resistant and susceptible plants. Defence reactions are always associated with increased demands for energy and carbon skeletons. In autotrophic tissue the metabolic situation is not well suited for defence: photosynthesis is less efficient, while the heterotrophic metabolism (e.g. respiration, OPPP), which supports defence, is suppressed. Obviously, autotrophic cells require a strategy to retain carbohydrates and activate carbohydrate-consuming pathways. To test, whether a shift to a more heterotrophic metabolism is a conditio sine qua non for a successful defence in autotrophic mesophyll cells we studied the primary and defence metabolism in source leaves after infection with Phytophthora nicotianae and compared two tobacco cultivars: highly resistant SNN with susceptible Xanthi. In SNN a shift from photoautotrophic to heterotrophic metabolism occurred. In contrast, in Xanthi defence related changes in primary metabolism like an early increase in cell wall invertase activity and callose deposition at cell to cell interfaces leading to a decline in sugar export as well as accumulation of carbohydrates were impaired and delayed. The formation of ROS and hypersensitive lesions were impaired due to the reduction of defence related changes in primary metabolism, supporting its importance for a successful defence.

P05-027 Auxin function in avirulent Pseudomonas syringae – poplar interaction

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Various microorganisms interacting with plants are able to produce the plant hormone auxin (IAA), e.g. Laccaria bicolor and Pseudomonas syringae. However, in many cases the function of microorganism-derived IAA in plants is unknown, as for IAA produced by P. syringae. In order to better understand the role of IAA during avirulent interactions, we established a novel model pathosystem between P. syringae and poplar. Although elevated IAA levels could previously be measured upon P. syringae infections in Arabidopsis it is not yet clear whether the IAA was of bacterial or plant origin. We addressed this question by an expression analysis of bacterial IAA biosynthetic genes during avirulent interactions. Furthermore, we monitored plant IAA response (GH3::GUS) and microRNA regulation of the poplar IAA receptor TIR1 during infection with different P. syringae pathogens, including a IAA biosynthesis mutant. An indication that also IAA transport is altered during pathogenesis came from our metabolomics data; the flavonoid pathway, which produces various inhibitory compounds, was shown to be increased in proximity of the site of bacterial infection. Metabolomics data further revealed major changes in salicin derivatives at the site of infection. Currently, we are testing the effect of the identified salicin derivatives on IAA response as well as their role in inducing the synthesis of phytoxins.

P05-028 Mutations in nitrogen transporters can influence on Arabidopsis resistance to Pseudomonas syringae

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It has been largely discussed that nitrogen status of the plant can influence on pathogenic resistance or susceptibility. The NRT2 families are thought to encode HATS for NO3- (Filleur et al. 2001), while the AMT1 family includes genes encoding high-affinity transporters participating to the HATS for ammonium (Kaiser et al. 2002). Upon normal nitrogen fertilization, the mutants Amt1-1:T-DNA insertion mutant in the gene AMT1 and atnrt2 (a deletion of the gene NRT2.1/2.2) mutant have wild type levels of nitrogen, however their response to Pseudomonas syringae seems to be altered since Amt1-1:T-DNA is hypersusceptible while atnrt2 is more resistant. Interestingly, when the atnrt2 mutant is inoculated with a coronatine less Pst strain shows a susceptible phenotype while Ws remains resistant. Looking into the hormonal responses upon infection, Amt1-1:T-DNA shows a lower SA accumulation while atnrt2 displays a higher SA accumulation compared with Ws which could explain the lower resistance of Amt1-1:T-DNA and the reduced susceptibility of atnrt2. Taking into account that in our experimental conditions total nitrogen levels in both mutants remain the same than in controls can be speculated that there exists a gene set link between nitrogen transporters and pathogenic resistance mechanisms.


P05-029 Intracellular dynamics of EDS1 immune regulatory complexes

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Arabidopsis ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and its interacting partners PAD4 and SAG101, are essential for basal defense against invasive pathogens and for resistance and cell death conditioned by TIR-type NB-LRR immune receptors. Previously, it was shown that EDS1 forms molecularly and spatially distinct complexes with PAD4 and SAG101 in the cytoplasm and the nucleus of healthy plant cells. Analysis of triggered tissues by confocal fluorescence microscopy and biochemical cell fractionation revealed no obvious redistribution of EDS1 and its partners. We are now studying the possibility of constitutive nuclear-cytoplasmic shuttling of EDS1 by treatment with nuclear export inhibitors. In order to determine the relevance of the subcellular distribution in EDS1 signal relay, we have generated transgenic plants with altered EDS1 nucleocytoplasmic partitioning through the fusion to a nuclear export signal (NES), a nuclear localization signal (NLS) or the glucocorticoid hormone-binding domain (GR). Analysis of transgenic plants expressing EDS1-mYFP-NES fusions under the native promoter showed a mild reduction of basal and TIR-NB-LRR conditioned resistance to Hyaloperonospora parasitica and Pseudomonas syringae. Studying the effect of impaired intracellular localization on defense activation should allow us to assess whether EDS1 exerts its function primarily in the nucleus or in the cytosol, or has distinct roles in the two compartments.

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P05-030  Towards purification of potato virus a viral protein complex from infected plants  
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Positive-sense ssRNA viruses, including potyviruses, replicate in association with virus-induced intracellular membrane compartments. The co-ordination of replication is believed to involve in addition to viral proteins and viral genomes, also host proteins, together forming functional viral ribonucleoprotein (vRNPs) complexes. We approach the composition and function of PVA protein and vRNPs complexes by fusing affinity-tags to PVA proteins. To this end, Strep-III affinity-tags were fused to the RNA-dependent RNA polymerase (NlbSIII) and the viral genome-linked protein (VpgSIII) encoding sequences in the infectious cDNA clone of PVA. The affinity-tag fusions did not interfere with the infection process. From infected plant leaves we conducted cell fractionation to prepare a heavy-membrane fraction that was subjected to step-tag-based purification. Three samples were prepared in parallel, wt PVA as a control and both NlbSIII and VpgSIII separately. Proteins detected in NlbSIII and VpgSIII samples by silver staining were analysed by LC-MS/MS. The specific presence of proteins was further analysed by immunodetection. We found that Vpg, Nlb, nuclear inclusion protein A (Nia), a cylindrical inclusion protein polypeptide intermediate (CH-K2) and a poly-A binding protein were specifically present in both strep-tagged samples, whereas coat protein (CP), the helper component protease (HC-Pro) and CI were found also from the control sample.

P05-031  Response of Arabidopsis thaliana to a necrosis-inducing protein from Phytophthora sojae  
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The family of NLPs (necrosis- and ethylene-inducing protein-like proteins) exists in a wide range of phytopathogens including Oomycetes and bacteria. These proteins are discussed as positive virulence factors during the necrotrophic phase of pathogen attack by triggering cell death possibly as a toxin. However, these proteins also induce plant defense responses, such as callose deposition, reactive oxygen species (ROS), ethylene, camalexin and salicylic acid production and might therefore be recognized by the plant as pathogen-associated molecular pattern (PAMP). For this work, the necrosis-inducing protein from Phytophthora sojae (PssoNip) was heterologously expressed in Escherichia coli resulting in inclusion body formation. A collection of Arabidopsis thaliana mutants impaired in different defence signaling pathways was treated with the refolded protein and several plant defence reactions were analyzed, such as ROS production and MAP kinase activation, salicylic acid, jasmonic acid as well as camalexin. The results obtained with PssoNip were compared to the results from PaNie, a highly homologous protein from Pythium aphanidermatum.

P05-032  Functional characterization of an OsWRKY involved in the defense signaling and transcriptional activation of NPR1 in rice  
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WRKY protein is a key regulator of SA- and pathogen-mediated defense-signaling pathway. We identified a pathogen- and SA-inducible WRKY that was early induced and reached a maximum at 6 h after SA treatments. By transgenic approach, over-expression of the OsWRKY resulted in strong induction of pathogenesis-related (PR) genes and enhanced disease resistance to Xanthomonas oryzae pv. oryzae (Xoo). RNA interference-mediated knock-down of the OsWRKY (OsWRKY-RI) caused to abolish inducibility of PR genes in response to SA and enhance the susceptibility to pathogens. NPR1 was also constitutively expressed in OsWRKY-OX lines. Therefore we suggest this OsWRKY plays as a positive regulator in defense-signaling pathway and its downstream genes are NPR1 and PR genes. We further confirmed that NPR1 and PR genes are direct target genes of this OsWRKY by transient assays with their promoters. All together, this OsWRKY plays as a positive regulator a role in defense signaling pathway and transcriptional activation of NPR1.

P05-033  ERD15 – a negative regulator of ABA responses in Arabidopsis  
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ERD15 (early responsive to dehydration 15) is rapidly induced in response to various biotic and abiotic stress stimuli in Arabidopsis. Transgenic plants overexpressing ERD15 accumulated increased amounts of abscisic acid (ABA), but simultaneously the sensitivity of the plants to this phytohormone was decreased, which was seen for example as reduced tolerance to drought. However, it seems that the observed insensitivity to ABA improved the resistance of Arabidopsis to the bacterial necrotroph Erwinia carotovora. Plants overexpressing ERD15 were more resistant to this pathogen, accompanied by enhanced induction of SAR-marker genes PR1 and PR2. This is supported by the observation that the tolerance of the abscisic acid insensitive 1 and 2 (abi1 and abi2) mutants for E. carotovora infection is also enhanced compared to wild-type plants. In contrast, RNAi silencing of ERD15 resulted in plants that were hypersensitive to ABA and showed improved tolerance to both drought and freezing as well as impaired seed germination in the presence of ABA. Interestingly, recent evidence suggests that control of abiotic and biotic stress tolerance overlap significantly. We propose that ERD15 is a novel mediator of ABA-signalling in both types of stress responses in Arabidopsis.

P05-034  Development of the marker closely linked with Xa3 resistance gene and evaluation in various population collected in Korea  
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P05-035 Phosphorylation in plant-phytophthora interactions
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Protein phosphorylation is a key biological process regulating many reactions in plant-microbe interactions. We demonstrate the use of titanium dioxide resin (TiO₂) for enrichment of phosphoproteins as well as a method to derivateize TiO₂, purified phosphopeptides to facilitate the determination of the exact site of phosphorylation. The use of these methods is exemplified by the identification of two plant proteins that were shown to be phosphorylated after elicitation of Arabidopsis cells with Phytophthora infestans zoospores and xylanase. Both identified proteins contain a universal stress protein domain of unknown function with conserved residues for ATP binding. Other new putative resistance factors have been identified by bioinformatics. For one of these a phospho-mimic mutant has been created and transformed into potato with the goal of increasing the resistance against P. infestans.

P05-036 Two arabidopsis peroxidases are necessary for the oxidative burst following perception of type three secretion system products and salicylic acid
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The first resistance gene associated with rice bacterial leaf blight (BB) caused by Xanthomonas oryzae, pv. Oryzae (Xoo) was discovered in 1967 by Sakaguchi. Now more than 20 BB resistance genes are known. The Xa3 resistance gene provides resistance to Xoo races K1, K2 and K3 which cause serious disease in southern Korea. We made a mapping population crossing HR13723 resistant to Xoo races K1, K2 and K3 with In390 susceptible to three races. HR13723 which carry Xa3 resistance gene is a near isogenic line of In390. We draw a map which show closely linked Xa3 resistance gene with some SSR, InDel and SNP markers in rice chromosome 11. We also developed Xa3LD514 (PCR-RFLP) marker which is digested with restriction enzyme of Rsal. This marker is located in rice chromosome 11 with the distance of 5.5cM to Xa3 gene. Twenty two resistant and seventy four susceptible varieties were used to compare this marker with the phenotype of Xa3 resistance gene. We can use this marker in MAS system to increase the selection efficiency of Xa3 resistance gene in rice breeding.

P05-037 Functional characterization of cytosolic and peroxisomal isoforms of ascorbate peroxidase in rice (Oryza sativa L.)
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Reactive Oxygen Species (ROS) are continuously produced by aerobic metabolism. In plants, the production of ROS is drastically increased in response to biotic and abiotic stress, disturbing the normal balance of superoxide radicals, hydroxyl radicals and hydrogen peroxide in the intracellular environment. Ascorbate peroxidases (APx) catalyze the conversion of hydrogen peroxide into water using ascorbate as a specific electron donor. Previously, we identified the presence of eight APx genes in the nuclear genome of rice, encoding isoforms that are located in different subcellular compartments. To address the functional role of the OsAPx isoforms, we generated transgenic rice plants silenced for APx-encoding genes by RNAi strategy. The reduction of cytosolic APx function correlates with a global reduction of APx activity, which strongly impacts the whole antioxidant system regulation. APx1/2 silenced plants showed increased hydroperoxide accumulation under control and stress situations. Also, transgenic plants presented higher tolerance to toxic concentration of aluminum when compared to wild type plants. Taken together, the results strongly suggest that an increased intracellular hydrogen peroxide, mediated by the silencing of cytosolic OsAPx genes, modulate the antioxidant system, contributing to stress tolerance in plants. (Supported by: CNPq, CAPES, UNESCO and ICGEB).

P05-038 Characterization of early defense responses in plant immunity
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One of the first layers of defense is the accumulation of reactive oxygen species (ROS), referred to as the oxidative burst. In plants,
ROS production is mediated by the respiratory burst oxidase homologues (Rboh) of the mammalian gp91phox, a catalytic subdomain of the phagocyte NADPH oxidase. Arabidopsis thaliana contains 10 Rboh paralogues that are involved in developmental processes and immune responses. AtRbohD, the major constitutive active form, facilitates ROS production in response to microbe-associated molecular pattern (MAMPs). MAMPs trigger immediate early defense responses. These include changes in ion distribution and early phosphorylation events that in turn activate the RbohD oxidase to produce ROS. Neither ion channels nor signaling components responsible for the MAMP-stimulated accumulation of ROS have been reported to date. We established a survey for A. thaliana mutants exhibiting alterations in the MAMP-triggered oxidative burst. We will describe the initial identification and characterization of such mutants. This includes a mutant defective in the gene coding for the NADPH-oxidase AtRbohD, which we could demonstrate being responsible for the MAMP-triggered ROS production.

P05-039 Analysis of Arabidopsis mutants that exhibit the abnormality of chitin elicitor signaling
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Detection of pathogens based on the perception of microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) plays important roles for basal resistance in plants. Chitin oligosaccharide, a representative fungal MAMP elicitor, induces various defense responses in Arabidopsis and rice. We recently showed that a rice plasma membrane glycoprotein, chitin elicitor binding protein (CEBiP), functions as a cell surface receptor for chitin oligosaccharide elicitor (Kaku et al. 2006). From the structural prediction, however, CEBiP seemed not to have any intracellular domain, indicating the presence of unknown partner protein(s) that mediates the signaling through the plasma membrane. To identify such a component, we used reverse genetic approach using Arabidopsis KO mutants and identified a novel receptor-like kinase, CERK1 (Chitin Elicitor Receptor Kinase1), which is essential component for chitin oligosaccharide elicitor signaling in Arabidopsis (Miya et al. 2007). To further identify the downstream components of these receptors, we developed a high-throughput screening method for signaling mutants based on the measurement of elicitor-induced ROS generation (Albert et al. 2006). Screening of T-DNA insertional mutants of Arabidopsis gave several candidate mutants in which the chitin elicitor responsiveness was decreased or suppressed. Characterization of some of the mutants will be shown.

Kaku et al. (2006) Proc Natl Acad Sci USA 103: 11086
Miya et al. (2007) Proc Natl Acad Sci USA 104: 19613

P05-040 Subcellular quantification of glutathione and its precursors during pathogen attack in susceptible and tolerant Cucurbita pepo hybrids
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To study the importance of glutathione in the development of tolerance and resistance and to gain a deeper insight into possible limitations of glutathione synthesis during pathogen attack glutathione and its precursors were quantified with cytohistochemical methods in single cells and organelles of leaves and roots in both a highly susceptible and highly tolerant Cucurbita pepo hybrid (styriaca GREB. and cultivar quine) during Zucchini Yellow Mosaic Virus (ZYMV) infection. Only the susceptible cultivar is characterized by the development of strong mosaic symptoms. During ZYMV-infection glutathione contents were much stronger increased in leaves of the tolerant cultivar than in leaves of the susceptible one. The weaker increase of glutathione in the susceptible cultivar was found to be caused by low levels of cysteine and glutamate in younger leaves and low levels of glycine in older ones. In roots, glutathione contents do not appear to be affected by the availability of glutathione precursors during ZYMV-infection as they remained in general unchanged. The present study demonstrates (1) a strong correlation between elevated glutathione contents and the development of tolerance and resistance during ZYMV-infection and (2) that the availability of glutathione precursors limits glutathione synthesis during pathogen attack and therefore the plants ability to fight against the dangerous invader. Acknowledgement: This work was supported by the Austrian Science Fund (P18976).

P05-041 Microorganisms as symbionts of carnivorous pitcher plants: bacteria, fungi and protozoa in traps of Nepenthes ventrata and Sarracenia purpurea
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Carnivorous pitcher plants use cone shaped, fluid filled leaves to trap and digest animals. However, no pitcher plant is able to kill all organisms in its traps. Resistant organisms like bacteria, protozoa and even insect larvae survive in the traps and contribute to prey digestion. This study deals with the influence of the plant on the pitcher communities. We compare organisms colonising the traps of Nepenthes ventrata (Nepenthaceae) and Sarracenia purpurea (Sarraceniaceae), kept in the greenhouse under the same conditions. Identification was accomplished by full cycle rRNA approach, FISH, microscopic analysis and cultivation techniques. In addition, the pitcher-fluid was analysed. In the traps of N. ventrata, bacteria (22 x 10^7/ml, 1,4% cultivable; e.g. Serratia, Rhizobium) and moulds (Mortierella, Acremonium) were found. Traps of S. purpurea host a higher diversity, including bacteria (15 x 10^7/ml, 3,6% cultivable; e.g. Actinobacteria and Lactobacillus), moulds (Penicilium, Cladosporium), or protozoa (Metachaos, Bodo, Vorticella). Both pitcher fluids exhibit high oxygen saturation, independent of prey capture. pH is 4.9 ± 0.6 in N. ventrata and 6.2 ± 0.6 in S. purpurea. Furthermore, N. ventrata evidence various digestive enzymes. The aggressive pitcher fluid of N. ventrata seems to be a stressful habitat. Thus, only a few species survive in the trap. S. purpurea, on the other hand, depends on a high diversity of pitcher inquilines, as it is not able to digest prey by itself.

P05-042 The role of isoflavonoids in plant-pathogen interactions revealed by LC/UV/MS profiling
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Flavonoids consist the group of phenolic compounds broadly occurring in plant kingdom and play the essential roles in protection against UV light, in the regulatory processes and defense response to microorganism attack. This last feature characterize mainly isoflavonoids acting as phytoalexines or phytoanticipines in plant-pathogen interactions. These secondary metabolites occurs in nature in a great number of isomeric forms. They are usually accumulated in plant tissues as glycosides, frequently acylated with organic acids. The analysis of complex extracts is possible using LC/UV and LC/MSn systems. We used two LC/MS systems: HPLC/IT/MS and UPLC/q-Tof/MS for the extracts analysis. We monitored the changes in profiles of isoflavone conjugates and aglycones in leaves of blue lupine (Lupinus angustifolius) seedlings infected with Colletotrichum lupini. This pathogenic fungus causes anthracnose resulting in serious losses in crop yields of lupine worldwide. Various infection procedures were applied to compare their efficiency. Changes in profiles of isoflavonoid conjugates and free aglycones were observed in different time points after the infection. Statistical analysis of quantitative changes in LC/UV profiles revealed the role of prenylated isoflavones in the defense reaction. Furthermore we investigated the changes in malonylated isoflavone glycoconjugates content after infection in order to determine the role of malonylation process in the plant response to the pathogen attack.

P05-043 Intricate interaction between pectins and pathogens
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The cell wall is one of the most important structural components of plants. The wall defines cell shapes, provides strength to withstand the turgor pressure and serves as the last physical barrier against invading pathogens. Pectins constitute ca. 30% of the cell wall polysaccharides but only a few examples are known about the defense roles of the pectin polymers. Previously studies have identified that oligogalacturonides released after digestion of homogalacturonan elicits a defense response in the host, thereby functioning as an endogenous signal for the host defense activation. We have recently identified a pectin mutant of Arabidopsis thaliana, arabinan deficient 1 (ara1), defective in the pectic arabinan biosynthesis. Detailed cell wall composition analyses identified that ara1 has 70% less arabinoxyllose in the pectic rhamnogalacturonan I fraction. The mutant did not show a visible growth phenotype distinct from the wild type, indicating that arabinan is not essential for plant growth. However, ara1 mutants showed increased susceptibility to the necrotic fungal pathogen Botrytis cinerea, while they appear to show the wild type level of susceptibility to the bacterial pathogen Pseudomonas syringae. Preliminary results suggest a possibility that arabinan may be involved in signaling and elicitation of the host defense. Exact molecular mechanisms responsible for the observed pathogen responses are currently under investigation and will be presented.

P05-044 The involvement of nitric oxide in the development of Oidium neolycopersici on the leaf discs of Lycopersicon spp.
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Nitrile oxide (NO) is a widespread signalling molecule with a broad spectrum of regulatory functions in plant physiological processes. Model pathogenesis system Lycopersicon spp. – Oidium neolycopersici was used to study the role of NO in defence mechanisms. The pathogen development was investigated on the leaf discs of L. esculentum cv. Amateur (susceptible), L. chmielewskii (moderately resistant) and L. hirsutum I. glabratum (highly resistant genotype) during 8, 24, 48 and 72 h after the inoculation. More intense development of pathogenic structures was observed in the presence of NO scavenger PTIO and NO/ROS scavenger rutin. Competitive inhibitor of animal NO synthase, L-NAME, significantly retarded the pathogen development on sensitive genotype L. esculentum cv. Amateur whereas no significant changes were observed on resistant genotypes L. chmielewskii and L. hirsutum. NO donor sodium nitroprusside inhibited the pathogen development on resistant genotypes while it has stimulating effect on pathogen growth on sensitive genotype. The obtained results confirm the involvement of NO in plant defence mechanisms during infection of Lycopersicon spp. by O. neolycopersici. Our results also suggest NO is likely involved in variable extent in different defence mechanisms affecting the resistance of tomato genotypes to this pathogen. This research was supported by grants MSM 6190859215 and 522/08/H003 from Czech Grant Agency.

P05-045 Photosynthetic response of pepper plants to wilt induced by Verticillium dahliae and soil water deficit
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A greenhouse experiment was conducted to compare stress effects caused by either Verticillium dahliae wilt or soil drought on photosynthetic gas exchange and fluorescence of pepper plants. Three treatments were conducted: (1) plants inoculated with V. dahliae, (2) uninoculated control plants and (3) uninoculated plants subjected to progressive drought. Leaf gas exchange, chlorophyll fluorescence and pigments were measured along a gradient of RWC and gs. Initial decreases of gs (150–50 mmol m$^{-2}$ s$^{-1}$) were accompanied by decreases of Pn and Ci, while ETR and Fv/Fm remained almost unaffected in both Verticillium and drought-induced wilt. Stomatal closure appeared to be the main limitation to photosynthesis in this range. When gs dropped below 40 mmol m$^{-2}$ s$^{-1}$, both Pn and ETR decreased significantly and Ci increased sharply. The ratio ETR/Pn + RD + RL (accounting for photosynthesis, dark and light respiration) was low in this range, indicating that alternative electron sinks, such as the Mehler reaction, were still low. The photosynthetic response of pepper plants to Verticillium wilt was almost mimicked by drought until values of gs close to 50 mmol m$^{-2}$ s$^{-1}$. However, when gs dropped below 40 mmol m$^{-2}$ s$^{-1}$, Fv/Fm and Oexc. decreased in plants subjected to drought, while remained constant in inoculated plants. This could reflect a differential effect between Verticillium and drought on the photosynthetic apparatus. Results are discussed in relation to the xanthophyll cycle interconversions.
PO5-046  The impact of primary metabolism on pathogen defence in tobacco leaves—light versus dark

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An important feature of plant resistance is the generation of ROS. NADPH-oxidases, mainly driven by the cytosolic G6PDH reaction, are considered to be main sources for this oxidative burst. Other carbohydrate consuming pathways (e.g. OPPP, respiration) are required to metabolically support defence. During photosynthesis, however, these pathways are tuned down. Thus photosynthesising cells are not well suited for defence. Otherwise ROS can be produced in chloroplasts and peroxisomes during photosynthesis and photorespiration. Surprisingly little is known about the role of these light driven ROS species during plant defence. It has been speculated, that intracellular interruption of photosynthetic pathways could stimulate ROS generation and thus Hypersensitive Reaction (HR). Taken together, there is no clear answer how light and plant primary metabolism influence defence. Does photosynthesis support or does it even interfere defence? Does plant defence require a transition to a heterotrophic state? As a model system we study the interaction between source leaves of tobacco with the oomycete Phytophthora nicotianae and compare infections at the beginning of the light and dark phase. In general, a metabolic shift to a carbohydrate consuming state seems to be required to facilitate oxidative burst and hypersensitive cell death. But different sources for carbohydrates are used during light and dark, leading to differences in the velocity of the ROS mediated HR.

PO5-047 Manipulation of host gene expression by Phytophthora during a compatible interaction with European beech

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The pathosystem Fagus sylvatica – Phytophthora citricola represents a compatible pathogen-host interaction. The oomycete P. citricola is a hemibiotroph that infects the roots. Plants were infected in a liquid in vitro system to avoid infections with additional microorganisms or fungi and to ensure harvesting without wounding signals. Local and systemic gene expression was analyzed in a time-series of five time-points post infection (6 h – 3 days) extending into the necrotrophic phase of infection. For this purpose an Agilent Oligo Microarray was constructed using 927 sequences of a subtractive library of infected root tissue. During the time-series 149 root ESTs were differentially expressed (P > 0.05) 133 of those were regulated more than two-fold. Differentially expressed root ESTs were clustered into 10 clusters by k-means clustering. For clustering validation figure of merit was used. There are two main expression patterns: early and late responsive. In the systemic reaction of the leaves 493 ESTs were regulated and 104 of those more than two-fold. Pathogen-responsive leaf ESTs were clustered into 11 clusters by k-means clustering. These clusters can be divided into two major groups, those up-regulated at 24 h and those down-regulated at 24 h. On the basis of predicted functions of the regulated genes the manipulation of host gene expression by Phytophthora is discussed in context of the biotrophic and necrotrophic phases of pathogen growth.

PO5-048 Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves

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Caterpillars and spider mites are herbivores with different feeding mechanisms. While caterpillars as chewing herbivores remove larger amounts of photosynthetic active tissue, spider mites as piercing-sucking insects remove the cell content and gradually destroy the chloroplasts. We investigated the local and systemic effects of caterpillar and spider mite herbivory on photosynthesis and leaf growth of cotton plants as well as on concentrations of total nitrogen, defence-related amino acids and soluble sugars and starch. Neither 48 h of caterpillar feeding nor spider mite infestation for 5 days influenced photosynthesis or transpiration of cotton plants but increased the dark respiration of the affected leaf. Spider mite infestation did not affect leaf growth, the relative water content and concentrations of defence-related free amino acids. Total nitrogen and sucrose concentrations were increased in leaves in response to spider mite infestation. In contrast, caterpillar feeding reduced the relative growth rate and the relative water content locally but concentrations of total nitrogen and soluble carbohydrates were not different from control plants. Altered concentrations of defence-relevant free amino acids may indicate plant defence responses to caterpillar herbivory. Systemic effects were neither significant in plants affected by caterpillars nor spider mites. The possible trade-off between defence induction and plant growth in cotton is discussed based on these data.

PO5-049 Cold resistance formation in potato plants infected by cyst-forming nematode under low temperature

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In the North low temperatures are often combined with nematode invasion. Globodera rostochiensis Woll. is one of the widespread potato cyst-forming nematode (PCN). Experiments were conducted in growth chambers. The nematodes were applied to potato plants at a rate of 2500 eggs and juveniles per plant. Before the application of PCN plants with three leaves were subjected to temperature treatments for 6 d: constant low hardening temperature (control), 2 h temperature drop at the end of night and optimal temperature (long-term treatment), 2 h temperature drop at the end of night and optimal temperature (control). Subsequent growth conditions were optimal. Cold resistance was estimated by LT50 method. The effect and aftereffect of low temperature treatments on cold resistance formation in non-infected and infected plants were studied. After temperature treatments before PCN application the increment in cold resistance was six times higher in drop-treated plants compared to that in long-term treated plants. In non-infected drop-treated plants cold resistance remained high for subsequent 3 weeks, while in long-term treated it dropped to the initial level in a week. PCN invasion caused an increase in cold resistance in control and long-term treated plants. Infected

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drop-treated plants had high level of cold resistance, which had been remaining constant during at least subsequent 30 d. It is assumed that response of infected by nematode plants to temperature drop involves both specific and non-specific reactions. Study was supported by RFBR 08-04-98833.

P05-050 Use of biotinylated ligands for the characterization of plant receptors
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A simple and non-RI method for the identification and characterization of plant receptors with biotinylated ligands is presented. Biocytin hydrazide conjugate of N-acetylchitootaoase (GN8-Bio) was synthesized and used for the characterization of chitin elicitor binding protein, CEBiP. Membrane fractions were treated with GN8-Bio and cross-linked with glutaraldehyde. CEBiP was successfully biotinylated and detected both in the plasma membrane and microsomal membrane fractions by Western blotting with anti-tylotin antibody followed by chemiluminescence detection. The binding characteristics of the GN8-Bio to the CEBiP showed a good agreement with the known specificity of CEBiP. The GN8-Bio-tagged CEBiP could also be purified from the membrane using an avidin column. For the identification of the purified protein as CEBiP, the purified protein was analyzed by MALDI-TOF/TOF after tryptic digestion. Four tryptic peptides were identified as the expected fragments of CEBiP by acquired searching algorithms based on GPS Explorer software and MASCOT. These results indicated the usefulness of biotinylated ligands both for the purification and characterization of putative receptors or binding proteins for the ligands of interest.

P05-051 New insights into P0 function in Arabidopsis/BWYV interaction
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The poleroviruses are an agronomically important genus of plant viruses, which can infect a wide range of hosts. Their genome is a single-stranded plus-sense RNA and they are restricted to the phloem tissue. The 5'-terminal ORF encodes P0, a strong suppressor of Post-Transcriptional Gene Silencing (PTGS), an important antiviral defence system in plants. P0 carries a F-box like-motif and in a previous study it was shown to interact with Arabidopsis SKP1-like proteins (ASK), components of the SCF class of E3 ubiquitin ligases involved in the protein ubiquitination and degradation pathway (Pazhouhandeh et al. (2006) PNAS 103, 1994-9). F-box proteins are the components of the SCF complex that specify the proteolytic degradation of target proteins. P0 was shown to promote degradation of ARGONAUTE1, an essential component of the silencing pathway, thereby, inhibiting the plant antiviral defence (Bortolamiol et al. (2007) Physiol. Plant. 133, 2008). Viral proteins have often several functions. To investigate further P0’s mechanism of action we performed a yeast two-hybrid screen of an Arabidopsis thaliana phloem cDNA library and we identified small heat shock proteins (sHSP) as cellular partners for P0. sHSPs are involved in cellular response to various forms of stress. In this congress we will present the results we obtained so far concerning this interaction and its putative role in viral infection.


P05-052 Modeling of plant Secondary metabolism for development of plants with improved pathogen resistance
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The amino acid-derived glucosinolates and indole alkaloid phytoalexins are important natural plant compounds characteristic for cruciferous plants. Upon tissue disruption glucosinolates are hydrolyzed by myrosinases to produce degradation products, typically isothiocyanates, thiocyanates, and nitriles, involved in plant defence. Indole alkaloids are induced in cruciferous plants upon pest attack and play a role in defence against specific fungi. Recent progress in the understanding of the biosynthesis of both indole phytoalexins and glucosinolates has revealed the central role of the cytochromes P450 of the CYP79 family. Very little is known about the biosynthesis of Brassica spp. indole alkaloids, and knowledge gained with camalexin in Arabidopsis thaliana is expected to be transferable to Brassica metabolites. The objective of this project is to determine the role of natural products, especially glucosinolates and indole phytoalexins in conferring resistance to different pests. The aim is to develop novel strategies for pest management in Brassica crops to reduce chemical input in the form of pesticides and provide an important step towards attaining a durable and sustainable agriculture. Oilseed rape (Brassica napus) is the number one oil crop of the Nordic countries. The beneficial fatty acid composition of the seed oil for human consumption and various technical applications of modified seed oil indicate that this crop will increase in use locally and worldwide.

P05-053 Seed treatment with jasmonates enhances plant resistance to herbivorous pests
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Plants have a range of natural defences against pests and diseases that could be exploited to improve crop production systems and minimise chemical inputs. Amongst the plethora of signalling molecules produced in response to herbivory, jasmonic acid (JA), is one of the most important. Several studies have investigated the use of foliar application of JA to activate natural plant resistance. Although this type of application does afford protection to the plant, it has some phytotoxic effects and would not be cost-effective on a large scale. We are investigating the potential for priming endogenous plant defence systems by using a novel JA seed treatment. We will present data to show that a range of different crop species grown from JA-treated seed display a significant increase in resistance to a number of arthropod pests, including aphids, caterpillars and spider mites. This resistance persists throughout the life of the plant.
Reactive oxygen species (ROS), originally considered merely cytotoxic compounds, fulfill important and tightly regulated roles as messengers in stress adaptation and development. ROS also regulate programmed cell death (PCD), such as the hypersensitive response (HR) during the defense against biotrophic pathogens. Clearly, the initiation of PCD as well as its containment to strictly defined areas needs to be tightly regulated. Therefore, it will be crucial to unravel the molecular mechanisms underlying ROS signalling and PCD. A novel insertion-mutant, rcd5, shows a severe lesion phenotype in comparison to Col-0 plants after O$_2$ exposure. On rcd5 plants the hemibiotrophic bacterial pathogen Pseudomonas syringae pv. tomato DC3000 shows a reduction of growth to the same levels as the avirulent isogenic strain DC3000 avrB. Most strikingly, plants overexpressing epitope-tagged RCD5 show similar phenotypes to the mutant line. Infiltration of wildtype leaves with a RCD5-peptide, corresponding to the insertion mutant showed a significant increase in cell death compared to controls, suggesting a role of RCD5 protein in the execution of PCD. Furthermore, rcd5 plants show severe defects in the transcriptional regulation of several markergenes associated with SA, ET and JA. This suggests a broader role of the RCD5 gene product in PCD regulation that is not limited to O$_2$-induced responses.

**P05-054** When proteins go postal – a new regulator in ROS induced cell death

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Host-pathogen interactions were investigated on Betula platyphylla var. japonica Tohoku and No.8 clone plants infected with a canker-root fungus, *Inonotus obliquus* IO-U1 strain in order to clarify the defensive mechanisms of those plantlets against the fungus. For time-course study, intact, wounded, and infected plantlets were collected from 2 h to 30 days after each treatment. Transverse sections were prepared from each sample, and they were stained with Fast blue BB for phenolics, Wiesner and Mäule color reagents for lignin, and Sudan III for suberin observation. Notable changes with Fast blue BB for phenolics, Wiesner and Mäule color reagents were observed between infected Tohoku and No.8 plantlets. Based on the results obtained, phenolics deposition and NP formation are considered to occur as infection-induced responses in Tohoku and No.8 birch plantlets infected with *I. obliquus* IO-U1 strain.

**P05-056** Systemic acquired resistance signals downstream from EDS1 – an omics approach

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ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), together with its interaction partners PAD4 and SAG101, is required for basal defence against virulent pathogens. It is also involved in signalling downstream from TIR-NB-LRR-type RESISTANCE (R) genes and is required to establish systemic acquired resistance (SAR). SAR is a long-lasting broad-spectrum disease resistance established in systemic tissues of locally infected plants. We show that EDS1 is essential for both SAR signal generation/transmission and for SAR signal perception in the systemic tissue. Unlike petiole exudates from infected wild type (wt) plants, those from infected eds1 mutants are incapable of inducing defence gene expression in healthy wt Arabidopsis. In reciprocal experiments, petiole exudates from infected wt plants fail to induce expression of the defence gene PR-1 in eds1 mutants. The exact composition of the mobile SAR signal is unknown, and we set out to map the nature of systemic metabolic defects in eds1 mutants. For this purpose, we are using conditional over expression of a bacterial effector protein, AvrRpm1, that activates the EDS1-independent CC-NB-LRR-type R protein RPM1. In this pathogen-free system, local defence signalling remains intact while SAR signal generation is defective in the eds1 mutant background. We are currently mapping EDS1-dependent protein, peptide, and small molecule accumulation in the apoplast and/or petiole exudates of SAR-inducing leaves.

**P05-055** Anatomical and histochemical characteristics of Japanese Birch Tohoku and No.8 plantlets infected with *Inonotus obliquus* IO-U1 strain


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Host-pathogen interactions were investigated on Betula platyphylla var. japonica Tohoku and No.8 clone plants infected with a canker-root fungus, *Inonotus obliquus* IO-U1 strain in order to clarify the defensive mechanisms of those plantlets against the fungus. For time-course study, intact, wounded, and infected plantlets were collected from 2 h to 30 days after each treatment. Transverse sections were prepared from each sample, and they were stained with Fast blue BB for phenolics, Wiesner and Mäule color reagents for lignin, and Sudan III for suberin observation. Notable changes were observed morphologically in the treated portion of wounded and infected plantlets. Phenolics first deposited at the cut margin and subsequently in vessels after 4 h of infection. Their deposition extended to other xylem elements, the cortex, and the pith with an increase in the infection period. Phenolics deposition was extensive at 10 days post-inoculation (dpi), when most of the cells were entirely filled with phenolics. A necrophylactic periderm (NP) was formed at the junction of the original periderm with a layer of 2–4 new phellem cells at 30 dpi. Almost same histochemical characteristics and trends were observed between infected Tohoku and No.8 plantlets. Based on the results obtained, phenolics deposition and NP formation are considered to occur as infection-induced responses in Tohoku and No.8 birch plantlets infected with *I. obliquus* IO-U1 strain.

**P06-011** A model for somatic embryo development in pine

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The aim of this study has been to develop a model for how embryogenic cultures of pine proliferate. By use of tracking of three embryogenic cell lines of Scots pine together with histological studies we have developed a fate map of somatic embryogenesis (SE). Embryogenic cultures of Scots pine are initiated from immature seeds, 1 or 2 weeks after fertilization, when zygotic embryos multiply by cleavage polyembryony. Both the initiation and the proliferation of embryogenic cultures take place on media containing the plant growth regulators (PGRs) auxin and cytokinin. Withdrawal of the PGRs for two weeks triggers the differentiation of somatic embryos. However, the embryos do not develop until they have been exposed to abscisic acid. Early somatic embryos either develop further or start a new round of proliferation. Somatic embryos at the stage of late embryogeny typically carry supernumerary suspensor cells, which are slowly degraded by programmed cell death. A striking difference between zygotic embryos and somatic embryos in some cell lines is the ratio between the embryonal mass and the suspensor. An unbalanced ratio between the embryonal mass
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and the suspensor at the stage of late embryogenesis affects further development of the embryos, resulting in embryos with two to three cotyledons and irregular cell division in the basal part. Based on these data, a fate map of SE has been constructed which includes a number of markers specifying distinct stages in the development.

P06-012 Oxidative stress in Mammillaria gracillis Pfeiff. (Cactaceae) tissues grown in vitro
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In vitro propagated M. gracillis plants of develop calli without any exogenous growth regulators. This habituated calli spontaneously regenerate morphologically normal and hyperhydric shoots. Since the habituation and hyperhydrycity are both part of a neoplastic progression, cactus cells were transformed with A. tumefaciens strain B653. The aim of this study was to find out if activated oxygen metabolism is involved in habituation, hyperhydrycity and tumourisation in in vitro propagated cactus plants. A higher MDA and carbonyl contents and H₂O₂ production were observed in callus, hyperhydric regenerants and tumour in comparison to normal shoots. LOX activity showed a similar trend, with a clear increase in activity in callus and hyperhydric regenerants. The activities of antioxidative enzymes P XP, APX, GR and CAT were also higher in the callus, hyperhydric regenerants and tumour while an increase in SOD activity was observed in the callus, the lowest in tumour. Total phenols content was also lower in callus, hyperhydric tissue and tumour in comparison to normal tissue. Our results revealed a prominent oxidative stress in callus, hyperhydric regenerants and tumour and a strong induction of the antioxidant system indicating that ROS and activated oxygen metabolism are involved in the processes of habituation and hyperhydrycity as well as in tumour transformation.

P06-013 Auxin export from auxillary buds
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The changes linked to the release of auxillary buds from apical dominance were studied on pea (Pisum sativum L.) cv. Vladač. Previously we have proved that polar auxin transport in the inhibited auxillary buds is not established and soon after decapitation export of auxin from auxillary buds was traced by the use of radioactively labeled [3H]-IAA and the establishment of polar auxin transport was visualized by immunolocalization of PIN1 protein. Now we show that also in the stem below and above the axillary bud there are dramatic changes in PsPIN1 and PsAUX1 gene expression due to canalization of the auxin exported from the outgrowing bud. In the above the stem above the bud the expression of both genes drops to zero in 6 h after decapitation
due to the absence of an auxin source. In the stem below the bud a slower decrease of gene expression and after 6 h an increase due to the auxin exported from the outgrowing bud could be observed. The changes occurring after auxillary bud outgrowth are further studied also on transgenic Arabidopsis plants PIN1:-PIN1-GFP and DR5rev::GFP. This work was supported by grants of the Ministry of Education CR - LC06034 and of the IGA MUAF - DP 2/AF.

P06-014 The BELL homeodomain proteins ATH1, PNY and PNF are redundantly required for shoot apical meristem function in Arabidopsis
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In plants, shoot apical meristem (SAM) function requires maintenance of a delicate balance between the depletion of stem cell daughters into primordia and proliferation of the central stem cell population. In Arabidopsis, the KNOX homeodomain (HD) protein SHOOTMERISTEMLESS (STM) is indispensable for proper shoot development. Lack of STM causes defective initiation and maintenance of the shoot apical meristem (SAM) and results in early developmental arrests. STM can heterodimerize with several members of a related class of TALE HD proteins, the BELL proteins. Here we show that three Arabidopsis BELL-class proteins, ARABIDOPSIS THALIANA HOMEBOX1 (ATH1), PENNYWISE (PNY) and POUND-FOOLISH (PNF), are redundantly required for initiation and maintenance of the SAM in conjunction with STM. All three BELL proteins physically interact with STM, and this interaction is indispensable for proper nuclear localization of the respective heterodimer. As a result, combined loss of ATH1, PNY and PNF results in a phenocopy of a strong stm mutation. We further demonstrate that the subcellular localization of these BELL-SAM heterodimers involves a CRM1/exportin-1-mediated nuclear exclusion mechanism that acts on the, among BELL proteins conserved, STM domain and that is probably generally involved in the subcellular localization of BELL and KNOX proteins. Possible evolutionary conservation of TALE HD protein activity regulation through controlled sub-cellular distribution is discussed.

P06-015 Nitric oxide-releasing compounds inhibit phytochrome dependent nyctinastic closure in Albizia lophantha Benth leaflets
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Nitric oxide (NO) is a cellular signalling molecule which affects the activity of ion channels. A. lophantha leaflets show both circadian rhythmic and nyctinastic movements that depend on the curvature of a specialized motor organ, the pulvinus, and are driven by turf changes associated to K⁺ and Cl⁻ ion fluxes. Red light (R) acting through phytochrome promotes nyctinastic closure and resets the internal clock when applied at the appropriate time. The present work investigates the effect of nitric oxide-releasing compounds (SNP, SNAP, NONOate) in the control of leaflet movements mediated...
by phytochrome. *Albizia lophanthra* plants were maintained under 16 h light/8 h dark cycles. Pair of leaflets were excised at 7 h of the photoperiod and floated for 1 h in light on 10 ml control or test solutions containing different concentrations (100–1000 μM) of NO-releasing compounds and then irradiated with a 15 min R pulse, a 5 min far-red (FR) pulse or 15 min R followed by 5 min FR, and then kept in darkness for 3 h. Leaflet angles were estimated at 30 min intervals. Albizia leaflets irradiated with a R pulse close to a greater extent than leaflets irradiated with a FR pulse. Application of several NO donors inhibited nyctinastic closure in both R and FR irradiated leaflets. SNP proved to be the most efficient of the three donors assayed. SNP, NONOate and SNAP did not affect phytochrome response photoreversibility.

**P06-016 Perturbations of RETINOBlastoma-RELATED PROTEIN (RBR) expression in Arabidopsis thaliana lead to altered shoot meristem activity**

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In animal cells the RETINOBlastoma protein (pRBR) plays a key role in cell cycle by regulating the transition from G1 to S phase. In *A. thaliana*, RBR down regulation has been reported to promote stem cell proliferation in the root meristem (Cell, 2005; Nature, 2007). Here we report that a constitutive down regulation of RBR expression in 35S::RBR co-suppression plants or by engineered RNA interference (RNAi) leads to defective shoot meristems already during embryogenesis. Based on the 17-beta-estradiol system (Plant Physiol, 2006) we constructed ectopically inducible systems to either down regulate RBR (via RNAi) or to promote RBR expression at different stages of plant development. Our results show that RBR is required for stem cell maintenance and continuous organ production in the Arabidopsis shoot meristem.


**P06-017 A possible role for the small GTPase AtRAC7 in auxin signaling in Arabidopsis**

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RAC are small molecular switches belonging to the Ras superfamily of small GTPases, and are found in most eukaryotic organisms. Arabidopsis contains a family of 11 RAC-like GTPases (AtRACs) that appear to be central regulators of signal transduction in plants, which lack Ras homologues. Previous work has shown that AtRACs are involved in diverse cellular processes, such as cell morphogenesis, plant defence, and stress responses. AtRAC GTPases have also been suggested to take part in responses to abscisic acid and auxin. The plant hormone auxin is a central regulator of cell identity, cell division and cell expansion. Polar transport of auxin, generated through the action of auxin efflux facilitators (PINs), produces auxin gradients that are necessary for processes such as embryogenesis, organogenesis, vascular tissue differentiation and root meristem maintenance. AtRAC7 is expressed in the embryo, root and shoot meristems and leaf provascular tissue. Several lines of evidence point towards a role for AtRAC7 in auxin signaling. The AtRAC7 promoter contains putative binding sites for auxin response factors (ARFs). AtRAC7 expression is reduced in the auxin mutants axr2 and axr3. A transposon insertion mutant of AtRAC7 shows defects in leaf vasculature and root growth; microarray studies of the atrac7 mutant indicate altered phosphate responses. The results presented will be discussed in view of a possible role for AtRAC7 in polar auxin transport and/or auxin responses.

**P06-018 Primary vascular development in Arabidopsis: identifying and characterizing mae mutants**

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The primary vascular pattern of the Arabidopsis root exhibits a diarch pattern of organization, i.e. it consists of a central xylem axis with two intercalating phloem poles diametrically opposed and intervening pluripotent procambial cells. *AHP6* is a central player in vascular cell fate regulation (Mähönen et al. 2006, Science). This gene is specifically expressed in two poles at protoxylem positions where it inhibits cytokinin signalling. Cytokinin has been implicated in regulating vascular cell fate, by inhibiting xylem cell identity and promoting procambial and phloem cell identity. Conversely, cytokinin signalling negatively regulates the spatial domain of *AHP6* expression. The identity of neither the negative regulatory (cytokinin mediated) nor promotive factors which converge on *AHP6* is known. To identify and characterize upstream factors controlling *AHP6*, a forward genetic screen was performed to identify modified expression patterns of *pAHP6::GFP* within an EMS mutagenized line. A set of novel mutants, the mae mutants (modified *AHP6* expression), were identified and a phenotypical and molecular characterization of these genetically interacting loci will be presented. Further functional analysis of those loci can reveal basic genetic mechanisms underlying Arabidopsis vascular development.

**P06-019 Interaction with auxin transport in Arabidopsis root tip suggests a possible morphogenic role of the ascorbate system**

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Polar auxin transport is the main regulator of post-embryonic root development. The hormone, mainly synthesized in young leaves, is conveyed to root tips by means of PIN efflux and AUX influx transporters and typically accumulates in the quiescent center (QC) and the columella, specifying the fate of those highly specialized cell populations. Environmental conditions may interfere with genetically-defined developmental program, but little is known about the molecular signals involved in root morphogenesis under stress conditions, although the involvement of reactive oxygen species (ROS) has been postulated (Potters et al., TIPS 12: 98–105).
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Here we report evidence of a possible involvement of the ascorbate (ASC) system in controlling root morphogenetic responses by interacting with polar auxin transport. Roots of Arabidopsis plants transformed with the auxin reporter gene DR5::GFP showed marked differences in auxin distribution upon treatment either with the ASC precursor L-galactono-gamma-lactone (GAL) or the ASC oxidized form dehydroascorbate (DHA), also resulting in an altered statolith pattern in columella cells. Interestingly, redistribution of PIN1 upon DHA treatment could be observed in an Arabidopsis line expressing the PIN1::GFP construct. We suggest that the ASC system, and in particular DHA, could have a key role in controlling root organiza-
tion by operating in a signalling module that regulates development in connection with environmental conditions.

P06-020 Changes of peroxidase activity in stem bases and leaves as a marker for determination of rooting phases in rhododendron leaf bud cuttings
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One method of rhododendron propagation is propagation by leaf bud cuttings. As peroxidases play a major role in several physio-
logical processes, the activity of this enzyme was used as a marker to identify different rooting phases. However, there is insufficient knowledge about the rooting phases of leaf bud cutting of rhodo-
dendron. The aim of this study was to investigate how changes of peroxidase activity in stem base and leaves correspond to adventi-
tious root initiation and development in the elepidote rhododen-
dron cultivar ‘Babîtes Baltais’ [‘Cunningham’s White’ X ‘Elisabeth’] leaf-bud cuttings during their rooting. The patterns of changes of peroxidase activity were similar in stem bases and leaves of leaf bud cuttings. Three phases of adventitious root formation were identi-
fied: induction, initiation and expression. During the induction phase the peroxidase activity decreased, but no anatomical changes were observed in the cuttings. During the initiation phase the per-
odxase activity increased parallel to the formation of adventitious root initials. A peak of peroxidase activity indicates the termination of the initiation phase. Reduced peroxidase activity was found dur-
ing the expression phase when the growth and the development of adventitious root primordia became visible. These results indicate the possibility to use peroxidase activity in leaves as a marker of rooting phases of elepidote rhododendron leaf-bud cuttings during their rooting.

P06-021 Identification of signalling components in the MAX pathway in the control of shoot branching in Arabidopsis
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Shoot branching is a highly plastic process in which auxillary meri-
stems laid in the leaf axes are activated to outgrow and develop into branches. It is regulated by a complex interplay of environmental and endogenous factors such as phytohormones. Three hormones have been implicated in regulating shoot branching: auxin, cytokinin, and a novel, carotenoid-derived hormone of yet unidentified chemical structure. Auxin moves basipetally to inhibit bud out-
growth indirectly. Cytokinin is a positive regulator, which is trans-
ported acropetally, while the novel hormone also moves upward but inhibits shoot branching. The activity of the novel hormone was proposed based on the phenotype of the max mutants, which exhibit increased shoot branching, and were shown to function in one pathway. The MAX-dependent hormone acts by modulating auxin transport capacity in the main stem, but little is known about the downstream targets of the MAX pathway and about the mecha-
nism of MAX-dependent modulation of auxin transport.

To identify components of the MAX signalling pathway we made use of the inducible MAX biosynthetic genes in their respective mutant backgrounds. By transcriptional profiling we identified sev-
eral candidates to function in MAX signal transduction, including transcription factors, and protein kinases. We will present prelimi-
ary results of the characterisation of selected genes in the context of their putative role in regulating shoot branching.

P06-022 Molecular control of bud dormancy in Poplar
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The shoots apical meristem of perennial plants cycles between an active and dormant state. The dormant state is characterized by the lack of responsiveness of cell cycle machinery to growth promotive signals. Transition from active to dormant state involves consider-
able modulation of gene expression. The aim of our project is to identify the molecular mechanisms underlying the loss of responsi-
FERTILIZATION INDEPENDENT ENDOSPERM (FIE) in dormancy since its expression is upregulated during transition to dormancy in hybrid aspen (Populus trichocarpa x Populus tremula) and down-regulated, during the release from dormancy. FIE is similar to genes that comprise the PcG- complex that has been shown to be involved in epigenetic modifications and stabile repression of gene expres-
sion. To investigate whether FIE is involved in dormancy, FIE RNAi plants were produced and analyzed under short day and long day conditions. Our results from these experiments shows that FIE RNAi plants are able to undergo growth cessation but do not establish dormancy.

P06-023 Identification of kinase genes regulating Xylem vessel differentiation in Arabidopsis Thaliana
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The plant vascular system functions to transport water, photosyn-
theses and signaling molecules. Also, it supports the plant body upright to finally facilitate dissemination of seeds. The plant vas-
cular system is made of xylem and phloem which develop from pro/vascular cambium. Xylem is the main tissue which is produced
during secondary growth and is made of several cell types including xylem parenchyma cells, xylem fibers, tracheids and xylem vessels. Transcriptome analysis conducted in our laboratory on Zinnia and Arabidopsis cell cultures, pointed out more than 1000 genes preferentially expressed during transdifferentiation of xylem vessels. Based on these candidates, we put the focus on kinase superfamily. Kinases are known to play crucial roles in plant growth, development as well as hormone responses. Particularly, leucine-rich repeat receptor-like kinases (LRR-RLK) are good candidates as key regulators involved in xylem vessel differentiation. Considering our transcriptomic databases, almost 100 kinase genes show differential expression in the course of transdifferentiation. Nine of those kinases belong to LRR-RLK family and three other are annotated as similar to receptor-like kinases. In order to investigate the involvement of these candidates in the vessel differentiation process, sense and antisense Arabidopsis lines as well as promoter-reporter lines (YFP and GUS) were generated. In parallel, T-DNA tagging mutant analysis has been initiated.

**P06-024** Effects of brassinosteroids on barley root growth, antioxidant system and cell division

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Brassinolide (BR), which is the most biologically active of the brassinosteroids, was used to examine the potential effect of hormone on barley (Hordeum vulgare L.) root germination, on the changes in activities of antioxidative enzymes and cell division. Barley seeds were germinated in between filter papers at dark 0.1, 0.5 and 1.0 μM BR supplemented distilled water for 48 h with their controls. BR application increased the root growth. The primary root length almost doubled 1.62 ± 0.6 cm (in control) to 2.49 ± 0.8 cm (in 1.0 μM BR treated materials) and obvious enlargements at the root tips were observed. Superoxide dismutase activity significantly increased at 1.0 μM BR concentration when compared with the control materials. However, no significant changes in catalase activity but decrease in peroxidase activity at the same concentration was observed. Treated and untreated control group roots were fixed in 1:3 aceto-alcohol and aceto-orcein smear preparations were made. For each experimental group, the percentage of cells showing abnormalities were calculated. Roots treated with BR showed the more abnormalities and mitotic activity. Data obtained could be beneficial for the understanding of BR effects on root development.

**P06-025** Contribution of MET1 to tissue specificity of the developmental regulator REVOLUTA

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DNA methylation is a key factor in the establishment of cell identities in plants and animals, which influences gene expression on a genome-wide level. Although an impact of DNA methylation on the regulation of specific plant developmental programmes is only evident in a few examples, a wide-spread contribution of DNA methylation to tissue specificity of gene functions is likely and remains to be unravelled. Here we demonstrate that the activity of the developmental regulator REVOLUTA (REV) depends on DNA methylation mediated by the DNA-methyltransferase MET1. In a met1 background, increased REV expression correlates with hypomethylation of the REV locus and phenotypic changes characteristic for effects generated by ectopic REV expression. Analysis of REV methylation in rev-10d mutants, generating a miR165/166 resistant REV mRNA, and in mutants generally affected in small RNA production reveals that miR165/166 but also other small RNAs are essential for REV methylation. Based on our observations, we suggest a model in which small RNA-mediated DNA methylation contributes to the tissue specificity of REV function. Analysis of tissue-specific DNA methylation at the REV locus together with tissue-specific modulation of MET1 activity is being used to test our hypothesis and will also address the interdependence of gene transcription and DNA methylation.

**P06-026** CLE41/CLE44 peptide regulates the plant vascular development

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The plant vasculature has two conducting tissues, xylem and phloem. Cells in both tissues are produced from the vascular meristem, called procambium/cambium, which locates between the two tissues. Stem cell fates in the procambium is spatially regulated, providing continuous growth and well-organized xylem/phloem pattern. This developmental process is thought to be regulated by cell-cell interactions. However, molecular mechanisms underlying this process are poorly understood. We previously identified a dodeca-CLE (CLE3/ESR-related) peptide as an inhibitory factor of differentiation from procambium cell to tracheary element (xylem vessel cell) in vitro, and designated TDF (tracheary element differentiation inhibitory factor) (Ito et al. 2006). TDF is a candidate of signaling molecule which controls the vascular stem cell fate. In this study, we attempt to elucidate in vivo role of TDF signaling. For this purpose, we first examined the expression pattern of Arabidopsis CLE41 and CLE44, which encode TDF. Furthermore we revealed the function of TDF in situ, and tried to isolate receptor gene(s) for TDF.

**P06-027** Leaf-air CO₂ gradient and/or CO₂ efflux as environmental signal regulating stomatal patterning on the leaf

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Stomata are responsible for efficient gas exchange between plant and environment and their behaviour responds to ambient CO₂ concentration. Stomatal number and patterning based on the one cell spacing rule (two stomata don’t adjoin each other) is controlled by
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genes and regulated by environmental signals during development. Molecular mechanism of the stomatal patterning in Arabidopsis thaliana and the role of many genes in this cascade was recently clarified. The phenotype of the mutants (e.g. trm, add, flp) involves clustering of stomata. Stomatal clusters were found with higher frequency on cotyledons and first true leaves in Lepidium sativum L grown in our experiment at low CO2, high humidity and in atmosphere with 2.3 x increased diffusivity when nitrogen was replaced by helium (He/O2) or under reduced pressure. These conditions modify CO2 gradient between peri/epidermis and atmosphere and/or accelerate CO2 efflux from epidermis. Epidermal CO2 is maybe a signal for proper distribution of stomata. Respiration and photosynthesis rates and isotopic ratio of carbon (δ13C) in plant mass were measured. As high relative humidity increased permeability of cuticle for water and presumably also for CO2, cuticle waxes on the leaf can be involved in regulation of stomatal patterning. The amount of cuticular waxes was increased. 

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P06-028 Probing the subcellular compartmentalisation of cytokinin conjugation
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Modulation of active cytokinin levels in plants involves many metabolic conversions like biosynthesis, degradation, interconversion and both reversible and irreversible conjugation. The subcellular compartmentation of this network determines the final levels of the active hormone inside the cell. The over-expression in transgenic tobacco of Zm-p60.1, a maize β-glucosidase capable of releasing active cytokinin from O-glucoside conjugates, leads to a disruption of zeatin metabolism and a hypersensitivity to exogenous zeatin. Over-expression of variants of this protein targeted to either the ectopically expressed enzyme accumulates as seen by histochemical staining. Over-expression of the vacuole-localized variant together. Molecular characterization as well as phenotypes during early seedling development of these plants will be presented. This work was supported by a grant (research centre for basic research LC 06034) from the Ministry of Education, Sports and Youth Affairs of the Czech Republic.

P06-029 Structural modifications of galactoglucomannan oligosaccharides and their activity in plant growth
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Biologically active galactoglucomannan oligosaccharides (GGMOs) influence the growth of intact plants. The aim of this work was to compare the effect of galactoglucomannan oligosaccharides (GGMOs), GGMOs-r (with reduced reducing end) and GGMOs-g (degalactosylated GGMOs) on elongation growth of hypocotyls and seminal roots, and formation and elongation of lateral and adventitious roots in hydroporons. Mung bean seedlings were cultivated hydroponically in Hoagland solution containing GGMOs, GGMOs-r or GGMOs-g (concentrations ranging from 10−10 to 10−6 M) solely, or in combination with IBA (10−6 M). Plants were grown in a growth chamber during 7 days in controlled conditions. GGMOs inhibited the hypocotyl elongation, and stimulated seminal and lateral roots elongation compared with the control. Similarly GGMOs + IBA inhibited the hypocotyl elongation, and stimulated seminal and lateral roots elongation compared with IBA. On the other hand, GGMOs-g stimulated hypocotyl and inhibited seminal root elongation in comparison with GGMOs. However, in combination with IBA these oligosaccharides inhibited the elongation of seminal and lateral roots compared with GGMOs+IBA. GGMOs-r influenced plant growth in the same range like GGMOs. It is likely that galactose side chains of GGMOs are responsible also for their biological activity in elongation growth of intact plants.

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P06-030 Production of antibodies against specific epitopes of hardwood 4-O-methylglucuronoxylan
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Hemicelluloses form an essential part of primary and secondary cell walls. In the secondary cell wall most dicotyledonous hardwoods, the main hemicellulosic component is xylan, which is substituted with (methyl) glucuronic acids and acetyl groups. The glucuronoxylan coats the cellulose microfibrils and potentially forms covalent linkages with lignin, but the exact nature of these interactions is not known. The substituents modify the properties of xylan, contributing to water solubility of xylans and to the swelling of fibers, and therefore to pulping quality. However, the substituents are not uniformly positioned and the functions of the different domains remain unknown. We study the hardwood cell wall properties that are related to the structure and quantity of glucuronoxylan by using chemical analyses combined with enzymatic fingerprinting techniques and immunoprofiling. For immunolocalisation, we have isolated, from hardwood glucuronoxylan, short oligosaccharides that are used as antigens to produce monoclonal antibodies against specific epitopes in woody cell walls, for example the methylglucuronic acid substituents. Together with commercially available antibodies, these will be used to characterise the distribution of different glucuronoxylan domains in the cell walls of wild type hybrid aspen

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(Populus tremula x tremuloides), as well as in transgenic lines in which candidate genes for hemicellulose biosynthesis have been modified.

**P06-031** Arabidopsis TCP transcription factors regulate gene network for differentiation of shoot lateral organs

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TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP) is a family of plant specific transcription factors and Arabidopsis genome contains 24 TCP genes. To identify the function of TCPs, we applied a novel gene silencing system called CRES-T, in which a transcription factor fused with EAR-motif repression domain (SRDX) dominantly represses the transcription of its target genes. Expression of the chimeric TCP3 repressor (TCP3SRDX) induced the formation of ectopic shoots on cotyledons and various defects in development of shoot lateral organs. TCP3SRDX induced ectopic expression of boundary-specific genes that include CUP-SHAPED COTYLEDON (CUC) genes in association with reduction of accumulation of miR164, whose product cleaves CUC transcripts. By contrast, the expression of mTCP3, in which the target site of miR319 was mutated, suppressed the expression of CUC genes and resulted in fusion of cotyledons and defects in formation of shoots. Furthermore, microarray analysis revealed an expression profile of the genes that TCP3 regulates. Because TCP3 acts as an activator of transcription, these results suggest that TCP3 appears to activate some unidentified factors that suppress the transcription of the CUC genes. We discuss about the gene expression network which leads to the negative regulation of CUC genes in shoot lateral organs.

**P06-032** Global analysis of the root hair morphogenesis transcriptome reveals new genes involved in root hair formation of barley

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Root hairs are specialized epidermal cells that play an important role in plant nutrition. They have become also a model for studies on cell differentiation in higher plants, however, there is a very little information available about the molecular basis of root hair formation in monocots. We previously used the cDNA RDA to identify qualitative differences between transcriptomes of the wild-type variety ‘Karat’ and its mutant rhl1.a (root hairless 1.a). Our analysis resulted in the discovery of HvEXPB1, a gene critical for root hair initiation in barley. Here we were interested in finding other genes involved in root hair development in barley, particularly in root hair initiation and primordia formation. To this end we compared the transcriptome of rhl1.a and rhp1.b (root hair primordia 1.b) mutants with the corresponding parent varieties ‘Karat’ and ‘Dema’, respectively. In this study we employed Affymetrix Barley1 GeneChips. We identified 25 genes differentially expressed between Karat and rhl1.a, and 72 genes differentially expressed between Dema and rhp1.b. The differential expression of candidate genes and correlation of their expression with root hair formation was confirmed by qRT-PCR. Additionally, we analyzed Arabidopsis knock-out mutants of genes orthologous to the most promising barley candidates. We propose that comparative analysis of root hair transcriptome in barley and Arabidopsis will reveal common mechanisms controlling root hair formation in plants.

**P06-033** Lignin forming cell culture of Norway spruce as a model for cell wall biosynthesis

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A Norway spruce cell culture that produces extracellular lignin is used as a model for cell wall synthesis. We have studied the enzymes involved in activation of lignin precursors and characterised the genes for enzymes needed for monolignol biosynthesis and their polymerisation. The removal of H2O2 from the culture medium diminished the amount of extracellular lignin, suggesting the importance of peroxidases in monolignol activation. This led to the question of the origin of H2O2 in the cell wall. This has been studied in lignin-producing cell cultures and compared to elicitor-induced H2O2-generation using cell wall fragments of Heterobasidion parviporum as an elicitor. At least two different mechanisms are involved in H2O2 generation: NaN3-inhibited enzyme having more important role in the initial elicitor-induced H2O2-generation and flavin-containing enzyme having that at the later phase. Purified plasma membranes were observed to contain redox active enzymes able to generate superoxide. Accumulation of phenolic dimers in both cells and in the culture medium was observed after removal of H2O2 from the medium. The other research interests have been the effect of ethylene on xylem and lignin formation, transport of monolignols into the apoplast and the interaction of polysaccharide matrix and lignification. Cell culture of spruce offers an excellent experimental material for resolving these kind of questions that are difficult to be studied in their native location.

**P06-034** Interaction of source and sink components in the green-white chimera Ficus benjamina CV. ‘Starlight’

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Ficus benjamina ‘Starlight’ is the periclinal chimera with green center and white edge in leaves. Proportion of green and white zones may vary whereas their arrangement is constant. The existence of green and white parts of leaves may lead to different sink-source relations in a leaf and in the whole plant. According to the morphometric data, the proportion of white zone in the leaf correlates with the leaf position in plant: the higher the branch order, the larger the rate of white zone. In mosaic chimeras the source-sink status appears to be dependent on leaf position in the axial system, and products of photosynthesis may act as metabolic signal for leaf primordium.
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Experiments with individual shoots have shown: the area of assimilating surface in a whole shoot affects the ratio of green and white parts in the leaf primordium. In young leaves the white zone contains chloroplasts with large starch grains. They are rare and small but appear functional activity of the photosynthetic apparatus (quantum yield of PSII averages 0.5 for white zone). In mature leaves white cells degrade: they have giant vacuoles, ‘myelinic figures’ obtained after degradation of plastid membranes and mitochondrial stacks. The given description is similar to the cell ultrastructure of the plant object under conditions of carbon deprivation. We suppose that degradation is caused by poor contacts between white and green cells or by age-specific switching of carbohydrate fluxes from inflow to outflow.

P06-035  dva4, a novel Arabidopsis mutant with root vascular defects

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The distorted root vascular pattern4 (dva4) mutant was isolated in a genetic screen designed to identify novel factors affecting vascular initiation, patterning and development. We have mapped the mutation to chromosome 3, and complementation analysis is under way. The mutant exhibits a short primary root as well as misexpression of the phloem marker SUC2:GFP. Both phloem and xylem cells are present in the dva4 root, however the number of vascular cells is considerably lower than in wild type. Although having fewer cells the mutant undergoes secondary development, indicating that DVA4 primarily controls early vascular-specific cell divisions. Furthermore, the leaves of dva4 are narrow and serrated, flowering is delayed, and the mutant exhibits low apical dominance, producing several inflorescences. An initial characterisation of the dva4 mutant phenotype will be presented.

P06-036  The role of APL as a transcriptional regulator in specifying vascular tissue identity

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The vascular system of higher plants confers efficient conduction and provides mechanical support. It consists of two kinds of conducting tissues, xylem and phloem. Phloem transports the products of photosynthesis and provides paths for translocation of proteins and mRNAs involved in plant growth and development. Although there are some reports of gene expression characteristic to phloem, the molecular basis of phloem development is still largely unknown. The APL transcription factor (Altered Phloem Development) was identified as the first gene specifying vascular tissue identity. Based on cell sorting coupled with genome-wide microarray analysis, we have been able to uncover phloem abundant regulatory genes dependent on APL. The results indicate that APL is a key node for transcriptional activation of gene expression characteristic to phloem development and for transcriptional repression of gene expression characteristic to xylem development. We are currently studying the possible functions of the identified genes in phloem development. Interestingly, some of the identified regulatory genes are related to each other, indicating subfunctionalisation of gene families related to phloem development.

P06-037  Arabidopsis temperature-sensitive mutant, long life span, displays altered cytokinin responses and sugar sensitivity

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A temperature-sensitive mutant, lls (long life span), has been isolated, which displays abnormal morphology at 22°C but can be partially recovered at 26°C. The mutant seedlings exhibit dwarf phenotype, reduced apical dominance, dark green curved leaves, delayed flowering and more axillary branches. A number of these features implicated that phytohormone related responses were altered. The lls mutant retains normal sensitivity towards auxin but less sensitive to auxin polar transport inhibitor, NPA. Additionally, the mutant shows less sensitivity towards cytokinin in roots inhibition assays and in anthocyanin accumulation assay. And long time treatment of 6-BA can induce wild type to form mutant’s phenotype to some extent. The mutant also shows hypersensitivity towards ACC and ABA. The mutant has a long life span more than 5 months and leaf senescence is significantly delayed in the mutant, which suggest that the alteration responses towards phytohormone could be responsible for the expanded life history of lls. Moreover, the mutant is hypersensitive to sugars. The lesion in the lls mutant was mapped to chromosome 4 where no other previously known temperature-sensitive mutant has been mapped, indicating that the LLS defines a novel locus involved in hormone, temperature, sugar signaling and senescence.

P06-038  Effects of indole-3-acetic acid on morphogenesis of AtCKX transformed potato (Solanum tuberosum L.) plants grown in vitro

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Cytokinins (CKs) control many plant developmental processes. Their pool in plant cells is down-regulated by irreversible degradation with cytokinin oxidase/dehydrogenase (CKX). CK-deficient transgenic plants overexpressing CKX genes represent suitable tool for studying interactions of CKs with other growth regulators in plant biology. With this regard, we investigated effects of exogenously applied indole-3-acetic acid (IAA) on morphogenesis in AtCKX transgenic potato plants grown in vitro under long-day (non-inductive for tuberization) conditions. The transgenics were obtained by A. tumefaciens-mediated transformation; the presence and expression of AtCKX genes were confirmed by PCR, Southern and RT-PCR analyses. Transformed Physiol. Plant. 133, 2008
plants showed enhanced CKX activity and declined CK contents. Compared to control, they exhibited reduced shoot length, increased branching and stolon initiation and occurrence of tubers. Although shoot length and branching were markedly influenced by IAA in control plants, they were not significantly affected in transgenics. A slight inhibition of tuber formation (occurring in transgenics only) by IAA was observed. On the other hand, IAA progressively enhanced rooting in both control and AtCKX plants. Possible changes of sensitivity to applied IAA in transgenic AtCKX potato plants are implicated. Supported by GA ASCR (I AA 600380701) and Ministry of Education, Youth and Sports CR (LC 06034).

P06-039 Auxin- cytokinin cross-talk in shoot branching
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In seed plants, during postembryonic development secondary meristems are formed along the primary shoot axis. They are located between the stem and leaf primordia, i.e. in leaf axils, and develop into axillary buds. The buds can then grow out to form side shoots or remain dormant. Thus the control of activity of these axillary buds has a great impact on plant architecture. The outgrowth of dormant buds is largely regulated by the plant hormones auxin and cytokinin. Whereas apically derived auxin exerts an inhibitory effect on bud activity, basally derived cytokinin promotes the outgrowth of buds into side shoots. To date little is known about the mechanisms underlying these antagonistic effects. Do the hormones act independently from each other to regulate bud outgrowth or is there a cross-talk between them? To elucidate this question we apply physiological as well as molecular genetics approaches and performed a transcriptional profiling of Arabidopsis buds.

P06-040 Suppressor of cobra gives new insights into the role of COBRA in cellulose biosynthesis
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COBRA is a glycosyl-phosphatidylinositol (GPI)-anchored protein involved in cell expansion that has been shown to be important for the deposition of cellulose microfibrils in primary cell walls. Cellulose synthase (CESA) motility in a cobra background appears greatly reduced, confirming the involvement of COBRA for normal cellulose synthesis. Cobra mutants display a sucrose-dependent phenotype. Sucrose treatment rapidly induces phosphorylation of two receptor-like kinases (RLK) belonging to the SRF family, which also appear co-regulated with genes involved in primary cell wall synthesis. Knock-out of these RLKs do not show any growth phenotype on their own but both reduce the growth defect and cellulose deficiency of cobra but not of other cellulose deficient mutants tested. Suppression of growth defect in cobra correlates with restoration of CESA motility and cellulose content in cob/rlk double mutants. Together these data suggest a role for these RLKs in the regulation of cellulose synthesis.

P06-041 Expression dynamic of a PIN homologous gene during Norway spruce (Picea abies) somatic embryogenesis
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Auxin and polar auxin transport have been implicated in controlling embryo patterning and development in seed plants. Directional auxin distribution appears to be controlled by active auxin transport from cell to cell by influx (AUX/LAX) and efflux (PIN-FORMED) membrane transporters. Hypotheses, based on work in Arabidopsis, propose that a network of PIN proteins create auxin gradients within the plant body and this, in turn, regulates various gene expression programs during development. Thus, the PIN network is important for coordinating proper plant development. Representatives of the PIN gene family have been found in many angiosperms, but information regarding PIN homologs from other seed plants, such as gymnosperms, is limited. In this study, a PIN homologous gene was isolated from the gymnosperm Picea abies and its expression dynamic was followed during somatic embryogenesis, a model system for embryo development in conifers. The aim was to determine at what stages, during embryo development, polar auxin transport is most critical. Studies using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) showed that the PIN gene was expressed in high levels in early staged embryos, reflecting the importance of polar auxin transport during these stages of development. Our results will thus provide additional data to correlate polar auxin transport to embryo patterning in a non-angiosperm seed plant.

P06-042 Proteomic approach to analyze dormancy breaking of sycamore (Acer pseudoplatanus L.) seeds
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Investigation of proteins, product of genes activated during a complex developmental process as is dormancy breaking was the aim of presented research. With seed dormancy breaking the plant hormones are associated: GA3 responsible for stimulation of dormancy breaking and germination, and ABA responsible for maintenance of dormancy and inhibition of germination. These studies were carried out on desiccation sensitive ‘recalcitrant’ seeds of sycamore (Acer pseudoplatanus L.) during their stratification and germination. After imbibition in water and in solution of GA3 or ABA, seeds were subjected to cold stratification, which breaks dormancy. Regarding the proteomic approach, proteins of the seeds were separated by 2D-gel electrophoresis and were analyzed by mass spectrometry. The influence of stratification and hormones was investigated and main protein variations were pointed out. Analysis of the proteins specific only for the water, GA3 or ABA was done. A total of 44 spots, showing significant changes in volume, were identified by MS. The classification of the proteins showed that the mechanism of seed dormancy breaking involves the proteins of many processes: protein destination, energy, metabolism, transcription and defense. The proteins whose activity changed during stratification and can be associated with sycamore seed dormancy breaking are glycine-rich RNA binding protein, calreticulin, annexin and proteasome proteins.
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P06-043 Control of lettuce germination rate and plantlet elongation by a xyloglucan and its oligosaccharides
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Fucogalactoxyloglans are building blocks used in plant cell wall assembly. In order to help to unravel the role of xyloglans in the control of plantlet elongation, we treated lettuce seeds with a galactoxyloglans extracted from seeds of Hymenaea courbaril. This xyloglans had a Glc:Xyl:Gal ratio of 4.4:2.7:1.0 and an average molar mass of 658 900 g mol⁻¹, according to high-performance size exclusion chromatography analysis. Lettuce seeds were treated with a mix of oligosaccharides, obtained from enzymatic hydrolysis of the xyloglans with endo1→4)-β-glucanase, and with combinations of the xyloglans and 2,4-dichloro-phenoxyacetic acid (2,4-D), as well. Treatment with the xyloglans resulted in increased germination rate, and, seedlings grown in the presence of 500 nM xyloglans presented a 28 % increase in elongation, compared to untreated seeds. However, oligosaccharides did not mimic the promotive effects of the native xyloglans. In addition, the xyloglans was not able to reverse a 2,4-D-driven inhibition of plantlet elongation. These results indicate that the xyloglans-induced enhancement of plantlet elongation might not rely on the xyloglans breakdown. Besides providing an insight on the molecular mechanisms involved in the control of plantlet elongation, this work also present practical applications, such as the stimulation of early germination and the improvement of plantlet establishment in lettuce, and, potentially in other species.

P06-044 Effects of low root temperature regimes on Ricinus communis L. leaf growth dynamics and root-shoot communication
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Low temperature is a limiting factor for plant growth and underlying processes. In laboratory and greenhouse experiments with potted plants, shoot and roots are usually exposed to the same temperature regime, while in the field they are often strong differences concerning amplitude and phasing of shoot and root temperature. It was the aim of this study to monitor leaf growth with non-invasive infrared imaging procedures in laboratory experiments with controlled root temperature. When R. communis root systems were kept at constant temperatures of 10, 15 and 20°C, respectively, a number of processes showed altered diel (24 h) patterns compared to plants grown without root temperature control: In plant with cooled roots, leaf growth preferentially occurred at night and was strongly inhibited at day. Also carbon allocation between root and shoot and hydrostatic pressures were affected, while transpiration was not affected. When root temperature was increased again, growth showed only a partial recovery. These results are necessary first steps for improved models of shoot growth control and for improvement of lab-based plant selection strategies for breeding or other applied processes.

P06-045 Quantitative estimation of IAA content based on histochemical staining of GUS-activity
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Modern research of plant physiology often requires the estimation of local hormone level on a scale of tissue. Transgenic plants with GUS reporter gene under hormone-sensitive promoter control are widely used as detection procedure is simple, but only qualitative detection is available now. We offer a method for quantitative estimation of indole acetic acid (IAA) based on histochemical staining of GUS-activity. The study was performed on 7 d.a.g. Arabidopsis thaliana DR5::GUS seedling roots. Plants were grown on a medium with IAA concentrations 10⁻⁸ ÷ 10⁻⁷ M for calibration. Histochemical staining was performed according to a standard protocol with X-Gluc. Root tissue samples were photographed with Biolam R-13 microscope and Canon EOS 350D camera mounted. Digital photo analysis was performed with Adobe Photo shop, statistical data were obtained from Microsoft Excel. The relationship between the luminance per digital image channel and the IAA content in medium was examined. The correlation of the light absorbance in channel R and IAA concentration was characterized by quadratic form equation. We have measured a set of IAA concentration profiles in control Arabidopsis roots and under gravistimulation. The IAA concentration in the lower part of root was 2–3 times greater than in the upper part. Thus the digital image analysis can be used for quantitative estimation of IAA content in DR5::GUS transgenic plants. The project was supported with RFBR Grant 08-04-00566-9.

P06-046 Principal growth directions of a dorsal petal in Antirrhinum majus flower
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Petals, like other plant organs, grow in a continuous and coordinated way. Such growth is of tensor nature. Its unique feature are principal directions of growth (PDGs), i.e. three orthogonal directions in which growth rates attain extreme values. The question arises whether PDGs are manifested in cellular pattern in petals. To answer this question, growth of dorsal petals of Antirrhinum majus was examined. Petals were stained in toto to visualize epidermal cell walls and cell packets were recognized. The packets develop generally along the proximo-distal axis producing a fountain-like pattern. In numerous packets oblique cell walls appear, usually as the oldest walls within a packet. Such pattern suggests that the petal development is controlled by the field of growth rates, in which PDGs trajectories are curvilinear and converge to the corolla tube base. This field was defined and a model of the petal growth was developed. It shows how the lobe increases its area and deforms. Assuming that cells divide perpendicularly to PDGs, we performed also simulations in which packets from different parts of the lobe were generated. They explain why some cell walls become oblique with respect to the parent cell walls. Such oblique walls had been formed perpendicularly to one of PDGs, but their orientation changed later due to shearing deformation of the whole packet. The
conclusion is drawn that tensor aspects of growth should be taken into account while studying petal morphogenesis.

P06-047 Dormancy release-proteins in the shoot apical meristem of Populus
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Perennials regulate their annual growth-dormancy cycle in response to changes in light and temperature. Short days trigger a sequence of processes, including growth cessation, bud formation, and dormancy. A distinctive feature of the dormant state is the symplasmic uncoupling of cells in the shoot apical meristem (SAM) by formation of dormancy sphincert complexes (DSCs) at plasmodesmata (PD). Additionally, cell walls become sealed. This obstructs intercellular signalling required for morphogenesis. Reversal of this non-communicative state during dormancy-release could be mediated by lipid bodies (LBs), minute protein-decorated organelles that arise in high numbers from the endoplasmic reticulum during dormancy induction. Chilling displaces LBs toward the plasma membrane, where they often contact PD, resulting in their restoration. To assess how LB-PD contact leads to restoration we are characterizing the LB proteome with MS/MS and CAF. Sequence analysis of eight major proteins indicated the presence of an oleosin and a 1,3-β-glucanase. Expression studies (qRT-PCR) of all poplar oleosins and some putative cell wall 1,3-β-glucanases were performed through- out the dormancy cycle to select genes for transformation with RNAi. We propose that 1,3-β-glucanase-production, its compartmentalization in LBs, and its chilling-induced displacement to DSCs constitutes a dynamic mechanism for dormancy breaking. Oleosins may restrict LB size thereby optimizing LB-PD contact.

P06-048 Function of the class III HD ZIP family in root vasculature patterning
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The vasculature of plants is of great importance not only because it provides rigidity and strength to the plant through lignified xylem cells and fibers, but also because the vascular tissue forms channels connecting all parts of the plant which are necessary for the transport and distribution of water, nutrients, and hormones. Yet, despite its importance, little is known of the developmental regulation that governs the formation of the vascular system. We have taken advantage of the simplicity in organization of the Arabidopsis root vasculature and identified a set of mutants with defects specific to the root vasculature. One of these mutants, distorted root vascular pattern1 (dva1), was shown to be a novel gain-of-function mutant of PHABULOSA (PHB), one of five genes in the HD ZIP class III family known for regulating adaxial identity in the shoot. The dva1 mutant displays cell proliferation and patterning defects affecting the pericycle, phloem and xylem tissues suggesting that normal PHB expression is required for proper root vasculature patterning. Interestingly, loss-of-function mutants of the HD ZIP III family display opposite vascular phenotypes and are accompanied by a reduced sensitivity to cytokinin.

P06-049 CENL1 in stem elongation and dormancy cycling in Populus
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We are interested in the mechanisms that underlie the annual growth-dormancy cycling in perennials. Our recent work shows that CENTRORADIALIS-LIKE1 (CENL1), poplar ortholog of the Arabidopsis thaliana gene TERMINAL FLOWER1 (TFL1), plays a crucial role in dormancy development. CENL1 is expressed subjacent to the shoot apical meristem (SAM), where the rib meristem (RM) regulates stem elongation. Under short photoperiod (SD) CENL1 was markedly downregulated in the apex coincident with cessation of elongation growth. In contrast, transgenic poplar overexpressing heterologous PHYTOCHROME A (PHYA) accumulated CENL1 in the RM area and accelerated stem elongation under SD. In SD-exposed heterografts, both PHYA overexpressor- and wild-type-scions ceased growth and formed buds, whereas only the wild type assumed dormancy and PHYA overexpressors showed repetitive flushing. This shows, firstly, that the transition is not dictated by leaf-produced signals but dependent on properties of the apex and, secondly, that the roles of RM and SAM are distinct. The suspension of indeterminate growth during dormancy, enforced by uncoupling of meristem cells by dormancy sphincert complexes (DSCs), may thus require down-regulation of CENL1 in the RM. The results suggest that the RM is sensitive to photoperiod and that CENL1 in the RM influences stem elongation and the transition to dormancy in poplar.

P06-050 Elongation of Scots pine (Pinus sylvestris) seedlings is independent of etiolation
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Two- and three-year-old Scots pine (Pinus sylvestris L.) seedlings were grown in plexiglass chambers and control plots at two study sites in northern Finland. The chambers were made of either orange or transparent plexiglass. The former removed the blue wavelengths (400–500 nm) of incoming sunlight, while the latter was transparent to all visible wavelengths. The removal of blue reduces the total amount of incoming light. This may enhance etiolation responses (i.e. increased stem elongation and reduced leaf development) in the seedlings grown under blue light depletion. Artificial removal of blue light did increase elongation of the main stem, although only at the sub-arctic latitude (69°N) of the experiment, not at the mid-boreal (64°N) latitude. However, elongation of the lateral branches, needle area and the weight of the new stem and needles were also increased in the seedlings grown in orange chambers at the sub-arctic latitude. In northern areas the sun
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remains continuously above the horizon in the summer time. Hence, it follows that in the evening the sub-arctic latitude exhibits a relatively long ‘end-of-day’-period during which the red (600–700 nm) to far-red (700–800 nm) light ratio is reduced. Thus, instead of attribution of the increased main stem elongation of the seedlings grown under blue light depletion to etiolation alone it may be an interaction between a long end-of-day far-red-rich period and an absence of blue wavelengths.

P06-051 Characterization of metacaspase 9 in Arabidopsis thaliana
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Programmed cell death (PCD) is intrinsic to various plant developmental processes such as xyleogenesis. Regulation of plant PCD is believed to be controlled by metacaspases, which show structural similarity with caspases; the metazoan regulators of PCD. We showed earlier in poplar that two metacaspases were specifically upregulated in xylem vessels and fibers approaching cell death. The homologous gene in Arabidopsis is the metacaspase 9 (AtMC9). Reporter gene analyses showed that AtMC9 was specifically expressed in the xylem vessels or vasculature of cotyledons, hypocotyl, root, sepals and petals, as well as in pollen, root cap cells, and root cortical cells adjacent to emerging adventitious roots, supporting a critical role of AtMC9 in a few different developmental PCD processes. T-DNA insertion in the coding sequence of AtMC9 seemed lethal as no homozygous lines were identified. Both RNAi as well as weaker insertion lines displayed phenotypic alterations including an increased size of the rosette leaves, thicker inflorescence stem and hypocotyl, increased size of the vascular bundles and the secondary xylem, delayed bolting and also a range of flower abnormalities. The larger size of the stem and the vasculature of the AtMC9 RNAi and insertion lines support the function of the AtMC9 in determining the size of the vascular meristem. We suggest that this is a result of delayed xylem differentiation due to early inhibition of the cell death program in the incipient xylem elements.

P06-052 Drug modulation of P-glycoproteins by immunophilins
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Active polar transport of the phytohormone auxin is essential for plant physiology and development. In plants, flavonoids are suspected to be negative regulators of this transport, however, the mechanisms by which flavonoids interfere with auxin efflux components are unclear. Recently, ABCB/P-glycoprotein/multidrug resistance (PGP1/MDR) ABC transporters, ABCB1 (PGP1) and ABCB19 (PGP19/MDR1), have been demonstrated to catalyze the cellular efflux and long-range transport of auxin. Both bind the synthetic auxin transport inhibitor, N-1-naphthylphthalamic acid (NPA) and are inhibited by NPA and flavonoids. Here we report that the C-termini of both PGP1 and PGP19 functionally interact with immunophilin-like FKBP42, TWISTED DWARF1 (TWD1). For PGP1, positive regulation by protein-protein interaction with TWD1 was demonstrated. Further, by using bioluminescence resonance energy transfer (BRET) we demonstrate specific disruption of PGP1-TWD1 interaction by NPA and flavonoids. In accordance, TWD1 was shown to mediate modulation of PGP1 efflux activity by auxin transport inhibitors and IAA. NPA binds to both PGP1 and TWD1 but was excluded from the PGP1-TWD1 complex, supporting a transient nature of interaction. Consequently, twd1 plants are NPA insensitive, with little effect on auxin fluxes and root gravitropism on NPA treatment. Our data support a novel model mode of drug-mediated ABCB/P-glycoprotein regulation via protein-protein interaction with immunophilins.

P06-053 Developmental defects and seedling lethality in apyrase mutants
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Previously, two apyrases or nucleoside tri- and diphosphate hydro-lases (NTPDases) were shown to be crucial for male fertility in Arabidopsis (Steinebrunner et al., Plant Physiol (2003) 131: 1638–1747). Mutant pollen (apy1-1; apy2-1) with T-DNA insertions in the two corresponding genes AtAPY1 and AtAPY2 could not germinate. In this study, pollen germination was restored and apyrase T-DNA double knockouts (DKOs) apy1-1/apy1-1; apy2-1/apy2-1 were generated by complementation with AtAPY2 under the control of a pollen-specific promoter. The DKO phenotype displayed developmental defects including the lack of functional root and shoot meristems. In cotyledons, morphogenetic and patterning abnormalities were apparent, e.g. unlobed pavement cells and stomatal clusters. Another set of lines was created which carried either AtAPY1 or AtAPY2 under a dexamethasone-(DEX)-inducible promoter as an additional transgene to the pollen-specific gene construct. Application of DEX did not reverse the DKO phenotype to wild-type, but some inducible lines exhibited less severe defects even in the absence of the inducer, probably due to some background apyrase expression. However, even these DKO mutants were seedling-lethal and shared other defects regarding cell division, cell expansion and stomatal patterning. Taken together, the defects in the DKO mutants demonstrate that AtAPY1 and AtAPY2 are essential for normal plant development.

P06-054 Analysis of cytokinin nucleotides by LC-MS/MS
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Cytokinins are plant hormones that influence many aspects of plant growth and development, such as cell division, shoot formation, apical dominance, sink strength and senescence delay. Formation of cytokinin nucleotides is the first rate-limiting step in cytokinin biosynthesis. Isopentenyl moiety from dimethylallyldiphosphate is attached to exocyclic amino group of ATP and ADP by action of isopentenyltransferases. However, occurrence of appropriate di- and triphosphates in plant tissue has never been confirmed by direct analytical method. We have developed a HPLC-MS/MS method for identification and quantification of these compounds in plant extracts. The method is based...
on proper chromatographic separation of cytokinin mono-, di- and triphosphates and sensitive detection by tandem mass spectrometry. Developed analytical method enabling detection of both isopentenyl-adenine-type and zeatin-type nucleotides may help to elucidate disputable parts of cytokinin metabolism. The work presented was supported by grant no. MSM 6198959216 from the Ministry of Education, Youth and Sports of the Czech Republic.

**P06-055 Epigenetic control of stomatal number in response to humidity environment**


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The formation of stomata is known to be influenced by environmental conditions including light, CO₂ and humidity, and the environmental signal has been shown to elicit both systemic and heritable responses. Plants, nevertheless, maintain plasticity in their capacity to moderate stomatal density during subsequent leaf growth. We hypothesized that the plastic element of stomatal formation could be faithfully copied cell-to-cell and inherited in a non-Mendelian fashion. Plant flowering response to extremes of temperature is known to be under epigenetic control. In this way gene expression patterns could be controlled in Arabidopsis by controlling relative humidity (RH) and investigated methylation of known genes in stomatal formation and patterning pathways, using methylation-specific qPCR and high resolution melt analysis following bisulphite treatment. Two target genes involved in stomatal formation pathways were differentially methylated between treatments. Furthermore, stomatal density in the following, seminal generation of plants exposed to both RH percentages varied according to parental treatment. We present these data and explore the implications of epigenetic control of stomatal density.

**P06-056 Stimulating effect of different cytokinin types and their concentrations on bulb formation of Muscari aucheri using bulb-scale segments**

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Muscari genus which is one of the important geophytes species belong to Liliaceae family and has valuable ornamental plants because of their attractive flowers which open in early spring and some species of the genus have a pleasant smell. There are 28 Muscari species in Turkey. *Muscari aucheri* (Boiss.) Baker are also important species with their attractive flowers. The species is also endemic and endangered species of Turkey and threatened by extinction. It is very important to cultivate these species of cultivation and propagation of both species by use of alternative techniques. We need alternative propagation systems for endangered and endemic species. In vitro techniques may result in production of large number of bulblets in a short period. In this study, endemic and endangered *M. aucheri* species was also cultured under in vitro conditions for bulblet production. Bulb-scale segments of *M. aucheri* were cultured on a medium containing different cytokinin analogues KIN (Kinetin), 6-benzylaminopurine (BA) 1-phenyl-3-(1,2,3-thiadiazol-5-yl) ura (TDZ) and N6-2-isopentenyladenosine (2-YP). BA and KIN were more effective than 2iP and TDZ and the highest bulblet production frequency was obtained from CHU (N6) medium supplemented with 2 mg/l BAP and 1 mg/l KIN.

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**P06-057 Leaf and root growth dynamics: How can plants reach their full growth potential in a dynamically fluctuating environment?**


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Plant growth occurs in an ever-changing environment. Prominent changes are the daily rhythms of atmospheric temperature and light intensity, to which leaves are exposed. Leaves of dicot plants cope with these rhythms by using the endogenous clock to adjust growth to predominant environmental fluctuations. In some species, such as Arabidopsis, the leaf growth rhythm shows a maximum at dawn, while in other species such as poplar, maximal growth happens at dusk. Both types of growth patterns ensure that maximal growth occurs, when water loss of the growing tissue due to transpiration is low and carbon availability is high. In leaves of monocot plants and roots, where the growing tissue is not subject to water loss via transpiration, growth is synchronized with the environment in a different way. There, growth is almost directly correlated with temperature of the growing tissue, leading to maximal growth at noon for monocot leaves and to an often constant diel growth pattern in roots. Leaves and roots live in completely different habitats, but are parts of the same organism. Thus, sudden alterations of environmental parameters in the root or the leaf habitat can affect growth dynamics of both organs strongly and unexpectedly. Elucidation of the mechanisms, how different plants manage to reach their full growth potential and optimal resource use efficiencies in a fluctuating environment, will hence require joint analysis of gene × environment and root × leaf interactions.

**P06-058 Effect of light on mitogen–activated protein kinase activity in etiolated Cucumis sativus cotyledons**

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The mitogen–activated protein kinases (MAPKs) are a subfamily of protein kinases (pKs) involved in the signal transduction of plant responses to environmental stimuli such as wounding, stress or light. We reported the effects of red (R) light and Ca²⁺ on protein phosphorylation in cucumber cotyledons and we...
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P06-059 Functional characterisation of TERMINAL EAR1-like genes in Arabidopsis thaliana

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Post-embryonic plant growth is mediated by meristems, which consist of well-organized pluripotent stem cells, able by division and differentiation, to maintain themselves and to initiate new tissues and organs. The relation between cell proliferation, cell differentiation and organogenesis is very complex and parts of the puzzle still remain unclear. The TERMINAL EAR1 gene has been proposed to regulate leaf initiation in maize, as well as its homolog, the PLA2/LHD2 gene, in rice. Evo/Devo analyses in Poaceae have revealed that TE1-like (TEL) genes may not only be involved in leaf organogenesis but more generally in all tissues and organ initiation within the plant. TEL genes belong to the Mei2-like gene family, encoding RNA-binding proteins with 3 RNA-Recognition Motifs (RRM). The A. thaliana genome contains 2 TEL genes, which harbour low expression level, mainly restricted to apical meristems. Molecular and phenotypic characterisation of AtTEL knocked-out and over-expressing mutants will be presented. Preliminary results suggest that the two AtTEL genes act, at least partly redundantly, regulating positively vegetative growth as well as floral transition, and negatively floral development.

P06-060 Role of VASCULAR-RELATED NAC-DOMAIN PROTEIN7 during xylem vessel differentiation

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We have shown that an Arabidopsis thaliana NAC domain transcription factor, VASCULAR-RELATED NAC-DOMAIN7 (VND7), plays a pivotal role in regulating root protoxylem vessel differentiation (Kubo et al. 2005, Genes Dev). In order to understand the mechanisms underscoring VND7 function in vessel differentiation in detail, we conducted extensive molecular analyses in yeast (Saccharomyces cerevisiae), Arabidopsis, and Nicotiana tabacum L. cv. Bright Yellow 2 (tobacco BY-2) cells. The C-terminal region of VND7 was required for its transcriptional activation in yeast and Arabidopsis. Expression of the C-terminus-truncated VND7 protein under the control of the native VND7 promoter resulted in inhibition of normal development of metaxylem vessels in roots and vessels in aerial organs, as well as protoxylem vessels in roots. The expression pattern of VND7 overlapped that of VND2 to VND5 in most of the differentiating vessels. Furthermore, a yeast two-hybrid assay revealed the ability of VND7 to form homodimers and heterodimers with other VND proteins via their N-termini, which includes the NAC domain. The heterologous expression of VND7 in tobacco BY-2 cells demonstrated that VND7 stability was regulated by proteasome-mediated degradation. Together these data suggested that VND7 regulates the differentiation of all types of vessels in roots and shoots, possibly in cooperation with VND2 to VND5 and other regulatory proteins.

P06-061 Inhibition of elongation growth in roots and hypocotyls induced by GGM-derived oligosaccharides is connected with peroxidase activity

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An inverse relation between the growth rate and peroxidase activity has been reported in many plant systems. Galactoglucomannan oligosaccharides (GGMOs) effect on the growth of intact plants was dependent on their chemical structure. The aim of this work was to ascertain the relation between GGM effect on elongation growth and cell wall peroxidase activity. Mung bean seedlings were hydroponically cultivated in Hoagland solution containing GGMOs or GGMOs-g (degalactosylated GGMOs) in 10⁻⁴ M concentration alone or in combination with IBA (10⁻⁸ M) during 7 days in controlled conditions. Modified method of Warneck et al. (1996) for cell wall-associated peroxidases extraction has been used. Peroxidase activity was determined spectrophotometrically; GGMOs and GGMOs-g affected the elongation growth of hypocotyls and roots with stimulation and inhibition effect in opposite way. However, this effect of GGMOs, as well as of the structurally modified GGMOs-g, on elongation growth in hypocotyls and roots was connected with changes of cell wall-associated peroxidases activity. From results obtained it can be concluded that the inhibition of elongation growth induced by both types of oligosaccharides is probably associated with the start of cell wall rigidification catalysed by peroxidase. This study was supported by the Slovak Grant Agency for Science (No. 2/7048/27) and APVV Agency (No. 51-013304).


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Biologically active galactoglucomannan oligosaccharides (GGMOs) are inhibitors of auxins-induced elongation growth of pea and spruce stem segments. This effect is dependent on the oligosaccharides concentration and timing of GGMOs and auxins application. The peroxidase activity showed inverse correlation to the growth in many plant systems. The aim of this work was to ascertain the relation between GGMOs effect on seminal root elongation and peroxidase activity. Mung bean seeds were cultivated (during 24 h in controlled conditions) in solutions containing GGMOs (10^{-10} to 10^{-4} M), or in combination with IBA (10^{-4} M). IBA was added after 3 h of preincubation with GGMOs, or both were added simultaneously at the beginning of the experiment. Modified method of Warneck et al. (1996) for cell wall-associated peroxidases extraction has been used. Peroxidase activity was determined spectrophotometrically. GGMOs were without significant effect on seed germination and seminal root elongation compared with the control. However, GGMOs inhibited seminal root elongation induced by IBA, and this inhibition was higher in the experiment with GGMOs preincubation. Cell wall-associated peroxidases activity in seminal roots was dependent on the GGMOs and/or IBA treatment. This study was supported by the Slovak Grant Agency for Science (No. 2/7048/27) and APVV Agency (No. 51-013304).


P06-063 Suppression of lateral root initiation occurs in response to transient root water shortage and reveals the presence a new checkpoint

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The plasticity of branching patterns of plants plays a crucial role in the capture of resources from unpredictable, heterogeneous and fluctuating environments. This plasticity is achieved by regulating the site and timing of initiation and controlling the further steps of the developmental program of new branches. We show in Hordeum vs seedlings grown in aeroponics that the distal seminal root segment formed during a transient mild root water shortage is devoid of lateral roots (LR). Since LR formation in barley occurs in the apical region of the seminal root (SR) and follows an acropetal sequence, this suggests that the treatment suppresses LR initiation. The response is highly reproducible and is not due to changes of mineral concentrations in the root zone. Tip excision experiments do not show that the SR meristem is involved in the response. In addition, the characterization of the developmental stages of LR primordia along treated SR indicates that the suppression occurs before the first divisions of LR founder cells. Interestingly, careful time lapse imaging of the SR reveals that the zone devoid of LR is localized about 8 mm proximal to the SR segment formed during the treatment. This study suggests the presence of a checkpoint operating in the early phases of LR initiation and allows to predict its site of action.

Abstracts

P07-011 Alkaloid production in *Catharanthus roseus* cell cultures elicited with cyclodextrins and methyljasmonate

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*Catharanthus roseus produces numerous alkaloids with high pharmaceutical importance such as vinblastine and vincristine which have antineoplastic activity. Because these alkaloids are produced at very low levels in plants, *C. roseus* cell cultures have been studied for many years as a potential way to produce these compounds. However, the alkaloid production from suspension cultures is relatively low. Among different strategies to increase alkaloid production, elicitation is one efficient strategy to provoke important increases in product yield. Thus, elicitation of grapevine cell cultures with cyclodextrins (CDs) induced the production of resveratrol, the stilbene unit characteristic from Vitaceae family. The effect of CDs on resveratrol production allowed the development of an innovative procedure where high levels of this metabolite were accumulated and were easily recovered directly from the culture media without cell biomass destruction. Moreover, the combined use of methyljasmonate (MeJA) and CDs provoked a synergistic effect increasing even more the levels of resveratrol in grapevine cell cultures. In the present research work, we tried to extrapolate this innovative technology focusing on alkaloid production improvement by elicitation of *C. roseus* cell cultures with a combination of both MeJA and CDs. L.A. and S.B.N. hold grants from the Fundación Séneca. This work has been supported by the MEC (BIO2005-0332) and by the CARM (BIO-BVA 07/001-003).

P07-012 An EST-SSR marker for yellow rust resistance at seedling and adult plant stage in Turkish bread wheat (*Triticum aestivum* L.) genotypes


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In bread wheat, ~500 000 ESTs are already available in the public domain and are being put to a variety of uses, including the development of molecular markers such as EST-SSRs. To date, no published results are available for studies on yellow rust resistance of Turkey bread wheat genotypes using EST-SSR markers. In the present study, F1 plants from the cross A2 zg2001 (resistant male parent) × ES14 (susceptible female parent) were screened at seedling and adult plant stage regarding yellow rust resistance. The most resistant and susceptible F1 plants, selected by yellow rust scoring, were used together with their parental lines for bulk segregant analysis to find out molecular markers linked to yellow rust resistance. EST-SSR analysis was performed on the constructed DNA pools, using 78 primer pairs to detect EST derived SSR markers that were present/absent respectively in the two pools and their parental genotypes. One EST-SSR marker (Pk54) which was present in the resistant parent and in the resistant bulk, but absent in the susceptible parent and in the susceptible bulk, was identified. The results obtained in our study showed clearly that EST databases would be a potential source of such markers and that potential will make them a valuable source of new generic SSR markers. The presence of Pk54 marker that is associated with yellow rust resistance may significantly enhance the success of selection for yellow rust resistant genotypes in future Turkey wheat breeding programs.

P07-013 A protocol for rapid micropropagation of endangered Apium repens
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Creeping marshwort (Apium repens) (Jacq.) Lag is rare and highly endangered species in many Central European countries, also in Slovenia. It is listed also in Habitat directive. Since it has been sporadically observed in this region, in vitro propagation allows the possibility of rapid propagation of this species and its reintroduction to its natural habitat. An efficient protocol for rapid micropropagation of A. repens through axillary shoot and stolon formation is described. Explants were cultured on solid Murashige and Skoog (MS) medium supplemented by different concentrations and combinations of (0–10 μM benzyladenine (BAP), dimethylallylaminothione (2-iP), thidiazuron (TDZ), with or without (0.5 μM) naphthalene acetic acid (NAA) and indolebutyric acid (IBA). Shoot and stolone multiplication was best when 2 μM BAP and 2 μM 2-iP were used. Regenerated shoots were successfully rooted on all media. More than 82% of the rooted explants survived transfer to in vitro greenhouse conditions.

P07-014 The study of hydrogen peroxide and ascorbate peroxidase in highbush blueberry (Vaccinium L.) shoots depending on in vitro treatment
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Different cultivation conditions were tested to study the growth of the highbush blueberry (Vaccinium L.) shoots during in vitro multiplication stage, as well hydrogen peroxide and activity of ascorbate peroxidase were determined. The growth of ‘Improved Stanly’ and ‘Woodart’ shoots had variable intensity under the influence of Anderson’s medium (1984) supplemented with 0.5 mg l−1 N−(2-isopenteny) adenine. However the application of fluoridine 1 mg l−1, pretreatment with + 4°C or using microcuttings without leaves stimulated shoot growth from microcuttings. It leaded to the suggestion that possible reason interfering shoots growth was accumulation of endogenous abscisic acid (ABA). The treatments suppressing possible ABA impact and stimulating growth influenced oxidative parameters – hydrogen peroxide and activity of ascorbate peroxidase. The results suggest a link between microshoots growth intensity and oxidative parameters. However the cause of growth wane has to be elucidated in further experiments.

P07-015 Transformation of tobacco plants with Na+/H+ antiporter (AtNHX1) gene isolated from Arabidopsis thaliana for salt tolerance
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Elevated Na+ concentration in soil is an indicator of soil salinity. Large, membrane-bound vacuoles of plants containing Na+/H+ antiporters compartmentalize ions. Na+/H+ antiporter expression induced by salt stress is necessary for gaining of salt tolerant characteristics. The Arabidopsis thaliana vacuolar Na+/H+ antiporter AtNHX1’s overexpression allows the transgenic plants to grow in 200 mM NaCl considered as a harsh condition for many plants. In this study, the AtNHX1 cDNA was cloned and transformed to tobacco plants via Agrobacterium-mediated gene transfer. Ten independent putative transgenic plants were obtained. Callus formation and regeneration under different salt concentrations were evaluated. Transfer of selected eight putative transgenic plants to soil provided the regeneration of T1 seeds. The 82% and 60% of the transgenic T1 seeds were germinated on 150, 200 mM NaCl containing media. In contrast, the germination percentage of wild type tobacco seeds under same concentrations were 39% and 21%. The germination rate of the transgenic T1 seeds were significantly higher (P = 0.001) especially under high salt stress conditions. Our results demonstrated that the germination efficiencies and growth of the plants transformed with AtNHX1 gene were higher than the wild type tobacco plants under high salt concentrations. Southern Blot and Northern Blot Hybridization of transgenic plants will be performed to analyse the presence and the expression of the gene as further studies.

P07-016 Effect of elicitors on stilbene biosynthesis gene expression and resveratrol production in Monastrell grapevine cell cultures
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Vitaceae phytoalexins constitute a group of molecules belonging to the stilbene family which are derivatives of resveratrol structure. Due to its potential benefits on human health, resveratrol have become one of the most thoroughly studied molecules, that is why Vitis vinifera cell cultures have been used in several studies to investigate the factors involved in the induction and regulation of stilbene biosynthesis. Using cyclodextrins (CD) as elicitors on grapevine cell cultures, we have developed an innovative procedure where high levels of this metabolite is accumulated and easily recovered directly from the culture media without cell biomass destruction. Moreover, methyljasmonate (MeJA), salicylic acid and ethylene which are involved in defence responses, were added to cell suspensions resulting in modified resveratrol accumulation positively or negatively. The effects of the different elicitor treatments in resveratrol production were analysed reaching a maximum accumulation after 120 h (1400 μmol/gDW) when cells were simultaneously elicited with CD and MeJA. We also analysed the relationship between levels of resveratrol and the expression of related biosynthetic genes by performing real-time qRT-PCR of stilbene biosynthesis genes. 

Acknowledgements L.A. and S.B.N. hold grants from the Fundacion genex by performing real-time qRT-PCR of stilbene biosynthesis genes.

Abstracts

P07-017 Characterisation of forage sorghum lines with altered levels of prussic acid and investigation of key regulatory genes involved in cyanogenesis

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Forage sorghum is an important pasture grass with high growth rates, providing good feed stock and is drought and heat tolerant. Thus, it is widely grown in dry, tropical regions worldwide. Sorghum plants produce a stable, non-toxic tyrosine-derived cyanogenic glycoside compound known as dhurrin. Dhurrin is a natural defence product that liberates prussic acid (HCN) when the leaf tissue is consumed. Young plants or those experiencing abiotic stresses, particularly drought, can be highly toxic, accumulating dhurrin to high levels. This is a major problem for farmers as the sorghum crop may be the only available source of animal feed during times of drought. The aim of our research is to generate sorghum lines with altered levels of prussic acid. Sorghum seed has been treated with the mutagen EMS and M2 plants are currently being screened for changes in prussic acid levels. Selected individuals are being analysed using Targeted Induced Local Lesions in Genomes (TILLING) to identify induced point mutations in a number of key genes in the cyanogenesis pathway. Lines with reduced or negligible cyanide levels can be used for feed whilst lines with high levels of dhurrin may be used in soil biofumigation. We will also report on the impact that environmental variables such as, drought and nitrogen fertilizers, have on the regulation of prussic acid levels in different sorghum varieties.

P07-019 Study of genetic erosion during conservation process of pea (Pisum sativum L.) genetic resources by microsatellite markers

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Ex situ conservation of crop genetic resources is a key element in long-term conservation of genetic diversity. The aim of work is to determine the hazard of genetic erosion during the conservation and regeneration in ex-situ seed collections of genetic resources of pea (Pisum sativum L.) registered varieties. In presented study, five accessions of pea (Pisum sativum L.) registered varieties (Bohatýr, Raman, Klatovský zelený, Arvika, Viktoria 75) were studied. Two temporally different samples composed of 20 individual plants for each accession, spanning the period of about 40 to 10 years and the most recent ones, were investigated and compared for 10 microsatellite loci. The aim of the study was: (1) to determine the effect of genetic drift during the process of maintenance and regeneration of accessions in ex situ seed collections of genetic resources of pea, and (2) study of intra-accession variation, mutability and speed of evolution of repetitive (SSR) sequences. In case of three accessions (Bohatýr, Arvika, Raman), evidence of genetic drift and intra-accession variation were clearly detected. Selection of these data will be presented and discussed. This work was financially supported by Ministry of Education of Czech Republic research project MSM 2678424601.

P07-020 Biotechnology applied to plant conservation: Micropropagation and organogenesis in Crepis novoana an endangered Spanish species

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In vitro culture and plant biotechnology is currently used in the conservation of germplasm of species with high conservation value.
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Micropropagation and organogenesis protocols are necessary for the development of a good ex situ plant conservation programme. Citokinins alone or combined with auxines are very useful promoting the development of preformed buds as well as the formation of new shoots from leaf explants. Crepis noveana is an endengered species endemic to a small area in the NW Spain. Studies of micropropagation have been developed using benzilaminopurine (BAP) and kinetin as cytokinins (0.2 mg/L, 0.5 mg/L, 1 mg/L) or naphthaleneacetic acid (NAA) as auxine (0.1 mg/L). For micropropagation, excised shoots from in vitro germinated seeds were used. Leaf explants from in vitro grown plants were used for organogenic propagation with BAP (0.25 mg/L, 0.5 mg/L, 1 mg/L) ± NAA (0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L). Rooting of shoots was obtained by basal immersion of the regenerants in a NAA or IBA solution (1 g/L, 2 g/L) for 30 s. The best combination for micropropagation was BAP (1 mg/L) + NAA (0.1 mg/L) with 49.77 viable shoots developed in average. NAA was necessary for the induction of an organogenic response in leaf explants, obtaining 2.48 shoots per explant with BAP 0.3 + NAA 0.5. However, NAA didn't improve cytokine micropropagation rates. The response of shoots to NAA exposure on root development was the best with nearly 100% success.

P07-022 Molecular aspects of drought tolerance in five Genotypes of Brassica napus, evaluated with callus cultures understress

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Brassica napus is an important widely cultivated plant in Iran in order to provide crude oil for edible oil industry. An efficient plant regeneration protocol of the Brassica napus from cotyledon was developed. Excised cotyledon explants from in vitro seedling after 6 days of germination were cultured on three different media implemented with plant growth regulators (BAP, NAA, 2,4-D and Kinetine) containing 30 g/L sucrose, and the media were solidified with 8 g/L agar for callus induction. The results showed that the medium containing BAP, NAA and 2,4-D was the best, compared with the medium containing 2,4-D with kinetin and the one with 2,4-D alone. Calli obtained from five genotypes of Brassica (SLM 046, ARC5, Shir, Rain and Gernimo) were exposed to different osmotic stress intensities. Relative growth rate (RGR), and molecular changes in proline contents were determined against four different osmotic potentials (0, -0.78, -1.24 and -1.69 MP). All genotypes showed a decrease in RGR and a significant difference among genotypes was recorded. Also, accumulation of proline contents occurred in different genotypes which was more marked in Shir than other genotypes.

P07-023 Carotenogenic gene promoter activity in transgenic Arabidopsis

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Carotenoids are synthesized from the plastidic glyceradehyde-3-phosphate/pyruvate pathway of isoprenoid biosynthetic systems in plants. It has been suggested that their biosynthetic genes express differentially at plant tissues and the corresponding enzymes have usually transit peptide (TP) sequence for localization to plastids. We focused these merits of carotenogenic genes to develop useful tissue-specific promoter and TP sequence for plant biotechnology. As results, the promoter functions of two Arabidopsis genes encoding beta-carotene hydroxylase (AtBCH) and carotenoid cleavage dioxygenase (AtCCD) have been elucidated as constitutive and vascular-specific promoters through Arabidopsis transgenic approaches, respectively. In addition, a 5′-deletion promoter (~367 bp → -31) of known chromoplast-specific gene encoding Capsicum capsanthin-capsorubin synthase (CaCCS) induces the ubiquitous and constitutive GUS expression in leaf, stem, root, siliqua and flower when being compared with dual 3SS promoter as a control. The cis-acting motifs are also predicted for ubiquitously spatial specificity by scanning of PLACE database. Its inducible activities against biotic and abiotic stresses are being characterized.

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The department performs basic research in areas that provide new knowledge on the molecular mechanisms that control life functions.
of plants and their interaction with the environment. Key objectives are the development of more robust plants producing high yields in spite of adverse growth conditions and plants that offer healthy quality foods for mankind and improved feed for livestock. An important aspect is to obtain an understanding of how to make plants more resistant to plant diseases and insect attacks in a rapidly changing environment. The department also favours vertical research where the knowledge gained is used in collaboration with industry to facilitate the implementation of new approaches and processes in the agricultural sector and to develop products with improved characteristics.

The Department is organized in four sections: Plant Biochemistry Laboratory, Molecular Plant Biology Laboratory, Laboratory for Plant Anatomy and Physiology, and Section for Plant Pathology. Innovations include the identification and development of novel uses for plants for molecular farming, biomedicine and bio-energy production. The department contributes decisively to the Biology – Biotechnology Education of the University of Copenhagen based on our strong research profile and state-of-the-art technology platforms within biotechnology research. The department has a strong international profile and houses three basic research centres and a Danida centre.

P07-025 RCC1 regulator of chromatin condensation protein and its putative role in Thlaspi caerulescens zinc tolerance
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Plants have evolved ways to survive in various demanding environments such as metal contaminated wastelands. In plants, controlling gene expression is the most important regulatory system. Regulator of chromatin condensation (RCC1) is a eukaryotic protein that binds to nuclear Ran to dissociate Ran-bound GDP and uptake fresh GTP. It can also bind to DNA via protein-protein complex. These interactions probably play an important role in nuclear import and regulation of gene expression. Homologue of RCC1–family gene, TrRCC1, was found from the metal hyperaccumulator plant Thlaspi caerulescens exposed to zinc. Interestingly, the same gene was found also from birch under copper exposure. Molecular function of RCC1 is unknown, however. Expression profiling of Arabidopsis in Genevestigator shows that RCC1 expression is elevated in flowers and senescent leaves. It also has an increased expression during senescence, anoxia and oxidative, osmotic and salt stresses. T-DNA mutants have been generated but they show no phenotype (Alonso et al. 2003). To study the role of TrRCC1, it will be transferred into yeast for yeast complementation analysis. Arabidopsis T-DNA mutants are available and their metal tolerance will be characterized. Quantitative PCR on different Thlaspi populations and zinc concentrations show that RCC1 is most highly expressed in the metal accumulator population Ganges and expression is higher in roots than in shoots.

P07-026 Induction of resistance to insect pests in pea plants
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Integrated plant protection is a modern trend against pest and diseases, and includes also creation of resistant varieties by biotechnological tools, especially transgenesis. For production of pea plants resistant to insect pests the strategy of expression of an insect protease inhibitor driven by a constitutive promoter was used. The protease inhibitor GmSpi2 was isolated by Nirmala et al. (2001) from Galleria mellonella. A construct pWell09 containing 35S promoter, GmSpi2, and OCS terminator was prepared in the binary vector pGreenII (John Innes Center, UK). Pea plants were transformed by the in vivo transformation method (Švábová et al. 2005). To avoid difficulties with the localization of protein expression a functional fusion p35S::GmSpi2- GFP was prepared (pWell1). Transgenic lines have been prepared for testing of transgene expression and insect resistance. This work was supported by a grant of the Ministry of education CR 1M06030.

Švábová et al. (2005) Biol Plant 49: 361–370

P07-027 Gentica transformation of flax (Kinum usitatissimum L.) and stability of the transgene
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Genetic transformation of plants generally results in a large variation in the transgene expression between individual transformants. DNA sequences with high binding affinity for the nuclear matrix (nuclear matrix attachment regions or MAR) have been shown to improve transgene expression levels and its stability. The present study deals with production of transgenic flax plants and is first to investigate the influence of MAR sequences on transgene expression in flax. The transformed lines were obtained by inoculation with Agrobacterium and subsequent regeneration from hypocotyl segments. For the MAR-containing population of plants, we observed a significant reduction in the variation of GUS gene expression. Analyses performed on the second generation (T1) demonstrate that the presence of MAR sequences in T-DNA did not interfere with meiosis and segregation ratio for kanamycin resistance and GUS activity showed inheritance in a Mendelian fashion. The expression of the incorporated transgene was stable in offspring population. Our results indicate that exploitation of MAR sequences may be an important strategy for stabilising transgene expression in genetically engineered flax.

P07-028 MARs as a ‘booster’ of flax genetic transformation
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Abstracts
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Genetic transformation of plants generally results in a large variation in the transgene expression between individual transformants. DNA sequences with high binding affinity for the nuclear matrix (nuclear matrix attachment regions or MAR) have been shown to improve transgene expression levels and its stability. The present study deals with production of transgenic flax plants and is first to investigate the influence of MAR sequences on transgene expression in flax. The transformed lines were obtained by inoculation with Agrobacterium and subsequent regeneration from hypocotyl segments. For the MAR-containing population of plants, we observed a significant reduction in the variation of GUS gene expression. Analyses performed on the second generation (T1) demonstrate that the presence of MAR sequences in T-DNA did not interfere with meiosis and segregation ratio for kanamycin resistance and GUS activity showed inheritance in a Mendelian fashion. The expression of the incorporated transgene was stable in offspring population. Our results indicate that exploitation of MAR sequences may be an important strategy for stabilising transgene expression in genetically engineered flax.

P07-029 Removal of plant volatiles in greenhouse


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Detection of stress associated emissions of plants can be used for non-invasive diagnosis of plant status in greenhouse. The feasibility of volatile based stress detection is hampered by the lack of knowledge about loss processes for volatile organic compounds (VOCs) in greenhouse. Removal of VOCs is required for measurement of time dynamics but fast removal might prevent detection. Thus, knowledge of processes determining VOC losses in greenhouses is imperative. The effect of ventilation was estimated based on tracer gas measurements. Colorimetric tubes were used to estimate the effect of oxidants in the loss process. To determine losses due to plants, we evaporated monoterpenes, a sesquiterpene, (Z)-3-hexenol, and methyl salicylate (MeSA) inside greenhouse with and without plants. GC-MS was used to measure the loss after evaporation. Fitting exponential decay curves was then used to calculate time constants for evaporated compounds. Tracer gas experiments resulted in time constant of 100 min due to ventilation. The time constants for monoterpenes, the sesquiterpene, (Z)-3-hexenol, and methyl salicylate was then used to calculate time constants for evaporated compounds. The time constant of (Z)-3-hexenol was then at least 10 times smaller. Results indicate that ventilation attributes to a large extent in the removal of monoterpenes, the sesquiterpene and MeSA. For (Z)-3-hexenol also the presence of plants attribute in the loss. The low ozone concentration indicates a limited effect of gas-phase reactions inside greenhouse.

P07-030 Forage and turf improvement through biotechnology

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ViaLactia Biosciences (NZ) Ltd is a New Zealand based biotechnology company that has a number of plant science programmes. These programmes are mainly directed at pasture and turf improvement. Our science platform has used GeneThresher® technology and ryegrass SAGE® technology for transcriptome analysis. Functional testing of promoters, genes and regulatory elements identified by these technologies was then conducted in ryegrass and rice. The genomic resource created using GeneThresher® technology consists of ~166 Mb of hypo-methylated sequence that assembles into 80 162 contigs and 18 969 singletons and is available to the international research community through the Gramene database. It is estimated that the ~25 000 genes, which ViaLactia has access to make up three quarters of the coding genes available in the perennial ryegrass genome. In order to understand the seasonal changes in gene expression the SAGE®™ technology used perennial ryegrass tissues sourced from active New Zealand pastures during autumn, winter, spring and summer seasons. Analysis of SAGE®™ tags revealed season-specific expression profiles for numerous genes that are likely to be involved in stress-tolerance and plant-growth and development. From these technology platforms we have developed forage and turf molecular marker-assisted breeding tools and identified an array of promoters and genes targeting drought, saline resistance and increased biomass.

P07-031 Phenotypic differences in transgenic tobacco plants expressing cDNA CYP11A1 mammalian P450scc

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The main difference between steroidogenic systems in plants and animals is the absence of cytochrome P450scc in plants. This enzyme catalyzes the conversion of cholesterol into pregnenolone – the precursor of all steroid hormones in animals. This process occurs only in animal mitochondria and proceeds with participation of the other two proteins of electron transfer chain – adrenodoxin and adrenodoxin reductase. The homologues of these proteins are also found in plant mitochondria. In order to study the synthesis of mammalian hormones and its possible influence on the plants phenotype the transgenic tobacco plants carrying cDNA of CYP11A1 gene encoding P450scc have been created. The plant expression vector was constructed by inserting the cDNA CYP11A1 into the binary vector pGreen0229 under the control of CaMV 35S promoter. The pregnenolone in the steroid fraction has been identified by gas chromatography mass spectrometry method. Its further conversion into progesterone was verified using the enzyme immunoassay method. The flowering time of the transgenic plant was about 2 weeks earlier than that of the non-transgenic plant. The protein and carbohydrates contents in leaves and seeds of transgenic plants exceed noticeably those in control plants. The results obtained indicate that CYP11A1 expression in transgenic tobacco plants makes considerable alterations in their regulatory system and it is accompanied by changes in phenotype.

P07-032 Molecular imaging for plant physiology: visualization physiological functions of photosynthesis and dynamics of competitive elements in intact plant

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We have developed two imaging systems for plant science research, which images biological processes in living systems noninvasively, quantitatively, and repetitively. One is the positron-emitting tracer imaging system (PETIS), which image a tracer dynamics of nutrients and pollutants in an intact plant. In addition, for the numerical analysis of plant physiological functions, tracer kinetics have analyzed with simplified physiological model of test plants. In this presentation, photosynthesis responsiveness to temperature were analyzed with a method of photosynthesis imaging using pixel-by-pixel compartmental analysis. On the other hand, we have been developing prototype of the multi-element imaging system for plant study using SiCdTe semiconductor detector, which has high-energy resolution and low noise-level analog ASIC. This system adopts a compact camera method as a new imaging instrument. The presented imaging methods will yield plant molecular imaging, which visualizes dynamics of some competitive elements in intact plant, non-invasively and quantitatively.

P07-033 Insights into subunit interactions in the heterotetrameric structure of Potato ADP-glucose pyrophosphorylase

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ADP-glucose pyrophosphorylase, a key enzyme involved starch biosynthesis in higher plant, is composed of pairs of large (LS) and small subunits (SS). We have identified critical amino acid residues of potato LS AGPase that interacts with potato SS AGPase in native structure taking both computational approaches and yeast two hybrid. Our results indicated that R28, R78, I321, T312, P310, I322, and I323 of LS directly participates interaction with SS. First we modeled LS of AGPase and then construct 3-D of heterotetrameric structure. Then, a list of important amino acids that play critical roles in the LS and SS interactions were identified by MM-PBSA method with combination of the molecular dynamics. Residues were converted into either Ser or Ala by site directed mutagenesis and tested with yeast two hybrid. Finally we have proposed the order of assembly for the formation of heterotetrameric structure by computational means.

P07-034 Organ-specific expression of Brassica promoters in transgenic Arabidopsis


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Korea Brassica Genome Project (KBGR) has been initiated since 2004. Using 100,000 EST information obtained from 20 different cDNA libraries by this project, we selected organ-specific candidate genes to find new and useful organ-specific promoters to be used in plant biotechnology. Their endogenous gene expressions were examined through RT-PCR with RNAs prepared from various tissues of Brassica rapa (B. rapa). About 2.0kb regions in the 5'-upstream of them were isolated by PCR from corresponding BAC clones of B. rapa. They were linked to the green fluorescent protein (GFP) and beta-glucuronidase (GUS) reporter genes into the Gateway binary vector pBGWFS7 and introduced into Arabidopsis via Agrobacterium tumefaciens mediated transformation. Expression of GFP and GUS activities are being analyzed at several organs including leaf, stem, root, flower and seed of transgenic Arabidopsis. Currently, some ubiquitous and flower-specific promoters are investigated in detail and their activities will be presented to compare with that of 35S promoter in the gene expression levels as well as histochemical and fluorometric GUS activities.

P07-035 Efficient transformation method of soybean using meristematic tissues of germinating seeds

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Efficient transformation method for soybean [Glycine max (L.) Merr.] using meristematic tissues of germinating seeds has been established. The embryonic tips were excised from 3 days germinating seeds. They were inoculated with Agrobacterium tumefaciens strain LBA4404 harboring a binary vector with the bar gene as the selectable marker gene and the GUSINT report gene, and then co-cultured for 7 days on CCM media. The meristematic tissues were cultured on SIMP6 medium supplemented with 0.4 mg/L BAP and 0.1 mg/L IBA in the presence of 6 mg/L phosphonitric (PPT) for 2 weeks and surviving explants were transferred to SEMP6 medium. Transformation efficiencies ranged from 1.2 to 3.5% and confirmed by Southern blot analysis. This method with refined co-cultivation conditions, tissue culture, and rooting medium is rapid, reproducible, and easily applicable to recalcitrant Korean soybean cultivars. (Supported by RDA Biogreen 21 and NICS grant).

P07-036 Joint expression of sncRNAs increases synthesis of target protein in plants

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The plant viral vectors became an efficient tool for transient expression of foreign proteins in plants. Viral infectious copy containing gene of interest replicates efficiently and provides target protein production in significant quantities. However, in many cases silencing of viral RNA is a serious obstacle for long-term stable gene expression. To prevent RNA degradation in transient systems, suppressors of RNA silencing are usually used. We developed an alternative system which allowed to control viral reproduction in plant cell without silencing suppressors. It comprises Agrobacterium-mediated delivery of viral vectors into cell and induction of artificial short non-coding RNAs (sncRNA) synthesis for nucleocytoplasmic transport modification. Thus we protected viral vectors from cell control at the nuclear stage and decreased competition of viral RNAs with cellular mRNAs. Different 35S-based construct expressing sncRNAs including (GAAA) multiple tandem repeats, tRNA and U6 snRNA sequences were tested in co-agroinjection experiments with viral vectors based on Tobacco mosaic virus or Potato virus X. We have shown that in contrast to non-replicating matrices the reproduction level of viral vectors is significantly increased by sncRNAs. The effect of sncRNAs depends
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On their lengths; best results were achieved with 32-120 nt sequences. Efficiency of our approach was proved in systems expressing tuberculosis vaccine proteins and monoclonal antibodies in plants.

P07-037 Biochemical characterization and functional analysis of AtPAP15, a purple acid phosphatase with phytase activity in Arabidopsis
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Purple acid phosphatase catalyzes the hydrolysis of phosphate monoesters and anhydrides to phosphate within an acidic pH range. Among the twenty-nine PAP-like genes in Arabidopsis, AtPAP15 (At3g07130) displays higher amino acid homology with the well known phytase-GmPhy than other AtPAPs. In the present study, AtPAP15 was expressed in transgenic tobacco and purified by a three-step purification scheme. The final enzyme preparation exhibited a phytase specific activity of 10 U/mg protein with an overall recovery of 2.41%. AtPAP15 displayed optimal activity at pH 4.5 and exhibited wide substrate specificities. It was also inhibited by molydate, fluoride and phosphate, but was resistant to tartrate. With the production of transgenic Arabidopsis thaliana that contained AtPAP15 promoter:GUS fusion protein, expression of AtPAP15 was found developmentally and temporally regulated, with strong GUS staining at early seedling growth stage and late stages of pollen development. The expression is also organ/tissue-specific, with strong expressions in the vasculature, pollen grains and roots. AtPAP15 was proposed to function in mobilizing phosphorus reserve in seeds and pollens during seed and pollen germination. AtPAP15 T-DNA insertion lines showed lower pollen germination rate when compared to the wild type and its complementary line. In situ GUS assay showed that AtPAP15 expression was also responsive to ABA, SA, salt and osmotic stress in root tips.

P07-038 Transgenic crops with enhanced tolerance to multiple environmental stresses using an oxidative stress-inducible peroxidase promoter
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Oxidative stress derived from reactive oxygen species (ROS) is one of the major damaging factors in plants exposed to environmental stress. Chloroplasts are especially sensitive to damage by ROS, because electrons escaping from the photosynthetic electron transport system under the stress conditions are able to react with high concentration of molecular oxygen in chloroplast. To maintain the productivity of plants under the stress condition, it is possible to fortify the antioxidative mechanism in the chloroplasts by manipulating the antioxidant enzymes and small molecular antioxidants. The use of a stress-inducible promoter which provides more precise regulation of expression might be useful for the development of stress-tolerant plants. In our previous study, we isolated a strong oxidative stress-inducible peroxidase (SWPA2) promoter from cultured cells of sweetpotato. Recently we developed various transgenic crops such as sweetpotato, potato and tall fescue expressing genes of both Cu/Zn superoxide dismutase and ascorbate peroxidase in the chloroplasts under the control of SWPA2 promoter (referred to SSA plants). In addition, transgenic potato plants with the ability to synthesize glycinebetaine in chloroplasts via the introduction of the bacterial choline oxidase (codA) gene under the SWPA2 promoter (SC plants) were developed. In the presentation, characterization of transgenic plants will be introduced in term of the usefulness of a stress-inducible SWPA2 promoter.

P07-039 Transgenic crops with enhanced tolerance to multiple environmental stresses for sustainable agriculture
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The dramatic increase in population accompanied by rapid industrialization in developing countries has caused imbalances in the supply of food and energy. To cope with these global crises over food and energy supplies as well as environmental problems, it is urgently required to develop new crop varieties to be grown in marginal lands. Oxidative stress derived from reactive oxygen species (ROS) is one of the major factors causing injury to plants exposed to environmental stress. In order to develop various transgenic plants with an enhanced tolerance to multiple environmental stresses, we are focusing on the manipulation of antioxidant genes in plant cells. Recently we developed several transgenic crops such as sweetpotato, potato, tall fescue expressing genes of both Cu/Zn superoxide dismutase (CuZnSOD) and ascorbate peroxidase (APX) in the chloroplasts under the control of an oxidative stress-inducible peroxidase (SWPA2) promoter (referred to SSA plants). Transgenic crops expressing nucleoside diphosphate kinase 2 (NDPK2) gene in cytosols under SWPA2 promoter (SN plants) were also developed. In addition, transgenic potato plants with the ability to synthesize glycinebetaine in chloroplasts under the SWPA2 promoter (SC plants) were developed via the introduction of the bacterial choline oxidase (codA) gene. In the presentation, characterization of transgenic plants will be introduced in term of enhanced tolerance to multiple environmental stresses in detail.

P07-040 dsRNAi-mediated resistance to rice stripe virus in transgenic rice with RSV-CP gene
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Rice stripe virus is a viral disease seriously affecting rice production in East Asia, especially in China, Japan and Korea. The rice stripe virus (RSV) is transmitted by small brown planthopper (SBPH), Laodelphax striatellus. It was generally known that most japonica
varieties are susceptible to RSV whereas indica and upland varieties are highly resistant. We generated highly resistant transgenic japonica rice plants to RSV using an dsRNAi construct to silence the sequence region of the RSV-CP gene. Eighteen transgenic T1 lines were obtained, and confirmed the stable integration of foreign RSV-CP gene by southern blot analysis. Transgenic plants were inoculated with a population of viruliferous SBPH, and their resistance was evaluated by ELISA test and infection rate. Nine independent lines out of eighteen lines showed high resistance to RSV when inoculated with the SBPH at an early stage of plant development. These plants will be used for further analysis of small RNA detection and stable transmission of the trait of the resistance to the next generation. (Supported by RDA Biogreen 21 and NICS grant).

P07-041 Cryopreservation of endangered species germplasm: somatic embryogenesis and embryogenic callus cryopreservation of Centaurea uteiae
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Biotechnological techniques are a useful tool to preserve the germplasm of threatened plant species. Somatic embryogenesis is a useful pathway for the ex situ conservation. In our study we induced somatic embryogenesis from zygotic embryos of Centaurea uteiae Silva Pando, an endanger endemism from the NW Spain with a low seed germination. Three concentrations of 2, 4 D (0.5 mg/l, 1 mg/l, 5 mg/l) plus BAP (0.5 mg/l) or TDZ (0.1 mg/l) were used to induce somatic embryogenesis of C. uteiae. Cryopreservation of regenerative embryogenic callus through vitrification in PVS2 was also checked. Five desiccation media were tested employing 0.3 M sucrose or 0.6 M glycerol supplied with or without 20 μM ABA. A combination of 0.25 M sucrose plus 0.25 M glycerol plus 10 mM ABA was also checked. Samples were pretreated with a loading solution consisted of 2 M glycerol and 0.4 M sucrose before their exposure to PVS2. Indirect somatic embryogenesis was obtained once transferred to expression medium. Cellular groups were isolated through suberification of peripheral walls developing proembryonal masses. Half development time of somatic embryos was similar in all media with a positive response, about 10–12 weeks. Nearly 100% of the plantlets survived when were transferred to soil. The presence of sucrose in the pre-culture medium plus a treatment with a loading solution prior to PVS2 exposure was shown as indispensable to recover 40% of embryogenic callus after cryopreservation.

P07-042 Production of candidate vaccine against edema disease in plant cells
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Edema disease (ED) occurs mostly in baby pigs, caused by Shiga toxin 2e (Stx2e) producing Escherichia coli and the lethality is as high as 50–90%. Because antibiotic administration has a risk of emergence of the drug-resistant strains, alternative methods including administration of a vaccine against ED are to be developed urgently.

Using plant factory (PF) is beginning to be one of the choices for the cultivation of food crops such as lettuce and tomato in Japan. PF is also suitable for the cultivation of GM plants, because it is possible to grow plants under tight controls, environmental control for the plant growth and safety control of them. Our research scheme is to grow transgenic plants of lettuce (Lactuca sativa) producing Stx2eB as an edible vaccine against ED in PF. In plant cells, the localization of a recombinant protein can be regulated by the translational fusion of localization signal peptide. Such signals generally affect an accumulation level of a target protein, too. We first confirmed that known signals for the localization to ER, apoplast, vacuole and chloroplast are functional in lettuce protoplasts by transient expression assay using GFP as a reporter. Next, the stx2eb gene fused with each signal was expressed in lettuce protoplasts. ER type construct attained the highest accumulation level. The ER type Stx2eB was successfully produced in stably-transformed cultured tobacco cells, too.

P07-043 Cryopreservation by encapsulation of Gentiana spp. cell suspensions maintains re-growth, embryogenic competence and DNA content
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A reliable technique for cryopreservation by encapsulation was developed for two suspension cultures of gentian species: Gentiana tibetica and G. cruciata of different ages and embryogenic potential. The effect of water content, aggregate size and the subculture time on viability was determined by the 2,3,5-triphenyltetrazolium chloride (TTC) test. Re-growth of a proembryogenic mass (PEM) on agar, liquid or agar/liquid media was assayed by measuring the increase in biomass. A water content of 24–30% (fresh weight basis) after 5–6 h dehydration of encapsulated cells of gentians yielded the highest survival (68% for G. tibetica and 83% for G. cruciata) after cryopreservation. Regardless of species, aggregate size and subculture time, the lowest PEM survival was 44%. The effect of water content, aggregate size and subculture time on the survival of G. tibetica PEM, but the survival of that of G. cruciata was higher when the smaller aggregates were cryopreserved on the 5th day of culture. Agar/liquid culture caused the greatest biomass increase. Cryopreservation did not affect the characteristics of suspension cultures and their re-growth after thawing, nor the number and dynamics of somatic embryos formed. Flow cytometry showed that cryopreservation did not change the genome size of the PEMs or regenerants.

P07-044 Property of fruit-specific 2A11 promoter from tomato and its effect on anti-sense transformation of V-ATPase
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To generate genetically modified crops, promoters which can induce target genes in proper tissues and timing are necessary. 2A11 gene is isolated from tomato fruits at red-ripe stage and its promoter has been used for fruit-specific gene expression. However, tissue specificity and timing of 2A11 expression had not been detailed. In this study, 2A11::GUS was transformed to tomato ‘MicroTom’ and GUS staining depending on 2A11 promoter was determined. GUS staining was observed in fruits at 0, 10, 20, 30, 40 and 50 days after flowering (DAF), but not in young leaf, mature leaf, stem and flower. Strong staining was observed in all parts of fruits at 20 and 30 DAF. In fruits at 40 and 50 DAF, staining was weaker in flesh, while strong in vascular tissue and seeds. V-ATPase is a proton pump on vacuolar membrane and considered to be important for fruit development. To confirm the importance of V-ATPase in fruit, V-ATPase suppressed tomato plants were generated using 2A11 promoter. The plants set smaller fruits without seed. Our results show not only the importance of V-ATPase in fruit but also the availability of 2A11 promoter for fruit-specific gene expression.

P07-045 Transformation of Kalanchoe with rol-genes of Agrobacterium rhizogenes changes plant morphology, resource allocation and improves ethylene tolerance
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In Kalanchoe blossfeldiana, root inducing (Ri-)lines were regenerated from hairy roots produced by inoculating leaf explants with an Agrobacterium rhizogenes wild-type strain. Transformants displayed alterations in plant morphology and resource allocation. Several lines were characterized and numerous morphological traits were affected. Compared to control plants, time to anthesis was unchanged in one Ri-line, but delayed in the other lines. In the transformants, an altered allocation of dry matter was evident, where the majority of dry matter was allocated into leaves and secondly into flowers. A higher amount of dry matter was allocated into the main shoot of Ri-lines relative to control plants. Apart from changes in morphology, the transformants exhibited distinctly better postharvest quality. Longevity and ethylene sensitivity offlowered varied among control, chemically growth retarded plants and Ri-lines. In response to ethylene exposure, the flowers of the plants transformed with rol-genes exhibited tolerance, while chemically growth regulated and control plants were sensitive. The improved postharvest performance of plants transformed with rol-genes can be assumed to be due to changes in hormone balance or an alteration in source-sink relations. A compact plant without delayed flowering and improved postharvest performance is valuable for future breeding programmes. Further research may explore the reasons for the improved ethylene tolerance of the transformants.

P07-046 Phylogenetic analysis of root endophytes along a primary succession gradient on a mid-boreal island
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A total of 38 endophytic strains were isolated from surface-sterilized, Empetrum nigrum (Empetraceae), Vaccinium vitis-idaea (Ericaceae) and the graminoid Deschampsia flexuosa (Poaceae) of a geographically isolated island area, Hailuoto. The goal of the current study was to identify the root endophytic fungi from a primary successional gradient by a sequence-based approach. Internal transcribed spacer region (ITS) was amplified and sequenced to identify the root endophytes (EU314675 – EU314712). Out of the 38 isolates, 27 were found to belong to Phialocephala fortinii, three to Mollisia minutula, four to Phialophora sp., one Ascomycetes sp. and three unidentified endophyte species according to the BLAST searches in the Genbank database. All the sequences were aligned using Clustal X and phylogenetic analysis was done with MEGA4 software. The phylogram of the 38 root endophytic fungi spread into five clades, irrespective of the sites and hosts from which they had been isolated. The strains originating from the early successional stage, the seashore dune ridge, seemed to host a distinct fungal taxon from all other stages of succession. Our results also suggest that roots of the ericaceous plants and grasses are colonized by the same endophytic fungi in this ecosystem.

P07-047 Manipulation of the composition of wheat endosperm cell walls to improve nutritional properties
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The cell walls of cereal endosperms are major components of the dietary fibre consumed by humans. In wheat they account for 4–9% of the whole grain and comprise principally arabinoxylan (70%) and 20% of (1–3)(1–4)-β-glucan and smaller amounts of glucomannan and cellulose. We aim to manipulate the fibre composition to improve its contribution to colon function and reduction in serum cholesterol. A transgenic approach is used to manipulate the expression of selected enzymes in the pathway of β-D-glucan biosynthesis and to determine the impact of these modifications on the composition and properties of the cell wall. Bioinformatics was used to identify rice homologues of Arabidopsis genes and corresponding ESTs of wheat. Sequences encoding putative glucan synthases were identified and wheat cDNA clones for candidate genes isolated using RT-PCR. These cDNA sequences were used to design vectors for overexpression and RNAi constructs for gene knock-out with biosciotics into the wheat variety Cadenza. T1 embryos of transgenic lines were rescued and segregation ratios of transgenes determined. Changes in cell wall polysaccharide composition resulting from down-regulation of genes, caused by RNA interference in the transgenic lines, were investigated. Enzymatic fingerprinting, a technique developed and used at the INRA laboratory, were used to analyse cell wall composition of the transgenic and control lines.

P07-048 Cloning of transgenic BY-2 cell lines – a simple method to reduce heterogeneity of transgene expression
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Characterization of transgenic cell lines often used in plant biology studies is complicated, since transgene expression is not usually well-balanced and stable in all the cells.

In our study, tobacco (*Nicotiana tabacum*) BY-2 cell line was transformed via *Agrobacterium tumefaciens* with a T-DNA containing green fluorescent protein (GFP) gene. Homogeneity of GFP fluorescence was studied in calli and individual suspension cells and their clones, prepared by a new simple cloning method introduced in this study.

GFP fluorescence was uniform in 35–50% of primary calli obtained after the transformation; cell populations with evidently different GFP levels were present in the majority of the primary calli in either sector or mosaic arrangement. Moreover, significant portion of suspension cultures (about 30%) derived from the seemingly homogenous calli also consisted of cells with different GFP fluorescence. Cloning of these heterogeneous suspension cultures resulted in secondary calli with predominantly uniform GFP expression on the callus level, however, on the cellular level (in suspension) the subclones of some lines remained heterogeneous. Neither secondary nor tertiary cloning gave rise to solely homogenous suspensions.

The introduced cloning procedure appeared as a suitable tool to increase a number of lines with homogenous GFP expression and it might be applicable even for other purposes.

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**P07-049 Scale-up and production of berry phenolics in arctic bramble suspension culture**


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Arctic bramble (*Rubus arcticus*) is a Nordic berry well known for good taste and flavour. In addition, the berries are rich in phenolic compounds with beneficial biological properties for human health. The demand of the berry is strong, but the crop of both wild and cultivated arctic bramble is very low. Therefore cell cultures of this plant are the potential choice for production of already characterized and novel secondary metabolites for food and pharmaceutical purposes.

Callus of arctic bramble was established from cuts of sterile in vitro leaves with plant hormones kinetin and NAA (α-naphtalen-acetic acid), and selected callus lines were maintained and used for initiation of suspension cultures. Growth characteristics were measured and cultivation conditions optimised for stable suspension culture line. Scale-up was performed well from 30 ml culture volume in shake flask to 1 l culture in Wave bioreactor. Non-elicitated and elicitated arctic bramble suspension cultures in Wave bioreactor were used in production of phenolic secondary metabolites. S-ABA (trans-abscisic acid) was used as the elicitor, and samples from both cultures were collected at different time points. The freeze-dried methanol extracts of cell mass were analysed by HPLC-DAD/ESI+/MS. The berry phenolics identified in both cultures were kaempferol, ferulic acid and sinapic acid, and kaempferol hexoside was detected only in elicited culture.

**P07-050 Enhanced tolerance to various abiotic stresses in transgenic rice by expressing PsAPX gene**

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Ascorbate peroxidases (APX) plays an important role for scavenging ROS by degrading H2O2 in plants. Transformation of genes corresponding to specific enzymes of the ROS-scavenging system can lead to the development of transgenic plants with enhanced oxidative stress tolerance. Transgenic rice (*Oryza sativa* L.) expressing pea ascorbate gene (*PsAPX*) in chloroplasts under the control of the oxidative stress-inducible promoter, sweet potato peroxidase anionic 2 (SWPA2) by *Agrobacterium*-mediated genetic transformation, and confirmed by Southern blot analysis. Transgenic plants were subjected to MV, drought, UV-B and ozone stress, and their tolerance was evaluated. High levels of *PsAPX* gene transcripts in the transgenic plants under stress conditions suggested that the *swpaPsAPX* gene was functionally induced by treatment with various abiotic stresses. Compared to control plants, significantly less ROS were generated in the leaves of transgenic plants exposed to abiotic stresses, resulting in decreased phenotypic damage, ion leakage, and chlorophyll degradation. Our results suggest that these transgenic lines can provide improved tolerance to various abiotic stresses. (Supported by RDA Biogreen 21 and NICS grant).

**P07-051 Usage of scalar and vectorial grayscale based invariant features for localization and classification of cell cycle events in Arabidopsis RAM**


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Details of cell cycle regulation are being uncovered in the case of model systems like suspension cells, but less progress are made in unraveling how these molecular events regulate growth processes at the whole organism level. The main problem is the absence of a set of analytical tools that are powerful enough to determine cell cycle events in whole organisms. Appropriate methodology has been pioneered in the last century and is now defined as ‘kinematic analysis’. This analysis requires an accurate and predictive model of the distribution of cell cycle events through the whole plant (in 4-D) and generation of mathematical models which will allow to predict and describe cell behavior in each cell file, including both generation of new cells, differentiation and cell maturation. Here we developed a 4-D model, which shows dynamics of S and M-phase of cell cycle. Detailed analysis of the architecture of the RAM revealed a number of important observations which were integrated in the model development. We show that the mitotic index of the epidermal cell file is significantly lower in comparison with other cell files. The data analysis showed a significant variation of nucleolus volume among different cell files in the RAM, thus we propose to use the size of the nucleolus as a novel marker to distinguish different cell files. The proposed model allows to correctly quantify cell cycle events in the RAM and demonstrates changes in nucleolus sizes during cell maturation.

**P07-052 Field experiment with transgenic non-flowering birches**

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Non-flowering birches (*Betula* spp.) demonstrate changes in nucleolus sizes during cell maturation.
Abstracts

In July 2005, we established a field experiment of non-flowering transgenic birches. Cytotoxic barnase gene with an inflorescence specific promoter was introduced to an early flowering wild type of German origin, which flowers already at the age of 3–4 months instead of usual 5–10 years. The experiment consists of three transgenic lines, wild type and half-sib native seedlings. Set up is randomized complete block design (12 blocks, 8 plants/line within each block). All wild type plants were flowering already in the summer 2006, whereas none of the transgenic plants have produced flowers so far. Growth, architecture and field damage have been monitored for 3 years. Condensed tannins, lignin and chlorophyll content were measured and small molecular phenolic compounds were analyzed by LC-MSn. Insect feeding assays were made with generalist and specialist herbivore species, and their relative growth rates and feeding preferences were monitored. Transgenic birches were shorter, more branched, and had more spider nests than the wild type, but during the last year the differences seem to be diminishing. The leaves of transgenic lines contained more lignin, and less condensed tannins, and chlorophyll than those of the wild type, but differences were statistically significant only in few cases. The results from insect feeding assays differed among the species, and significant effects were seen only in few experiments.

P07-053 Bacteriaemia caused by agrobacterium tumefaciens does not lead to GFP gene expression in mouse organs
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In the last decades, tremendous success has been made in the area of genetic engineering of plants. T-DNA fragment of Agrobacterium tumefaciens Ti plasmid could be used for modified genes introduction into plants. Recently, cross-kingdom transformation capacity of agrobacteria was shown. Under laboratory conditions it turned out to extend T-DNA transfer to non-plant eukaryotic organisms (Kunik et al. 2001 Proc. Natl. Acad. Sci. USA 98, 1871-1876). On the other hand, the list of known medical cases of Agrobacterium species isolation from bloodstream infections is constantly enriched. These facts put forward the assessment of biosafety-related risks of wide exploitation of agrobacteria-related approaches. We studied A. tumefaciens Ti plasmid could be used for modified genes introduction into plants. Recently, cross-kingdom transformation capacity of agrobacteria was shown. Under laboratory conditions it turned out to extend T-DNA transfer to non-plant eukaryotic organisms (Kunik et al. 2001 Proc. Natl. Acad. Sci. USA 98, 1871-1876). With some embryo germination after 30 days of culture. For embryo germination cultures where transferred to germination medium in the presence of 10 μM NOA, 1 μM BA, 20 μM IAA and 0.25% activated charcoal, where some embryo germination was observed after 30 days of culture. For embryo germination cultures where transferred to germination medium in the presence of 10 μM IAA, 1 μM GA3 and 0.25% activated charcoal until conversion to plantlets.

P07-054 Somatic embryogenesis from anthers and ovaries of autochthonous grapevine (Vitis vinifera L.) cultivars from Galicia (north-western Spain)
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Somatic embryogenesis has raised great importance for grapevine improvement, being an efficient regeneration system with a number of applications in fields ranging from biotechnology to genomics. Our objective is to develop reliable protocols of somatic embryogenesis for autochthonous grapevine (V. vinifera L.) cultivars from Galicia, a north-western region of Spain with an old viticultural tradition. Immature inflorescences were collected from field-grown plants of six grapevine cultivars (Albariño, Treixadura, Torrontés, Mencia, Brancellao and Merenzao) over a 3-week period during April to May 2007. All basal media used were based on NN salts plus MS vitamins. Initiation of somatic embryogenesis was accomplished from anthers and ovaries using several growth regulator combinations. Efficiency of initiation varied in relation to the collection date in all cultivars except for Albariño, which remained more constant. The combination of 4.5 μM 2,4-D and 8.9 μM BA allowed to obtain the highest embryogenic response, which was further improved by using casein hydrolysate. Embryos and/or embryogenic callus were transferred to proliferation medium in the presence of 10 μM NOA, 1 μM BA, 20 μM IAA and 0.25% activated charcoal, where some embryo germination was observed after 30 days of culture. For embryo germination cultures where transferred to germination medium in the presence of 10 μM IAA, 1 μM GA3 and 0.25% activated charcoal until conversion to plantlets.

P07-055 Biotechnology of gentians: the induction of embryogenic potential, its cryopreservation and utilization in somatic cell genetic manipulation
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The aim of the presentation is to show the evidence of biotechnology definition requirements compliance by the systems of gentians in vitro cultures. Single cell plant regeneration, interspecies somatic cell hybridization and transformation meet meaning of the biotechnology. The background of all performed somatic cell manipulations is tremendous embryogenic potential of gentians expressed by easy, non-limited plant regeneration via somatic embryogenesis in liquid and agar cultures. The developed cryopreservation systems help to maintain this potential for unlimited period of time. Protoplasts of cell suspensions were cultured in three different ways. Direct and indirect somatic embryogenesis was observed and resulted in production of 2C, 4C and 6C plants. Green leaf mesophyll protoplast culture with the help of similar media appeared to be more complicated. However, the obtained plants were different in the chromosome number and total nuclear DNA content, but did not pass 6C.

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Electrofusion of green leaf and cell suspension protoplasts helped to obtain the following somatic hybrids: \(G. \textit{kumoo} \ (2n = 26) + G. \textit{cruciata} \ (2n = 52)\) and \(G. \textit{cruciata} \ (2n = 56) + G. \textit{tibetica} \ (2n = 56)\). All obtained hybrids were different in appearance and possessed various chromosome numbers. In transformation experiments of leaf explants and cell aggregates among 10 studied constructs only one appeared successful. Transient expression and regeneration parameters on selection media was proved.

**P07-056 Production of secondary metabolites and extracellular proteins in \textit{Lycopersicon esculentum} elicited cell lines**

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The use of plant cell cultures for the production of secondary metabolites has limited commercial success due to the empirical nature of selecting high-yielding cell lines, the instability of cell cultures and the lack of knowledge about how these metabolites are synthesized or their synthesis is regulated. In order to improve this production, elicitation is the most useful biotechnological strategy. Bru and Pedreno (2003) have developed an innovative procedure to produce high levels of resveratrol with cyclodextrins (CDs) in grapevine cell cultures. Likewise, the addition of both cyclodextrin and methyljasmonate (MeJA) increased even more resveratrol production (Pedreno et al. 2008). For this reason, the main objective of this study was to extrapolate these strategies on secondary metabolite production by elicitation of \textit{Lycopersicon esculentum} cell lines using a combination of both MeJA and CDs. Elicited culture media were routinely used for extraction and identification of these metabolites by HPLC/MS and GC/MS. At the same time, a comparative analysis of the extracellular proteome has been carried out. Bru R, Pedreno MA 2003. PCT patent WO 03/062406. Pedreno MA, Almagro L, Belchí-Navarro S and Bru R 2008. ES patent 200800591 L.A. and S.B.N. hold grants from the Fundacion Seneca. This work has been supported by the MEC (BIO2005-00332) and by the CARM (BIO-BVA 07/01-0003).

**P07-057 Transplastomic tobacco plants overexpressing maize transglutaminase: modifications of the thylakoid appression pattern and photochemistry parameters**

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Looking at the functional role of transglutaminase (TGase) in plants, transplastomic tobacco chloroplasts over-expressing maize TGase were obtained from the cDNA of the first plant TGase cloned (tgz1). The TGZ13 over-expressed protein was immunodetected principally in chloroplant inclusion bodies. Compared to wt, thylakoid appression was greater in the youngest transformed leaves, and thylakoid membrane connection was less apparent or disappeared with leaf aging. A decrease in pigment content, and an increase in cell damage with increasing leaf age was observed. Chlorophyll fluorescence parameters, potential (Fo/Fm), effective quantum yield of PSII (O2PSII), number of open PSII centres (qP) and intrinsic efficiency of open PSII centres (Fv/Fm) were lower in the transformed plants. In contrast, non-photochemical quenching (NPQ) was higher in transgenic than in wt plants, indicating that the increase in thylakoid stacking may induce a higher photoprotective response by the xanthophyll cycle. The increase in bound polyamine (PA) content and TGase activity in transformed plants also account for the involvement of PA in the protection of the photosynthetic apparatus (2). The over-expression of TGase seems to disturb the structure of the antenna proteins affecting the regulation of thylakoid stacking via PA conjugation to these photosystem proteins, inducing abnormal grana structure, cell damage and increased participation of photoprotective mechanisms.

**P07-058 Heavy metal resistance of poplar plants transformed with a MRP-type ABC transporter of budding yeast**

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**P07-059 Ecotopic expression of BrD1, a plant defensin from Brassica rapa, improves the resistance to the brown planthopper in rice**

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Multidrug resistance-associated proteins (MRPs) play critical roles in drug resistance and detoxification process of a wide range of organisms. A member of yeast MRP subfamily, Yeast cadmium factor YCF1, is important for detoxification of cadmium and lead. We previously reported that YCF1-transgenic Arabidopsis plants are improved in cadmium and lead resistance. YHL035C has the highest homology to YCF1 in Saccharomyces cerevisiae. We expressed this gene in poplar tree to test whether this transporter could function in plant system and enhance the plant’s resistance to heavy metals. YHL035C-overexpressing poplars grew better than wild type in medium containing lead. We will report results that suggest a high potential for the YHL035C-transgenic plants for phytoremediation of heavy metals.
Abstracts

Plant defensins are small basic peptides of 5–10 kDa and thought to be an important component of plant defense against fungal and/or bacterial pathogens. However, very little is known about their modes of action and biological roles in insect resistance. In order to understand the role of plant defensins against insect herbivores, we isolated the BrD1 from Brassica rapa and expressed in rice (Oryza sativa L.). Insect bioassay and feeding studies with the transgenic rice plant resulted that BrD1 decreased survival, overall fecundity and deterrent feeding effect on brown planthopper (Nilaparata lugens). Sequence comparison of the encoded protein using BLAST analysis revealed significant homology to defensins from other plant species. The deduced amino acid sequence of BrD1 contains an endoplasmic reticulum signal sequence of 30 amino acids and a standard defensin domain of 50 amino-acid residues, but does not contain an unusual C-terminal domain. BrD1 seemed to belong to a different subgroup from other plant defensins based on the phylogenetic tree analysis carried out with 80 amino acid sequences of premature plant defensins. These results suggest that BrD1 has a different role with other plant defensins. Our results showed that BrD1 exhibit insecticidal activity and can be used for developing cereal crop plants with resistance to sap-sucking insects such as brown planthopper. (Supported by RDA Biogreen 21 and NICS grant).

P07-061 Effect of exogenous abscisic acid on stomatal characteristics during acclimation of in vitro-grown tobacco (Nicotiana tabacum L.) plants

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In vitro-grown plants developed specific anatomical structures as well as physiological activities due to the specific environment where they are growing. After ex vitro transfer, plants had to overcome the ‘transplantation shock’ and, for survival, they had to acclimate to the new non-sterile environment with lower relative air humidity, higher irradiance, changed carbon dioxide concentration, a substrate without sucrose, etc. This step used to be a critical one in micropropagation. With the aim to ameliorate acclimation of the plants, abscisic acid (ABA) which is known to close stomata, was used for decreasing water loss of the plants. ABA was applied into the substrate immediately after ex vitro transfer. Stomatal density and sizes were determined on replicas of both leaf sides. Ten images from each side were captured from light microscope with a digital camera and M.I.S. Quickphoto. After three weeks of acclimation with ABA, an enhancement of stomatal densities on the adaxial and abaxial leaf sides was found. Furthermore, the ratio of stomatal density on the upper to the lower leaf side was increased as well. Stomatal width and length showed only small differences between control plants and ABA-treated ones. Supported by the Grant Agency of the Czech Republic. (Project No. 522/07/0227).

P07-062 Generation of novel mosaic flower color in transgenic Petunia plants by transforming snapdragon yellow pigment genes

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Petunia is one of the most popular garden flowers. To breed novel flower color in Petunia, we have isolated two yellow pigment genes from yellow snapdragon flowers by RT-PCR technique: AmAS1 (Antirrhinum majus aureusidin synthase; key enzyme of aurone biosynthesis from chalcones) and Am4’CGT genes (A. majus chalcone 4’-O-glucosyltransferase; essential for aurone biosynthesis and yellow coloration in vivo). Subsequently, AmAS1 and Am4’CGT were cloned into plant expression Gateway vectors, and then transferred into Petunia by Agrobacterium-mediated method. Conditions for callus induction, plant regeneration, and dosage of antibiotics from different tissues of in vitro Petunia plantlets were determined. Insertion of foreign gene into plant genome was checked by PCR analysis and then confirmed by T0 progeny assay. In AmAS1 transgenic Petunia plants, some of the flowers showed part of white color in petal during the late stage of flowering. However, in Am4’CGT transgenic Petunia plants, petal color was changed from all purple to getting part of white and finally became whole white flower during the senescence. These results suggested that two genes may express in transgenic Petunia plants and alter the flower color. Mechanisms of altering flower color in transgenic Petunia plants are under investigation.
P07-063 Cryotreatment effects on morphogenic potential of Gentiana kurroo (Royle) cell suspension culture
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The aim of the presentation is to show the effect of the cryotreatment on somatic embryogenesis of cell suspension and plant regeneration in post-thawing culture. Experiments were carried out on embryogenic cell suspensions of Gentiana kurroo being in various age. One and 2 and half year old embryogenic hypocotyl callus derived cell suspension was used in the experiments. After 1 year of culture samples of suspension were frozen with the help of LN for 18 months long period. Alternatively 48 h LN cell suspension treatment was employed. For both experimental and control culture combinations only cell suspension aggregates closed in alginate beads were used. After NL treatment in post-thawing culture beads were transferred to agar medium at 14 days and later into liquid medium. After 3–4 weeks new cell suspension were formed. Cell aggregates from frozen and non-frozen culture were transferred on agar regeneration medium MS + 0.5 mg/l GA3 + 1.0 mg/l Kin + 80.0 mg/l adenine sulfate, to complete somatic embryogenesis. Later, obtained somatic embryos were transferred to hormone-free 0.5MS medium to achieve plantlets. The quality and uniformity of regenerants to initial plant material were assessed with application of the following methods: flow cytometry, chromosome counting and DNA molecular analysis.

P07-064 Searching mechanisms related to metal accumulation and tolerance in metal hyperaccumulator plant Thlaspi caerulescens
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Plant metal homeostasis has been under enthusiastic research during the recent years. Reason for this is the possible application of the mechanisms in improving the micronutrient content of food crops and in cleaning metal contaminated soils and waters. One of the most interesting plants used in metal studies is Thlaspi caerulescens, a close relative to Arabidopsis. Thlaspi can accumulate exceptionally high amounts of metals in its tissues without toxicity. Moreover it has several accessions which have variation in their metal tolerance and in accumulation capacities, which makes Thlaspi an attractive model. In spite of several advancements, knowledge of the molecular mechanisms related to metal homeostasis in plants still has many gaps. To characterize proteins underlying the enhanced metal uptake in Thlaspi, we have compared proteomes of metal-exposed Thlaspi accessions using two-dimensional electrophoresis (2-DE). Differences in the protein patterns were mainly seen between the accessions, whereas metal exposures played a minor role. The most interesting proteins were identified with the help of mass-spectrometric analysis and homology searches of databases. Function of the proteins are being studied further with Thlaspi crosses that have been phenotyped according to their metal accumulation and tolerance traits. Also Arabidopsis mutants for the selected genes are being characterized.

P07-065 PPO driven latex coagulation – new insights from the rubber-producing model plant Taraxacum kokssaghyz
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Due to an increasing demand for natural rubber new sources are needed. Beside the main commercial crop for caoutchouc Hevea brasiliensis, plants of many genera such as Ficus elastica, Parthenium argentatum, and Taraxacum kokssaghyz synthesize rubber within specialized cells. These cells – for some plants called laticifers – contain a milky cytoplasm, the latex where rubber appears as soluble particles. A major problem in rubber production is the latex coagulation upon harvesting, which acts as a limiting factor for rubber yield. Screening latex-producing plants we found a correlation between increased polyphenol oxidase (PPO) activity and the pace of latex coagulation. PPOs collectively refer to enzymes that catalyse the oxidation of diphenols to their respective quinones, which in turn spontaneously polymerize. This polymerization can be easily observed since the affected tissue turns brown. In our studies, latex producers with strong PPO activity simultaneously exhibit fast latex browning and coagulation. Therefore, we hypothesize that PPO activity is a critical factor for latex coagulation. Consistent with this idea, enhanced fluidity of latex of T. kokssaghyz could be achieved by inhibition of PPOs. We currently characterize PPO-knock down plants of T. kokssaghyz. We will report on the potential of PPO-knock down T. kokssaghyz plants as a new source for natural rubber due to enhanced latex fluidity and improved rubber extraction.

P07-066 In vitro protective activity of transformed root-derived benzy1- and 3-(methylthio)propyl isothiocyanates against cadmium oxidative toxicity
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Cadmium carcinogenicity is crucially mediated by the interference with the cellular antioxidant system. Several studies suggest an inverse association between human isothiocyanates intake and the risk of cancer. The aim of this work was to study the protective activity of transformed root-derived isothiocyanates by determination of some markers of pro- and antioxidant processes in Cd-stressed mouse fibroblasts WEHI 164. Benzyl isothiocyanate (BITC) and 3-(methylthio)propyl isothiocyanate (MtpITC) were extracted after endogenous hydrolysis of glucotropaeolin and glucobrassinin in homogenates of Tropaeolum majus and Arabis caucasica transformed roots, respectively. Oxidative toxicity protection was determined in the cells preincubated for 48 h with 300 ng/ml BITC or 700 ng/ml MtpITC (nontoxic conc. in MTT test) and then treated for 24 h with 1500 ng/ml Cd (IC50). Enhanced thioredoxin reductase, glutathione peroxidase and glutathione S-transferase activities in the cultures treated with BITC or with BITC and Cd in comparison to

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those treated with Cd alone suggested that hairy root-derived BITC could be a significant inducer of both antioxidant and xenobiotic detoxification processes in vitro. Treatments with MtpITC or with MtpITC and Cd increased only GST activity, thus suggesting potential role of hairy root-derived MtpITC in detoxifying reactions rather than in antioxidative defense. Partially supported by Ministry of Science and Higher Education grant No. 2P04C 003 29.

P07-068 The optimization of Agrobacterium-mediated and biolistic genetic transformation of flax (Linum usitatissimum L.)
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Various modifications of Agrobacterium-mediated and biolistic transformation protocol of flax/linseed (Linum usitatissimum L.) were studied in order to improve transformation efficiency. The effect of several treatments of initial explants, namely partial peeling of epidermis, sonication of hypocotyl segments and duration of explant preculture on de novo shoot regeneration capacity after transformation was tested as well as the effect of cocultivation additives application (acetosyringone, L-cysteine, proteolytic enzymes) on transformation efficiency was determined. Flax/linseed genotype sensitivity/recalcitrance to particular transformation treatments was demonstrated based on shoot regeneration capacity and transient/stable transformation efficiency. A. tumefaciens strain EHA 105 containing nptII selectable marker gene and several genes of interest (e.g. genes for heavy metal binding peptides amt and cp with potential for heavy metal phytoextraction utilization) fused with uidA/gus reporter gene was used in the experiments. Transient expression of β-glucuronidase (uidA/gus) gene served as a reporter of transformation efficiency (with Image Analysis DIA application) and early determination of transformed shoots. The transgene integration in putative, kanamycin selection-surviving and GUS-positive transformants was confirmed by PCR. The optimized protocol for flax/linseed genetic transformation will be presented.

P07-069 Improvement of gene transfer efficiency for the transformation of flower colour gene in Tibouchina semidecandra Cogn.
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Genetic transformation of Tibouchina semidecandra Cogn. with sense and antisense dihydroflavonol-4-reductase (DFR) genes using the Agrobacterium-mediated method was carried out. D-galactose, tyrosine, aluminum chloride and ascorbic acid were used to enhance the transformation efficiency. Plasmid pBETD10 and pBETD11, each harbouring the DFR gene at different orientations (sense and antisense) and the selectable marker nptII for kanamycin resistance, were transformed into the shoot and nodal explants of T. semidecandra using an improved transformation protocol. Putative transformants were selected in the presence of kanamycin at their respective optimized concentration. The results showed that approximately 5.3% of pBETD10-transformed shoots and 9.3% of the nodes regenerated whereas only 4.7% (shoots) and 8.3% (nodes) regenerated with pBETD11 transformation. The presence and integration of the sense and antisense DFR genes into the genome of T. semidecandra were verified by polymerase chain reaction (PCR) and nucleotide sequence alignment and confirmed by southern analysis. The regenerated putative transformants were acclimatized to glasshouse conditions. Approximately 69.4% of the pBETD10-transformed and 57.4% pBETD11-transformed T. semidecandra survived after transfer to the glasshouse. The colour changes caused by the transformation event were observed at the budding stage of the putative T. semidecandra transformants.

P07-070 Molecular diversity in Liquidambar orientalis Mill. assessed by sequence analysis of matK region of chloroplast genome
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Oriental sweet gum (Liquidambar orientalis Mill.) a relict-endemic species is naturally found in only southwestern Turkey, mainly in Muğla Province. The limited distribution of species with two disputed varieties (var. integriloba Fiori and var. orientalis) and increased anthropogenic threats to its genetic resources signify the importance of studying genetic diversity in the species to have better conservation and management programs. For this purpose, 18
different populations were sampled throughout the species range and matK region of chloroplast DNA (cpDNA) was sequenced to assess the genetic structure of the species. Based on the molecular diversity analysis, there were no significant differences among varieties as well as among geographic regions. The great amount of total variation was found within oriental sweet gum populations. The results of phylogenetic analysis indicated that Turkish sweet-gum has genetically closer to L. styrraciflua L. than to L. acyclina H.T Chang and L. formosana Hance. Due to high genetic diversity and differentiation, 10 oriental sweet gum populations were identified as important for conservation purposes. Eight of these located in MuBi province and sixth of them belonged to var. integriloba. Especially Fethiye-Günüklübaşı, Marmaris-Çetibeli and MuBi-Kiyra populations were suggested to include in an in situ conservation program in the future.

**P07-071 Site-directed mutagenesis of aspartate kinase and/or homoserine desaturase allows the accumulation of high levels of threonine in leaves of tobacco**

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Elevating leaf threonine (Thr) content depends on overcoming the negative feedback control on the synthetic pathway. The bifunctional enzyme aspartate kinase (AK) (EC 2.7.2.4) -homoserine desaturase (HSD) (EC 1.1.1.3) in Arabidopsis thaliana is controlled in this way. Where release of control has previously been shown, high Thr has been accompanied by extreme fitness costs. Our goal was to increase leaf Thr in tobacco without incurring these costs. Modification of the enzymes to prevent Thr-induced inhibition was performed by site-directed mutagenesis on AtAK, AtHSD or both, and the resultant constructs used to transform tobacco (Nicotiana tabacum). Expression of mutated Arabidopsis AK/HSD genes in tobacco under the control of a leaf-specific promoter had effects on both leaf Thr levels and plant morphology. The increased levels of Thr correlated with presence of mutated AtAK rather than AtHSD. The HSD mutation had less effect on Thr level and morphology. This mutagenesis approach on Arabidopsis did not yield the same results when performed in tobacco as in Arabidopsis. The development of increased Thr content in tobacco was accompanied by extreme fitness costs. Our goal was to increase leaf Thr in tobacco without incurring these costs. Modification of the enzymes to prevent Thr-induced inhibition was performed by site-directed mutagenesis on AtAK, AtHSD or both, and the resultant constructs used to transform tobacco (Nicotiana tabacum). Expression of mutated Arabidopsis AK/HSD genes in tobacco under the control of a leaf-specific promoter had effects on both leaf Thr levels and plant morphology. The increased levels of Thr correlated with presence of mutated AtAK rather than AtHSD. The HSD mutation had less effect on Thr level and morphology. This mutagenesis approach on Arabidopsis did not yield the same results when performed in tobacco as in Arabidopsis. The development of increased Thr content in tobacco was accompanied by extreme fitness costs.

**POSTER PRESENTATIONS, TOPIC 08: PHOTOSYNTHESIS AND RESPIRATION**

**P08-011 Transcriptional and post-transcriptional control on chloroplast acclimation: focus on mutants affected on plastoquinone redox state and ROS production**

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**P08-012 Growth and photosynthetic apparatus in *Brassica chinensis* L. plants grown under red and blue light-emitting diodes**

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It is generally accepted that mostly red and blue photons are effectively utilized in photosynthesis and used in regulatory pathways, although the optimal light spectrum differs for different species and cultivars of plants. Red and blue light-emitting diodes (LEDs) were used in LED lighting units for growing plants built up to date. However, an in-depth study of the photosynthetic apparatus under these lighting conditions has not yet been performed. We studied the growth (root and shoot fresh and dry weight), sugar content, photosynthetic pigment composition, and chlorophyll fluorescence in 15- and 27-days-old *Brassica chinensis* L. plants grown under two types of illumination: high-pressure sodium (HPS) lamps and red (650 nm) and blue (470 nm) LEDs with a red/blue photon ratio of 7:1. The plants were illuminated with two photosynthetic photon flux (PPF) levels: nearly 400 μmol/m²/s and about 100 μmol/m²/s. At PPF of 400 plants grown under HPS lamps and LEDs didn’t differ significantly in shoot fresh weight and photosynthetic apparatus characteristics; however, the shoot dry weight and sugar content were lower in plants grown under LEDs. These differences were even more pronounced in plants grown at PPF of 100. Our study suggests that the photons emitted by our light sources are not absolutely equivalent.

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Photosynthetic apparatus acclimation to different light conditions consists into changes in several components but mainly changes in the stoichiometry between light harvesting antenna and reaction centers. Chloroplast proteins are encoded by plastid or nuclear genes and a fine tuning of the two genomes expression is needed. The molecular mechanism of this cross talk is poorly understood. Here both transcriptional and post-transcriptional regulation were studied by mutants impaired in the reduction state of PQ (plastoquinone) or in ROS (Reactive Oxygen Species) scavenging. The vir zb63 barley mutant has constitutively reduced PQ and exhibits a constitutively reduced antenna system like high light acclimated plants. Nevertheless, transcriptomic analysis revealed that only for few genes the expression is affected. PQ redox state clary controls post transcriptional regulation. In a second experiment, the lut2npq1 Arabidopsis mutant was used. This mutant shows enhanced ROS production due to a decreased capacity for chlorophyll triplet quenching. Transcriptional analysis showed a down regulation of nuclear encoded photosynthetic genes under high light conditions at 4°C, while protein levels are less affected compared to vir zb63 plants. Nuclear-encoded photosynthetic proteins are regulated at least by two factors: PQ redox state and ROS concentration. The first controls post-transcriptional steps while ROS control transcription. Experiments with ROS scavengers support this view.
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Concerning the plants’ growth and development. Various reasons for this effect can be discussed.

P08-013 Responses of PS II to high light in LHC-deficient Arabidopsis thaliana mutants detected by two chlorophyll fluorescence techniques
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Recently, the role of LHCs in photoprotection is into focus. In our study, we used wild type plants (control), Lhcb2-1 and Lhcb2-12 mutants having 20% and 40% reduction in LHC content. Two chlorophyll (Chl) fluorescence methods were used to investigate the response of photosystem II (PS II) to high light (HL 2000 μmol m⁻² s⁻¹). For an early response, repetitive saturation pulses were applied in 10 s interval for 10 min and HL-induced change in effective quantum yield of PS II would be evaluated. The other approach was an analysis of fast Chl fluorescence kinetics (OJIPs) recorded before and after photoinhibitory treatment (30 min) and then after 60, 120, and 180 min of recovery under dim light. General response of quantum yield to a 10 min HL had a polyphasic character. It dropped immediately after HL initiation from 0.75 to almost zero. Then it exhibited curvilinear increase to a constant value (0.15) found at the end of HL treatment. After 30 min HL, OJIPs showed HL-induced decrease in Chl fluorescence values indicating strong photoinhibition of PS II. After 180 min recovery, Chl fluorescence increased towards pre-HL values indicating high capacity of photoprotective mechanisms. Simultaneously, content of antioxidants (zeaxanthin, glutathione) was evaluated in samples taken before and after 30, 60, 180, and 600 min recovery. The results showed that LHC-deficient A. thaliana mutants were more sensitive to photoinhibition than control. GACR52206097.

P08-014 From RACE to walking through the spinach Lhcb1 gene family
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In higher plants the light harvesting complex protein Lhcb1 is encoded by multigene families, whose members are variable in number from plant to plant. By RACE analysis, we identified the full-length cDNAs corresponding to three isoforms of Lhcb1 polypeptides in spinach (Rea et al. 2007). The three isoforms appear to be differentially expressed in response to long-term white light exposure. In order to identify regulatory regions of the three genes, we have developed a genome walking strategy, which is independent of the use of specific restriction enzymes and does not require the use of random primers or ligation of single- or double-strand linkers (Leoni et al. 2000). Analysis of 5' flanking sequences of cDNA coding regions allowed the identification of regulatory elements commonly found for genes coding for light harvesting proteins, such as GATA and I-box motifs and the circadian expression element CAANNNNATC. Interestingly, the genome walking procedure also produced sequence data for other genes of the Lhcb1 gene family.

Data from the sequence analysis of the spinach Lhcb1 gene family and characterization of regulatory regions, together with a discussion on the critical steps of the genome walking procedure and their optimisation will be presented.

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Leoni et al. (2008) Biotechniques 44: 229–235

P08-015 The use of complex compounds (ammoniates) for intensifying photosynthesis and plant productivity
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Assimilate export from leaves and plant productivity under the treatment of plants with complex compounds (ammoniates) was studied. Ammoniates were supposed to suppress cell wall inverase activity through alkalization of the apoplastic medium. Feeding ammoniate solution (10⁻⁶ M) through the transpiration water stream resulted in an increase of photosynthesis and the ratio of 14C-labelled sucrose to labeled hexoses. Spraying of sugar beet plants with ammoniate solution (2·10⁻⁵ M) augmented root sugariness and weight. The effect was more prominent on the background of lower nitrogen fertilization. As a result, a possibility to increase crop yield with concurrent decrease of mineral nutrition level appeared. N balance in the plant-soil system was calculated and it indicated that plants treated with ammoniates draw much more nitrogen out of soil. Changing carbonate anion of the complex compounds for malate, treated with ammoniates draw much more nitrogen out of soil. Participating in potassium circulation in nitrate translocation from roots to leaves has significantly increased the preparation efficiency. If carbonate ammoniates stimulated growth of the plant above ground part by 15–20%, malate ammoniates did that by 35–45%. The ammoniates were efficient in catalytic amounts (10-5M) and their action was not species specific. Favourable action of ammoniates was shown also for flax, pea, bean, piper and cucumber.

P08-016 Effect of growth media on some leaf gas exchange parameters of highbush ‘Blueray’ grown in pots
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Sixteen experimental variants of 2-year-old blueberry (Vaccinium corymbosum L.) plants cv. Blueray were organized on pots with different percent of peat, waste, sawdust, green manure and distillation residues in order to elucidate the relationship between different ingredients of the substrata on the dynamics of leaf gas exchange parameters, during the vegetation period, as a means to characterize the best conditions for efficiently young plants stabilizing. Photosynthesis

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P08-017 Lutein epoxidation: carotenogenesis or true cycle?
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Two xanthophyll cycles have been described in higher plants: the violaxanthin (V) and the lutein epoxide (Lx) cycles. Each one involves the light-induced de-epoxidation of the epoxidated xanthophylls (V or Lx) to the de-epoxidated forms (A, Z or lutein, L). The epoxidation reaction of L to Lx in the dark is slower (or even inactive) than the epoxidation of Z. Snyder et al. 2005 found that Lx recovery in the dark was not accompanied by a decrease in L, which was interpreted as synthesis de novo of Lx. Therefore, the aim for this work was to study Lx epoxidation in order to know whether it is part of a true cycle or it is due to de novo carotenogenesis.

Three woody plants were used: Laurus nobilis, Persea americana and Ocotea foetens. We obtained a complete overnight recovery of Lx pool only in O. foetens, suggesting the operation of the Lx cycle only in this species. Violaxanthin recovery was faster (after 16 h of dark) than Lx (after 40 h of dark). To test whether Lx formation in the dark was due to synthesis de novo or to interconversions with L, illuminated leaf discs were treated with the herbicide norflurazon (that blocks carotenoids biosynthesis at the level of phytoene desaturase) and allowed 18 h to recover in the dark. We observed the same pattern of light-induced de-epoxidation of V and Lx and dark recovery of the initial Lx and V pools. This result demonstrates that, at least in O. foetens, the light-dark interconversions between Lx and L represent a true cycle.

P08-018 Xanthophyll cycles in fruits and stems of avocado
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In addition to violaxanthin (V) cycle, avocado leaves posses the lutein epoxide (Lx) cycle. This species, apart from leaves, present other photosynthetic tissues as is the case of stems and fruits, which are also important for the overall carbon balance of the plant because of their contribution to fixation of respiratory released CO2. Therefore we aimed to study whether both cycles are functional in non-foliar photosynthetic structures in avocado. In chlorophyllous stems we found higher concentrations of Lx than V. Lx photoconversion was almost nil, compared with V that displayed a complete cycle. In the case of fruits Lx content was also higher than V, and both xanthophylls deepoxidised upon illumination, but none recovered in the dark, indicating a one-way conversion of Lx and V. An unusual pigment composition was observed in deep chlorophyllous fruit layers, with absence of neoxanthin, and high content of cis-V. The same composition has been described in photosynthetic stems of Cuscuta reflexa (Snyder et al. 2005). Interestingly, the absence of gas exchange in this tissue may be an adaptation to recycle respiratory CO2, as occurs in fruits. We conclude that, at least in stems, the dynamic regulation of photosynthetic activity does not depend on Lx cycle.

P08-019 Respiratory pathways ratio in young and mature leaves of plants with different phenological strategy
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The contribution of the cytochrome oxidase pathway (COP) and the alternative oxidase ‘wasteful’ pathway (AOP) to the total respiration in young and mature leaves of the field growing spring wheat, winter rye and wintergreen bugle plants is analyzed. To study the AOP and COP activity the specific inhibitors, 25 mM salicylhydroxamic acid and 5 mM NaN3 respectively were applied. The total respiration in young leaves with area 10–30% of final value was 1.5-2 higher than mature leaves. The contribution of the AOP to the young leaves total O2 consumption was 40–50%. Respiratory pathways ratio in mature leaves depended on species. The contribution of AOX in winter rye mature leaves respiration was the same as that in young leaves and 25% higher in spring wheat. The bugle overwintered leaves respired through the COP only. The results are discussed in a view of differences in the plants phenological strategy. The relation of mature leaves respiration with activity of maintenance processes in spring wheat, increasing in cold resistance of winter rye and ATP-dependent export of vacuolar sugars in bugle overwintered leaves is supposed.

P08-020 Effect of different light intensities on resolution of the three components of non-photochemical quenching in Spinacia oleracea
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Plants absorb more sunlight than they can really use for photosynthesis. The excess of absorbed light energy is mainly dissipated by thermal processes in order to minimize photodamage to photosystem II (PSII), through the production of damaging reactive oxygen species. Non-photochemical quenching (NPQ) of chlorophyll fluorescence is an index of non-radiative energy dissipation in the light-harvesting antenna of PS II. NPQ is ascribed to three major processes: qE, the feedback de-excitation process; qT, caused by state transitions and
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P08-021 CO$_2$ permeability and significance of the Arabidopsis thaliana aquaporin AtPIP1;2
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We provide evidence that AtPIP1;2 facilitates CO$_2$ membrane transport in the heterologous expression system yeast and in planta. Water transport was not observed to be increased by AtPIP1;2. The role of AtPIP1;2 in plant-physiology was studied by comparison of AtPIP1;2 T-DNA insertion lines to controls. The studies revealed that AtPIP1;2 is necessary to assure optimal photosynthesis efficiency. Stomatal conductance, quantum use efficiency of photosystem II and electron transport rate was found to be reduced, while internal CO$_2$ concentration in the stomatal cavity was not different from wild type plants. Because no differences in leaf growth, morphology, stomatal density and size were found, we suggest that the mesophyll conductance for CO$_2$ was affected by the absence of AtPIP1;2.

P08-022 The use of chlorophyll a fluorescence as a tool to assess a status of endangered coastal species Eryngium maritimum
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Eryngium maritimum L. is a diminishing species in Northern Europe. The aim of the present study was to search for environmental factors affecting physiological status of E. maritimum in two Latvian populations in comparison with other North European populations. Chlorophyll a fluorescence was used as an indicator of plant vitality and photosynthetic productivity. Maximum photosynthetic efficiency of photosystem II (Fv/Fm) and chlorophyll content in leaves of E. maritimum showed a development-related characteristic trend during a growth season. Photoinhibition of photosynthesis due to unfavorable growth conditions was indicated by a severe decrease of Fv/Fm. Performance of photosynthesis was affected by local environmental conditions. It is concluded that individuals of E. maritimum in conditions of Latvia are negatively affected by increased atmospheric precipitation and decreased number of sunny hours per day together with a low average summer temperature.

P08-023 The unusual step in chlorophyll fluorescence induction of lichens can be observed also in some higher plants
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Recently, an unusual step appearing after the P step in chlorophyll fluorescence induction (Fl) has been detected in Trebouxia possessing lichens and their native photobionts (Ilk et al. 2006, Biochimica et Biophysica Acta 1757: 12–20). It was shown that this step (in contrast to the P step) does not reflect a traffic jam of electrons at photosystem I and is not associated with cyclic electron flow around photosystem I. The step is probably associated with a transient reduction of plastoquinone, but its origin has not been explained yet. In this work we demonstrate that this unusual step in Fl can be observed also in some higher plants. While we have found it in Fl of mosses, ferns and many conifers, we did not observe this step in Fl in leaves from flowering plants (Magnoliophyta). Interestingly, this unusual step in Fl appeared in barley (a representative of flowering plants) that was grown at very high light (1000 μmol photons m$^{-2}$ s$^{-1}$) or that was stressed by rapid mild heating. Possible phylogenetic implications of these results will be presented.

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P08-024 Formation of end products of photosynthesis from ‘own’ and ‘foreign’ assimilates
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In a plant of flax distribution of 14N among end products of photosynthesis in leaves and stem in upper (acceptor) and lower (donor) part of shoot was investigated after uptaking of 14N2 by leaves in lower part of shoot. In leaves from own assimilates (synthesized from 14N2) there were produced more low-molecular compounds and products extracted by acetone rather than polymers. From ‘foreign’ assimilates (transported from lower leaves) there were produced more polymer products. Among high-molecular compounds produced from ‘own’ assimilates the polysaccharides predominated and among compounds produced from ‘foreign’ assimilates – nonsoluble proteins. Similar changes among high-molecular compounds were observed in shoot tissues (wood and bast). The same patterns were found in leaves and leaf sheaths of wheat plants, were these plant parts were used either as donor or as acceptor of the assimilates. 14N2 was uptaken by leaves or by leaf sheaths and 14N-assimilates were analyzed in both cases. Distinction in the ratio of different fractions of proteins synthesized either from ‘own’ products of photosynthesis (synthesized from 14N2) or from exogenous amino acid was also detected on green callus culture of tobacco. Proteins of cytoplasm were produced in larger rate from 14N2 and cell wall
proteins were produced from exogenous amino acids. A conclusion is made that cell endogenous and exogenous compounds are differently used, and this process is strictly regulated by cell.

P08-025 A stressful situation – consequences of less NPQ
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The Arabidopsis thaliana mutant npp4 is deficient of the PsbS protein, which is an essential protein for the major photoprotective process the qE type of non photochemical quenching (NPQ), also named feedback de-excitation. Plants lacking PsbS are therefore suffering an increased photooxidative stress. We have been growing npp4, wt and PsbS overexpressing plants in the field (in Umeå, northern Sweden) and studied their performance under natural conditions. Metabolomic studies indicated a metabolic shift and transcriptomic studies – using DNA microarrays – suggested that an increased jasmonic acid (JA) signaling may be involved. JA levels are also elevated in plants lacking PsbS. Experiments with herbivores have shown that a specialist and a generalist insect herbivore had different preferences when it came to these plants, consistent with an hypothesis that the metabolic shift induced by lack of PsbS resulted in an increased chemical defence against herbivores. We therefore believe that there is a connection between light harvesting in photosystem II, photooxidative stress, and herbivore preferences.

P08-026 Effect of ozone on respiratory and photosynthetic parameters in relation to the development stages of poplar leaves
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Ozone is known as a major phytotoxic air pollutant causing disturbances in physiological and biochemical processes. Related to carbon metabolism, it is considered that catabolic processes are generally up-regulated with a stimulation of respiration. However, some regulatory aspects of catabolism are far to be well known, and photosynthesis which could be involved in energy dissipation and photoprotection, has been poorly studied. In our work, young poplar trees (P. tremula Michx. × P. alba L. clone INRA 717-184) were subjected to 120 ppb of ozone for 35 days in phytotrons. Treated trees displayed precocious leaf senescence and visible symptoms of injury exclusively on fully expanded leaves. In these leaves, ozone reduced parameters related to photosynthesis and photosynthetic CO₂ fixation as well as the rate of photorespiration, estimated from chlorophyll fluorescence. The activity of photorespiratory related enzymes (rubisco and glycolate oxidase) and the amount of serine hydroxymethyltransferase also decreased. With the stimulation of PEPc activity, a higher mitochondrial respiration and a lower stomatal conductance, leaves reaching full expansion under ozone exposure showed potential responses of protection. By contrast, leaves in the early period of expansion were exempt of visible symptoms of injury and remained unaffected for all measured parameters.

P08-027 Structural and kinetic properties of UDP-glucose pyrophosphorylase isozymes in Arabidopsis
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UDP-glucose pyrophosphorylase (UGPase) is an important enzyme in the production (and conversions) of UDP-glucose, a key precursor for carbohydrate biosynthesis in all organisms. cDNAs corresponding to two UGPa isozymes in Arabidopsis were overexpressed in E. coli and the proteins purified/characterized. Both proteins were highly conserved, sharing 93% identity. Based on crystal structure-derived images, the main amino acid differences mapped to N- and C-termini domains, but not to central active site region. The two proteins existed mainly as monomers, and they had similar molecular mass of ca. 53 kDa. However, comparison of molecular masses of UGPases from Arabidopsis root and leaf extracts revealed that the root protein was slightly larger, suggesting a posttranslational modification. Specific activity of the purified UGPa-1 was ca. 10–30% lower than that of UGPa-2, depending on direction of the reaction, whereas its Km values with all substrates in both directions of the reaction were consistently ca. twice lower than those of UGPa-2 (0.03–0.14 mM vs. 0.07–0.36 mM, respectively). Both proteins were ‘true’ UGPases, and had no activity with ADP-glucose/ATP or galactose-1-P. Equilibrium constant for both proteins was ca. 0.3, suggesting preference for the pyrophosphorylation direction of the reaction. The data will be discussed with respect to potential roles of UGPase in carbohydrate synthesis/metabolism in both source and sink tissues of Arabidopsis.

P08-028 Participation of ferredoxin in light-induced oxygen reduction in chloroplasts
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Photosynthetic electron transport chain (PETC) of plants reduces oxygen concurrently with NADP⁺. In spite of the important physiological functions of the electron transport to oxygen, - the additional ATP synthesis, the protection against photoinhibition, and the generation of ROS, signal for the system of gene expression, - the mechanism of the process remains unclear. The acceptor side of Photosystem I (PSI) is usually considered as the main place of O₂ reduction. However, till here there is no common opinion about the component operating as the immediate reductant of O₂, either the membrane-bound carriers of PSI, or ferredoxin (Fd), the autoxidizable protein, an electron carrier between PSI and Fd-HADP-reductase. We compared the Fd-dependent oxygen reduction rate found from the measurements of Fd redox changes, with the total rate of oxygen reduction in PETC of plant chloroplasts.
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the electron flow to oxygen. The latter was found from the measurements of either the oxygen concentration change, or Photosystem II quantum yield. The results derived from both methods closely agreed. In the absence of NADP⁺ the Fd-dependent oxygen reduction amounted to 40–60% in strong light, and 70–80% in weak light, of total electron flow to oxygen; the contribution decreased to 5–10% in the presence of NADP⁺. These data are the first indicating the relationship between concurrent electron flows reducing oxygen and leading to production ROS in the different phases; the latter can be the basis to reflect the different PETC states.

P08-029 Reversible phycobilisome coupling and variable phycobilisome fluorescence in the marine diazotrophic cyanobacterium Trichodesmium
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The non-heterocystous diazotrophic cyanobacterium Trichodesmium performs both photosystem II (PSII)-mediated photosynthesis and nitrogen fixation in the same cells in the light period. Energy for nitrogen fixation is delivered, and nitrogenase protected, by the Mehler reaction. In previous studies we found that this regulation of photosynthesis for nitrogen fixation involves various activity states involving reversible coupling of photosynthetic components. We now investigated the interactions between photosynthesis and nitrogen fixation in Trichodesmium in more detail. Spatially and spectrally resolved fluorescence kinetic measurements of single cells revealed that changes in photosynthetic activity were related to alternate uncoupling and coupling of phycobilisomes from and to the photosystems, changing the effective cross-section of PSII. By fitting the in vivo fluorescence spectra with those of isolated phycobilins we are quantitatively investigating this reversible coupling of individual parts of the phycobilisome antenna. Further, we found that phycobilisomes can be so closely coupled to PSII that their fluorescence emission exhibits strong photochemical and weak non-photochemical quenching. Again, the contribution of different parts of the phycobilisome antenna to fluorescence quenching changes during the daily activity cycle. Thus we propose that variable phycobilisome coupling plays a key role in the regulation of photosynthesis for nitrogen fixation in Trichodesmium.

P08-030 Impact of elevated CO2 on Norway spruce needle structure – anatomical study based on confocal microscopy and stereology
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Elucidation of leaf anatomical changes connected with elevated CO2 may help to understand the potential of conifers in carbon sequestration. This study is focused on proportion of intercellular spaces and internal surface density in mesophyll of Norway spruce needles.

Experimental trees were grown for 8 years in glass domes with adjustable windows with following CO2 concentrations: 350 µmol CO2 mol1; and 700 µmol CO2 mol1; control trees were grown in adjacent open-air stand at Experimental Ecological Study Site Bílý Kož, Czech Republic. Samples of sun-exposed and shaded needles were collected in August 2004. Initially, technical aspects of using free-hand sections of frozen needles for three-dimensional analysis of mesophyll by stereology and confocal microscopy were tested and specific specific constraints of this method were established.

As far, no effect of elevated CO2 concentration on needle volume and proportion of individual needle tissues was detected, however, anatomical alterations are expected at more subtle level, which is under investigation: measurements of internal surface density of mesophyll are now in process. Working hypothesis suggests higher internal surface density as response to elevated CO2 concentration and more pronounced changes in internal surface density of shaded needles are expected.

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P08-031 Characterization of leaf-targeted FNR isoforms in Arabidopsis thaliana
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Ferredoxin-NADP+-oxidoreductase (FNR) catalyses the reduction of NADP⁺ to NADPH as the final step of linear photosynthetic electron transfer chain. In Arabidopsis thaliana two distinct nuclear genes encode leaf-targeted isoforms of FNR, AtLFNR1 and AtLFNR2, which appear in chloroplasts in soluble and thylakoid membrane-bound forms. We are currently studying the specific functions of these FNR isoforms using mutant plants lacking either AtLFNR1 or AtLFNR2. Since both mutants are viable both FNR isoforms are functional, although reduced growth of the mutant plants implies that both isoforms are needed to guarantee optimal photosynthetic capacity. In addition to reduced carbon fixation the amount of the photosynthetic protein complexes is reduced in both mutant lines when compared to wild type. Absence of AtLFNR1 protein leads to dissociation of FNR from thylakoid membrane whereas AtLFNR1 can be found both as thylakoid-bound and soluble form when AtLFNR2 has been knocked out. This implies that AtLFNR1 is needed for thylakoid membrane association of AtLFNR2 protein, most probably by forming FNR1-FNR2 heterodimer, which is supported by structural model. Analyses of the thylakoid membranes by blue native gel electrophoresis revealed that both isoforms are also needed in formation of big protein complexes consisting FNR and some unknown proteins in thylakoid membrane. The binding partners of FNR in thylakoid membrane are still under study.

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Organ-specific effects of two-day dark treatment on photosynthesis and expression of related genes in Arabidopsis cotyledons and primary leaves

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The differential effect of 2-day dark treatment on the photosynthetic apparatus in cotyledons and primary leaves of Arabidopsis plants grown under 16/8 h photoperiod was studied. Dark stress was applied on whole plants as well as on individual intact leaves and cotyledon pairs. In cotyledons, a slight inhibition in the total chlorophyll content and a significant drop in the actual PSI efficiency (about two-fold) accompanied by an increase in the nonphotochemical quenching were registered, regardless of the type of darkening. A drastic decrease (up to 5-fold) was observed in the mRNA levels of psbA and rbcL while those of psbA remained unaffected. In contrast to cotyledons, the changes in the photosynthetic parameters and the chloroplast mRNA levels in primary leaves were strongly dependent on the light status of the rest of the plant, the inhibition being higher when leaves were individually darkened. Similarly to cotyledons, the psbA transcript levels in dark-stressed leaves did not differ from the control. The effect of darkness on D1-protein degradation processes was studied following the changes in the transcript levels of the two plastid proteases FtsH5 and Deg1. The analysis of their relative expression rates showed that, in contrast to the expected simulation, darkness led to a decrease in the FtsH5 and deg1 mRNA levels, thus supporting their involvement only in D1-repair during photoinhibition.

Leaf-type ferredoxin-NADP-oxidoreductase isofoms in Arabidopsis thaliana

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Ferredoxin-NADP-oxidoreductase (FNR) is an enzyme catalysing the final step of linear electron transfer reducing NADP+ to NADPH. In Arabidopsis thaliana, the chloroplast targeted FNR enzyme exists as two isofoms, AtFNR1 and AtFNR2, encoded by two distinct nuclear genes. Both isofoms are evenly distributed between the thylakoids and soluble stroma, and they can be separated by 2-D electrophoresis in four distinct spots, suggesting post-translational modification. We have characterized knock-out mutants of both isofoms in order to reveal their functional specificity. Absence of either one of the isoforms resulted in reduced size of the rosette with pale green leaves, which was accompanied by a low chlorophyll and LHC protein content. Also the PSI/PSII ratio was significantly lower in the mutants, although the PSI activity, measured as FV/FM ratio or DMBQ-dependent oxygen evolution, remained nearly unchanged. Slow re-reduction rate of P700 measured in the mutant plants suggests that both isoforms are involved in PSI-dependent cyclic electron flow. Impaired function of FNR also resulted in decreased capacity of carbon fixation whereas nitrogen metabolism was up-regulated, detected as changes in the levels of nitrate transporter and nitrate reductase transcripts and as increased accumulation of nitrite in the leaves.

Influence of Cu²⁺ on the valence and spin state of the non-heme iron of Rhodopseudomonas sphaeroides photosynthetic reaction centers

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We investigated the influence of copper ions on electron and energy transfer in reaction centers (RC) prepared from Rhodopseudomonas sphaeroides (Okamura et al. 1974) using fluorometer with a dual modulation. We applied Mössbauer spectroscopy to monitor spin and valence electronic states of the non-heme iron in native and treated with Cu²⁺ reaction centers. For these studies we cultivated bacteria in a 57Fe- enriched medium.

In intact reaction centers we observed the non-heme iron in a reduced state Fe²⁺ but at two different spin states: in a high and low spin state. Copper ions modified the non-heme iron binding site and transferred the iron into a new diamagnetic state. A similar action of Cu²⁺ on photosystem II was observed in algae (Burd et al. 2003). We found that copper ions within the range of the applied concentrations did not remove the non-heme iron from its binding site. However, the new arrangement of the quinone-iron complex caused by Cu²⁺ resulted in slowing down the electron transport within the modified bacterial reaction centers.

Chloroplastic ultrastructure, chlorophyll levels and photosystem II efficiency in horseradish (Armoracia lapathifolia Gilib.) tissue culture

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In vitro grown horseradish (Armoracia lapathifolia Gilib.) plantlets, tumour and teratoma tissues were compared with regard to chloroplastic ultrastructure, chlorophyll level and efficiency of photosystem II (PSII). Tumours were induced on the leaves with an octopine strain R653 of A. junéaciens. From the same primary tumour two tissue lines were established: unorganised tumour without any morphogenic capacity (TN) and teratoma composed of malformed shoots (TMS) and unorganised tissue (TMn). For electron microscopy tissue was fixed with...
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P08-036 Turnover of PSII reaction center D1 protein in C4 maize plants
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The PSII repair process is affected by various factors: PSII supercomplex monomerization, dephosphorylation, degradation and synthesis rate of the D1 protein. Two types of chloroplasts are involved in photosynthesis in maize, mesophyll chloroplasts (MC) develop grana thylakoids, while bundle sheath chloroplasts (BSC) are agranal. Up to date limited data are available concerning photoinhibition in C4 plants and the D1 protein turnover in MC and BSC. We investigated the D1 protein turnover in the presence of a lincomycin in maize. Illumination of whole leaves caused a decrease in chlorophyll fluorescence parameters as it was previously noticed for C3 plants. The D1 protein was dephosphorylated during illumination of plants similarly in MC and BSC but D1 protein degradation varied significantly between the two types of chloroplasts. It was much faster in BSC than MC, and was independent of light intensity during treatment. Simultaneously, accumulation of PSII monomers was observed in MC, whereas the degree of PSII monomerisation decreased in BSC. The Reaction Center devoid of its inner antennae of PSII was found in both MC and BSC isolated from lincomycin-treated plants. These observations indicate that the rate of PSII photodamage is similar in C3 and C4 plants. However, the D1 protein degradation rate differs between MC and BSC. Damaged and dephosphorylated D1 protein accumulates in MC during photoinhibition, however, the origin of this phenomenon is yet to be established.

P08-037 Environmental engineering based upon bamboo: possibilities and prospects
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To enhance the diversity of possible biofuel sources, we propose the use of bamboo plants, in addition to using Miscanthus, Salix and Populus. Bamboo offers several advantages: the plants are tolerant to Western European climate, and require little maintenance. Also, bamboo plants seem to do well in preliminary tests concerning heavy metal tolerance, offering a possibility to use the plant for both green energy production and phytoremediation. On the negative side, bamboo requires a high initial investment.

To provide data on the performance of bamboo in Western Europe, a plantation was set up in Ballyboughal (Co. Dublin, Ireland) in 60–80% gley soil, in April 2005. This plantation consists of four Phyllostachys species (P. decora, P. humilis, P. bissettii and P. aurea), planted at regular intervals of 1 m, with rows spaced 1 m apart. After 3 years of undisturbed growth, the bamboo plants were analysed in terms of photosynthetic rate, chlorophyll fluorescence and biomass production, both at the beginning of spring and of summer. In addition, a combustion model was constructed for bamboo biomass using data from differential scanning calorimetry and thermogravimetric analysis.

Altogether, results will be presented regarding the performance of these species under field conditions, as well as indicative calculations regarding the energetic yield and the economic viability of bamboo cultivation.

P08-038 Assessing of photosynthesis tolerance to heat and high illumination by fluorescence imaging
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Fluorescence imaging represents a non-invasive tool for revealing and understanding spatial heterogeneity in leaf performance caused by external factors, such as abiotic stress. Sun (Rosa sp. and Chrysanthemum sp.) and shade (Spathiphyllum wallisii) plants were used to study their tolerance to heat and high illumination. Fluorescence, effective PSII quantum yield and non-photochemical quenching were analysed in leaves by fluorescence imaging. The control plants showed homogeneous images of the fluorescence parameters in all the leaf. The fluorescence value was less 0.1, the effective PSII quantum yield around 0.75 and non-photochemical quenching less 0.3. The two sun plants showed high tolerance to stress conditions, the images of the fluorescence parameters being similar to those of control plants. Shade plant showed low tolerance, and irreversible damage was observed after the first photoperiod, particularly at the base of the leaf and in the areas adjacent to the ribs. The centre and top of the leaf were less damaged because the leaf was doubled to reduce the incident radiation. Incubation with herbicides led to differences in the fluorescence parameter images. The effect of DCMU (0.1 mM) was visible after 30 min incubation, beginning at the ribs and adjacent areas of the leaf. Paraquat (0.2 mM) had a visible effect after 4 h (sun plants) or 9 h (shade plant) incubation, the leaf surface showing several damaged regions. Work supported by the Spanish MEC (BFU2005-09243-C02-01).

P08-039 Novel application of interferometry to monitor volume changes during photosynthesis
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P08-040 Changes of nitrogen metabolism in cucumber MSC16 mitochondrial mutant
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We describe main features and applications of a novel interferometric apparatus constructed primarily for the photosynthesis research, which enables to measure very precisely changes in sample dimensions from tens of nm to hundreds of um. Because the photosynthetic light energy utilisation is accompanied by processes resulting in dynamic volume changes of photosynthetically active samples (due to the O2 evolution, CO2 uptake, heat propagation, transpiration etc.), we constructed a new instrument based on the Michelson interferometer with a fixed reference-path mirror and a movable sample-path mirror connected to the sample using an optomechanical device. HeNe-laser (632.8 nm) is used as a source of a coherent measuring beam. A cuvette type sample holder for liquid samples or ventilated pre-darkening chamber for plant leaves can be applied. The apparatus is used for measurements on isolated chloroplasts, photosynthetic bacteria, and higher plants in vivo. Main advantage of this method is the possibility to quantify non-photochemical processes in photosynthetic samples measured in vivo using parallel recording of interferograms and Chl a fluorescence induction kinetics. Using this double-detection method, transversal sample volume changes and fluorescence induction kinetics during photosynthesis are demonstrated and discussed in dependence on external conditions (temperature, irradiance). Theoretical model for heat evolution and propagation in samples is described and verified.

P08-041 Respiration of illuminated leaf: gas exchange determination and light response
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An illuminated green leaf, as a net CO2 consumer driven by photosynthesis, also produces CO2. Both photosynthesis and the tricarboxylic acids cycle (TCA) respiration are involved. However, TCA respiration in light (day respiration rate, RI) is inhibited when compared to dark respiration. The role and regulation of RI still remains a puzzle of present photosynthesis research. This is partly due to methodical difficulties in distinguishing the components of the complex CO2 exchange between atmosphere and the leaf. We designed and tested a gas exchange technique suitable for measurement of RI in photosynthesizing leaf or the whole plant. The method is based on two subsequent measurements of net photosynthesis rate in 12CO2 and 13CO2 atmosphere. IRGA sensitivity to both stable isotopomers differs due to overlap of the absorption bands and the 13CO2/12CO2 sensitivity ratio has to be determined. Photosynthesis rate was manipulated by variable oxygen (2% or 21%) and CO2 concentration in the atmosphere. An adapted closed Li+6400 photosynthesis system was used. Preliminary measurements with water hyacinth (Eichhornia crassipes) showed that the proportion of RI in net photosynthesis rate rose from 21 to 58 % with light (PPFD) decreasing from 3000 to 100 μmol m-2 s-1. At this PPFD range, RI showed saturated light response amounting to 21 % of gross photosynthesis rate at 3000 μmol m-2 s-1. Day respiration in several C3 and C4 species will be presented.

P08-042 Reaction kinetics of photoinhibition of photosystem II
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Photoinhibition is light-induced damage to photosystem II (PSII). Photoinhibition resembles kinetically a first-order reaction but slight deviations have been reported in vivo. These deviations have been explained by assuming that photoinhibited PSII centres protect the remaining active centres. An alternative explanation is that photoinhibition proceeds via a reversible intermediate. In the present study, thylakoids and leaves of pumpkin (Cucurbita pepo L.) were illuminated with different PPFD’s (900, 1600 or 2300 μmol m-2 s-1). In vitro illumination was provided within three wavelengths (400–500 nm, 500–600 nm or 600–700 nm) of light, white light was used for leaves. In vivo experiments did not reveal any consistent deviation from first-order kinetics but a small deviation was found in photoinhibition of isolated thylakoids. The kinetics was analysed by fitting in vitro data to models representing either the presence of a reversible phase in photoinhibition or protection by already photoinhibited PSII. Additional data were obtained from experiments with randomized thylakoids and PSII core complexes, which have less energetic connectivity between PSII centres than thylakoids. Data from these in vitro experiments were
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P08-043 The different nuclear-organelle combinations change the chromosome behavior and the photosynthetic parameters of barley alloplasmic lines
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We studied here the effect of organelle genome on the nuclear genes transmission and recombination with the help of 3 barley SSR-markers. The objects of analysis were barley reciprocal hybrids between the cultivar and five alloplasmic lines. For one of hybrids the segregation ratios for these markers were 12:40, 14:38, and 16:36. All these distortions from the expected 1:1 were significant. We found also the effect of organelle genomes on the recombination process: we observed the decrease of recombination rates for some hybrids. We also studied changes of photosynthetic parameters of nine alloplasmic lines as a result of the periodic heat shock. The most of lines, but not their parent forms, have demonstrated stable chlorophyll and carotinoides contents in the heat shock conditions. Some of these lines have showed the increase of NPQ parameter (nonphotochemical fluorescence quenching). Perhaps it proves that there is some disturbance between light energy which was caught and utilization of this energy in synthetic reactions. On the other hand most of alloplasmic lines have demonstrated the increase of quantity ofQB-nonreducing centers. In summary, we proved the effects of definite organelle genomes on the transmission and recombination of nuclear markers in barley hybrids. We conclude that the alien organelle genomes affect mostly the male gametes forming. The above-mentioned effects prove that the new sources of variability should be drawn into the breeding process.

P08-044 AtCYP38 ensures early biogenesis, correct assembly and sustenance of photosystem II
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AtCYP38 is a thylakoid lumen protein comprising the immunophilin and phosphatase inhibitor domains. Here we show the association of AtCYP38 with photosystem (PSII) monomer and address its functional role using AtCYP38 deficient mutants. The greening process of etiolated leaves and the early development of seedlings under short photoperiod failed in ΔAtCYP38 plants, due to problems in biogenesis of PSII. Detailed biophysical and biochemical analysis of mature AtCYP38 deficient plants from favourable growth conditions (long photoperiod) revealed (1) intrinsic malfunction of PSII, which (2) occurred on the donor side and (3) was dependent on growth light intensity. AtCYP38 mutants also showed decreased accumulation of PSII, which was shown not to originate directly from impaired D1 synthesis or assembly of PSII core but rather from the incorrect fine-tuning of the oxygen evolving side of PSII rendering PSII centers extremely susceptible to photoinhibition. AtCYP38-deficiency also decreased the in vivo phosphorylation of PSII core proteins, probably related to the absence of AtCYP38 phosphatase inhibitor domain. It is proposed that during PSII photoinhibition-repair cycle the AtCYP38 protein first assists the dephosphorylation of PSII core proteins, thus enhancing the degradation of damaged D1 protein, and then guides theproper folding of D1 (and CP43) into PSII thereby making the correct assembly of the water-splitting Mn4-Ca cluster feasible even upon high turnover of PSII.

P08-045 Expression and functional analyses of the two PsbO isoforms in Arabidopsis thaliana
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The PsbO protein, stabilizing the water-oxidizing complex in photosystem II (PSII), is represented by two isoforms expressed in Arabidopsis thaliana. To understand the functional difference between the two isoforms, we have analysed mutant plants with T-DNA insertions in either of the two genes (psbO1 and psbO2). Both type of mutants were retarded in growth in comparison with the wild type, while differed from each other phenotypically. We have recently demonstrated that the plant PsbO is a GTPase, and also proposed that in Arabidopsis the PsbO2 is the main isoform responsible for an efficient degradation of the PSII D1 reaction center subunit following photoinactivation. In this work, we have further analysed the two psbo mutants using blue native gel electrophoresis, biophysical techniques as well as gene expression and radioactive assays. Our data show that the psbO1 mutant has a distinct composition of PSII monomers, dimers, PSII supercomplexes and CP43-less PSII complexes, from the psbO2 mutant and wild type plants. Also the various biophysical techniques indicate distinct properties of the electron transport in the psbo1 mutant. The measurements of GTPase activities in PSII membranes from the wild type and mutants indicate PsbO2 as a better GTPase than PsbO1. The relevance of these findings and the expression analysis data will be discussed.

P08-046 Abscisic acid affects stomatal development in Arabidopsis thaliana
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Plant regulates its transpiration by controlling the movement of stomata. The phytohormone abscisic acid (ABA) is known to regulate stomatal closure in response to the changing water status. It is reported that ABA-induced stomatal closure is impaired in Arabidopsis ABA-insensitive mutant, abi1-1. In addition to rapid response, the number of stomata is also regulated by environmental factors such as CO2 concentration and light. In this study, we
found that in addition to the increase in transpiration rate in aba1-m, the density of stomata was increased in the aba1-m mutant. We also found that ABA-deficient aba2-2 mutant also showed the similar phenotype. As the stomatal density in aba2-2 was decreased by ABA application, we supposed that ABA suppresses stomatal development. As the high stomatal density was also observed in ethylene-over-producing mutant eto1-1, we investigated the effect of ethylene on stomatal development and supposed that, as ethylene inhibits ABA-induced stomatal closure, ethylene inhibits the effect of ABA to suppress stomatal development.

P08-047 Core protein phosphorylation facilitates the release of damaged D1 protein from PSII complex upon photoinhibition
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Phosphorylation of photosystem II (PSII) reaction center protein D1 has been hypothesised to function as a signal for the migration of photodamaged PSII core complex from grana membranes to stroma lamellae for concerted repair. Recently, this theory was challenged by a study with mutants incapable in phosphorylation of PSII core proteins. Here, by using the same mutants, the role of PSII core protein phosphorylation in PSII photodamage and repair was investigated. We show that the lack of PSII core protein phosphorylation disturbs the disassembly of PSII complexes at high light, which is a prerequisite for removal of damaged D1 protein from PSII complexes. This results in accumulation of photodamaged PSII complexes, which in turn result, upon prolonged exposure to high light, in general oxidative damage of photosynthetic proteins in the thylakoid membrane.

P08-048 Relationship between leaf internal conductance and CO2 concentration evaluated by simultaneous measurement of gas exchange and 13CO2 discrimination
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Present biochemical models of photosynthesis assume infinite and invariable leaf internal conductance to CO2 (g). Recent research has shown that this is often not the case. Especially in perennial plants is g sufficiently small to reduce concentration of CO2 at the sites of carboxylation thus significantly limits rate of photosynthesis. Moreover, there is evidence of rapid variation of g in response to several environmental conditions. The effects of short-term changes of CO2 concentration on g in abscisic acid (ABA) treatment and control leaves were evaluated by simultaneous measurements of gas exchange and 13CO2 discrimination. Used method turned out to be very sensitive to precise assessment of 13CO2 discrimination and CO2 leakage within the leaf chamber of gas exchange system. We found out strong correlation between g and CO2 concentration in substomatal cavity (C) varying along the range typically used in photosynthesis CO2-response curves. After ABA treatment stomatal conductance decreased and its sensitivity to variable C appeared.

P08-049 Effect of CO2-enrichment and fertilization regimes on CO2 uptake and growth of the CAM cactus Hylocereus undatus
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During the last 40 years, research into plant responses to increasing atmospheric CO2 levels focused on C3 photosynthetic pathway plants, which exhibited enhanced net CO2 uptake and growth. Data concerning Crassulacean acid metabolism (CAM) responses to elevated CO2, however, are limited, variable, and usually recount research conducted under low fertilization (~0.1 strength Hoagland). This study examines the response of the commercial CAM vine-cactus fruit crop Hylocereus undatus to CO2 enrichment (1000 ppm) under high and low fertilization regimes, 0.5 and 0.1-strength Hoagland, respectively. On average, CO2 enrichment increased net CO2 uptake, stem elongation, dry biomass accumulation, and malate accumulation by 1.5, 1.2, 1.1 and 1.1 times, respectively. However, plants exposed to high fertilization regimes and elevated CO2 showed 2.0, 1.2, 2.4 and 6.5-fold increases in net CO2 uptake, stem elongation, dry biomass accumulation and malate accumulation, respectively, relative to plants tested in the low fertilization regime. In conclusion, highly fertilized CAM crops may benefit from elevated CO2 more than CAM plants grown under the low fertilization regime, results that pave the way to test this best treatment on fruit yield and quality.

P08-050 3-D gas exchange pathways in pome characterised by synchrotron X-ray computed tomography
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Gas exchange of plants with their environment is essential for metabolic processes such as photosynthesis and respiration. Our understanding of the exchange mechanisms critically depends on insights in the structural arrangement of cells and, specifically, gas spaces in tissues. Here we report the successful use of synchrotron X-ray computed tomography for 3-D microscopic imaging of plant organs in their natural state. Fruits of apple cv Jonagold and pear cv ‘Conference’ were considered, as their gas exchange properties have been shown to be very different. The storage life of these fruit and their year-round availability to consumers critically depends on their gas exchange properties. Fresh samples from parenchyma cortex tissue of optimally picked fruits were imaged. We obtained for the first time high contrast 3-D absorption images of in vivo fruit tissue at micrometer resolution and improved image contrast between voids and cells. 3-D phase contrast imaging of cell assemblies at a resolution as low as 0.7 µm also enabled visualization of individual cell morphology, cell walls and void networks. In terms of facilitating gas exchange, the network pattern of the voids found in pears is by far not effective to compensate for the large size and volume fraction difference with the unconnected void structure we find in apple. These results will significantly contribute to explain the large sensitivity of pears to physiological disorders.
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P08-051 Bio-optical modelling as an approach to balance the energy use from light to plant biomass and to identify the loss processes in quantitative terms

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Bio-optical models measure the amount of photosynthetic radiation taken up by a photosynthetic unit (single cell, chloroplast, or leaves) and follow the fate of the absorbed energy through the different processes of energy conversion. Therefore, the efficiency of all following processes can be related to the number of absorbed quanta. PAM Fluorescence together with simultaneous oxygen production is used to quantify linear and alternative electron transport, whereas respirometry follows the losses during the night. The resulting biomass can be qualitatively analysed by elemental analysis (CHNO) and by FT-IR spectroscopy. The latter can reveal the relative proportion of carbohydrates, to proteins to lipids present in the newly formed biomass. The application of such a complete energy analysis shows that the energetic losses under stress in most cases are not due to changes in the photosynthetic efficiency but to other metabolic regulations, which became obvious at most either by increased dark respiration and changes in the macromolecular composition of the biomass. The results clearly show that PSHI fluorescence is a good tool to test photosynthesis but not to predict changes in the biomass production under stress.


P09-012 Functional analysis of promoter activity of sugar beet NHX1 gene

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Expression of the sugar beet (Beta vulgaris) NHX1 (BvHNX1), encoding a vacuolar Na+/H+ antipporter, was shown to be highly induced by salt stress. A 2.5 kbp genomic sequence of BvHNX1 upstream the translation start codon was cloned. Two 5’ serial deletions of the BvHNX1 promoter sequences fused to GUS were constructed. One series contained the 5’ UTR and intron sequences within 5’ UTR, whereas the other series did not. GUS was assayed, both histochemically and enzymatically, in transgenic Arabidopsis plants containing single insertions. While BvHNX1 was expressed in most tissues; highest expression levels were observed in apical meristems, shoot and root vascular tissues and roots branching, but not in root tips. Application of salt-stress, osmotic stress or ABA doubled the BvNHX1 promoter activity. The 5’ UTR and intron are not necessary for expression levels and/or salt induction of the reporter gene. Moreover, the 336 bp promoter fragment was sufficient to drive gene expression in a salt dependent way. The DNA sequence lacks ABRE and DRE, major cis-acting elements involved in ABA-dependent and ABA-independent regulatory pathways. A number of putative cis-acting elements were identified. To study if these cis-acting elements have a role in the salt-induced activity of this promoter, these sequences were mutated and introduced into Arabidopsis plants. The activities of constructs of the shortest BvNHX1 promoter construct containing mutated cis-acting sequences were assayed.

P09-013 Chlorophyll fluorescence as a practical tool to assess the response the perennial halophyte Batis maritima to long-term salinity

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We investigated the effects of two-month long exposure to a wide range of salinities (0–1000 mM NaCl) on the growth and chlorophyll fluorescence of the halophyte Batis maritima. Biomass production was significantly stimulated up to 300 mM NaCl, with an optimum at 200 mM NaCl, and the plant was able to survive, even when challenged with 1000 mM NaCl. Neither shoot nor root water content were affected by increasing salinity. While F0, NPQ and qP in salt-treated plants remained generally constant, PSII maximal efficiency (Fv/Fm) and ETR were transiently increased in the salinity...
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P09-014 Analysing and elucidating the role of Arabidopsis Thioglucosidase Glucohydrolase 1 (myrosinase) towards dehydrogenation and methyl jasmonate

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The presence of Glucosinolate-Myrosinase system is a classical characteristic of plants of the order Capparales that includes Arabidopsis and other crucifers. The enzyme β-Thioglucosidase Glucohydrolase 1 (TGG1) hydrolyzes glucosinolates into toxic compounds, that deter herbivory. TGG1 is expressed in stomatal guard and phloem cells and in above-ground organs. To analyse TGG1 expression against methyl Jasmonate (MeJA) exposure, transgenic Arabidopsis plants carrying [β-glucuronidase (GUS) fused to 2.5 kb TGG1 promoter]; (pBITGG1-GUS)] were used. PBITGG1-GUS plants were exposed to MeJA for 3 and 6 days, respectively. Six days MeJA treated leaves showed very weak GUS staining in older leaves. In parallel, MUG-(4-methyl umbelliferalone glucuronide) assays showed lower activity in MeJA treated plants. In order to observe the effect of dehydration stress, we exposed wild type (wt) and TGG1 knockout (KO) plants, which had been conditioned for growth on MS-agar plates, to dehydration, we exposed wild type (wt) and TGG1 knockout (KO) plants, and above-ground organs. To analyse TGG1 expression against methyl Jasmonate (MeJA) exposure, transgenic Arabidopsis plants carrying [β-glucuronidase (GUS) fused to 2.5 kb TGG1 promoter]; (pBITGG1-GUS)] were used. PBITGG1-GUS plants were exposed to MeJA for 3 and 6 days, respectively. Six days MeJA treated leaves showed very weak GUS staining in older leaves. In parallel, MUG-(4-methyl umbelliferalone glucuronide) assays showed lower activity in MeJA treated plants. In order to observe the effect of dehydration stress, we exposed wild type (wt) and TGG1 knockout (KO) plants, which had been conditioned for growth on MS-agar plates, to dehydration under laminar flow conditions for 30 min. Transcriptional analysis of dehydrated TGG1 (KO) vs wt plants showed upregulation of Aquaporin TIP2.3, Osmotin 34, Dehydration-responsive element-binding protein, Dehydrin XERO2, and Dehydrin Rab 18 genes; reported to be abscisic acid and cold responsive. The results from MeJA experiments highlight that long term exposure of MeJA lowers TGG1 expression, while dehydration experiment shows TGG1 (KO) plants to be more stressed as compared to wt plants.

P09-015 Baptism by osmotic stress: post germination seedling immersion alters ABA relations and improves establishment and crop yield. ABA biosynthesis and signalling are involved in the control of specific phases of development with a large protective effect against environmental stresses via expression of protective proteins (e.g., dehydrins, LEA). It has been hypothesized that, at the early post-germination stage, seedlings monitor the osmotic environment with physiological consequences manifested during the transition to vegetative growth. The application of osmotic stress (~0.5 MPa PEG) to young tomato seedlings (termed ‘osmopriming’) just after germination by complete immersion for a 5-day period, induced adaptation to both drought and salinity stress, allowing greater vegetative biomass production and maintenance of a better water and photosynthetic status. ABA analysis of the young leaves revealed important differences between control and osmoprimed plants when cultivated under optimal and stress conditions. In the absence of stress, the ABA levels in the adult osmoprimed plants were increased by 7-times when compared to the non-osmoprimed plants, while an additional 4-fold increase was registered under high salinity. The physiological and molecular roles of the ABA changes of osmoprimed plants are under investigation. These phenomena may facilitate plant adaptation to harmful conditions and could result from the retention of a stressful memory.

P09-016 Influence of drought stress on photosynthesis in representative species of the different C4-subtypes and in a C3 species from the genus Panicum

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Drought stress is one main environmental factor limiting photosynthesis (A) of plants and due to the climate change drought problems in the world’s agriculture will increase. C4 plants have a higher water use efficiency than C3 plants and great economic significance as crops and weeds. Panicum is one of the few plant genera, which include C3-, C3/C4- and all three subtypes of C4-plants and is thus a good model to compare drought stress effects in phylogenetically related species with different metabolic types. Drought stress was induced both in soil grown plants and by the application of different PEG concentrations in hydroponically grown plants. In all examined species (C3: P. bisulcatum, NADP-ME: P. bulbosum, NAD-ME: P. miliacum, PKC: P. maximum), drought stress lead to a decrease of growth and of net photosynthesis. The photochemical PSII chlorophyll fluorescence quenching parameter qP decreased also in drought stressed plants compared to the controls. The cellular origin of chlorophyll fluorescence is different in the investigated metabolic types and was made visible by fluorescence microscopy. ACO2-curves indicate a nonstomatal limitation of A and hence explain the decreasing qP under drought stress conditions. However, in vitro activities of PEPCase and several other C4 enzymes were measured in control and drought stressed plants and can be excluded as limiting factors for A, indicating feedback or regulatory inhibition of A in vivo in the drought-stressed plants.

P09-017 Do plants and animals share a similar pathway in the mitochondria for the onset of stress-induced PCD?

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The mitochondrial electron transport chain involves two pathways: the cytochrome pathway and the alternative oxidase (AOX) pathway. In plants, heat stress induces the release of cytochrome c (cyt-c) from the respiratory chain, which in turn leads to elevated levels of Reactive Oxygen Species (ROS) and induction of AOX. Moreover, the activation of caspases and the onset of PCD have been reported in animals, but are not yet identified in plants. We investigated the above pathways under salt stress. Fully grown tobacco plants were treated with 250 mM NaCl for 24 h. Northern and western blot analysis revealed an induction of AOX, coinciding with elevated levels of ROS. Also the immunoreactive cyt-c protein in mitochondrial fragments was significantly reduced. Addition of salicylhydroxamic acid (SHAM), an inhibitor of AOX, reduced respiration of stressed plants. Furthermore the release of cyt-c coincided with the activation of caspases and the onset of PCD as indicated by TUNEL analysis. A change in the mitochondrial transmembrane potential was also evident in stressed plants. In conclusion, we propose that stress metabolism was also evident in stressed plants. In conclusion, we propose that animal and plant systems share a similar model for the onset of PCD under abiotic stress conditions consisting of the change in the transmembrane potential of the mitochondrion, exit of cyt-c, ROS accumulation and activation of caspases.

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**P09-018 Ascorbate-dependent cytochromes b561, new players in plant iron metabolism and oxidative stress metabolism**

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Cytochromes b561 (Cyt b561) are a newly identified class of transmembrane proteins, using ascorbate as an electron donor. These proteins have been demonstrated to transfer electrons across the membrane in which they are embedded, but their physiological role remains unclear. We have identified four Cyt b561 isoforms (AtCytb1-4) in Arabidopsis and are characterizing their mechanism of action using biochemical, molecular biological and physiological approaches. Several lines of evidence suggest that the plant Cyt b561 are involved in iron metabolism and in oxidative stress responses (1) a knock-out in one of the four Cyt b561 identified from Arabidopsis demonstrates a particular phenotype under iron deficiency; (2) the recombinant AtCytb1 protein can be oxidized by iron chelates; (3) the AtCytb1 gene appears upregulated under iron-deficiency conditions; and (4) in vivo experiments with AtCytb1 expressed in yeast demonstrate its ferric-reductase capability. Recent experiments however also demonstrate that the AtCytb1 knock-out plants show a particular phenotype under oxidative stress conditions. Strongly reduced root development is observed in the mutant plants when treated with pararquat. These results suggest that Cyt b561 may provide a link between plant iron metabolism and oxidative stress phenomena, using ascorbate as the electron donor.

**P09-019 The regulation and evolution of the chloroplast antioxidant network**

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The chloroplast antioxidant system is a combinatorial network of low molecular weight antioxidants and antioxidant enzymes, which protects plants from photooxidative damage. Evolutionarily, the genes involved are of endosymbiotic and heterotrophic origin. In present day plants, all enzymes are nuclear-encoded and are post-translationally targeted to chloroplasts. Analysis of over-expressors and silenced lines demonstrated that the balance of the antioxidant system is highly delicate and demands for gene-specific regulation. Based on genome analysis, we hypothesize that the regulation of the chloroplast antioxidant system evolved under the selective pressure of photooxidative stress in a gene- and plant group-specific manner. In Arabidopsis the thaliana nuclear transcription of genes encoding chloroplast antioxidant enzymes responds to chloroplast signals. Light- and ABA-responsive motifs in the promoter cores are combined with redox-, sugar- and ABA-responsive peripheral cis-regulatory elements. The integration of various signals on each promoter enabled a fine-tuning mechanism. Especially the antagonistic regulation of genes encoding enzymes with complementary catalytic properties supports the stability of the antioxidant system. Exemplarily, data on 2-Cys peroxiredoxin-A and ascorbate peroxidases will be shown. The antioxidant network of Arabidopsis thaliana will be compared with that of cryptophytes and basic eukaryotes.

**P09-020 Effects of Cd, Pb, chilling and drought treatments on activity of five antioxidant enzymes and free proline level in Albizia leaves**

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Cd and Pb induce oxidative stress with overproduction of ROS. To combat oxidative damage, plants possess antioxidative defence enzymes that remove, neutralize and scavenge the ROS. Drought and low temperature may interact with oxidative stress. We have investigated effects and interactions of such stresses in the legume Albizia julibrissin. Four-month-old plants grown in sand cultures with a modified Ingestad medium were subjected to various single or combined treatments involving exposure to 0.05–0.25 mM Cd, 1–5 mM Pb, periodic chilling (CH) at 4°C, or drought (DR), for 1 to 6 weeks. Upon harvest, leaves were extracted and assayed for activity of catalase (CAT), glutathione-disulfide reductase (GR), glutathione peroxidase (GSHPxs), guaiacol peroxidase (GPxs) and ascorbate peroxidase (APxs). CAT activity decreased 50–80% with increasing Cd or Pb exposure and in CH and DR treatments. GR activity was upregulated 50–300% in all treatments, most strongly at high Cd or Pb, and in combination with CH or DR. GPxs showed a similar trend of increase. Only minor changes were observed for GSHPx and APxs. Massive accumulation of free proline in highly stressed plants started in week 2 of Pb, and in week 3 of Cd treatments. It was indicated that GSH-linked redox systems replaced CAT under stress. Increased foliar proline may alleviate water stress, act as an antioxidant, and maintain higher levels of glutathione and phytochelatin synthesis.
**P09-021** Characterization of a SOS3-like calcium binding protein and SOS2-like protein kinase genes from tomato (Solanum lycopersicum)

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SOS1 plasma membrane Na⁺/H⁺ antiporter constitutes an essential element in plants for ion homeostasis under saline conditions. In the SOS pathway, previously described in Arabidopsis, a calcium-binding protein, SOS3, senses cytosolic calcium changes elicited by salt stress. SOS3 physically interacts with and activates the protein kinase, SOS2. The SOS3/SOS2 kinase complex phosphorylates and activates the transport activity of the plasma membrane Na⁺/H⁺ exchanger encoded by the SOS1 gene. We have identified the genes encoding the regulatory proteins in the SOS pathway in tomato: SISOS2 and SISOS3. On the basis of amino acid comparisons, SISOS2 and SISOS3 show 71% and 70% identity respectively with the Arabidopsis genes AtSOS2 and AtSOS3. The system has been functionally reconstituted in yeast proving to be true ortholog of AtSOS. Gene expression associated to salt stress was studied in the tomato wild species Solanum pimpinellifolium and the cultivated species S. lycopersicum cv Moneymaker known to be salt tolerant and salt-sensitive, respectively. SISOS2 is strongly and constitutively expressed in all tissues in S. pimpinellifolium whereas gene expression increased remarkably under salt stress in leaves and especially in roots in the cultivated variety. SISOS3 is only expressed in roots and its transcript content was much lower under saline conditions in the tolerant species. These results suggest a relevant role of the genes in the SOS pathway on halotolerance in tomato.

**P09-022** Redistribution of Ca²⁺ in wheat root protoplasts under anoxia-reoxygenation: involvement of the mitochondrial uniporter

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Isolated wheat root mitochondria and Ca²⁺-sensitive dye Arsenazo III were used to monitor the properties and kinetics of mitochondrial Ca²⁺ transport. Confocal microscopy was employed to assess the relationship between cytosolic Ca²⁺ and the physiological state of mitochondria under oxygen deprivation. Ratiometric Ca²⁺-sensitive probe Indo1-AM and TMRM were co-loaded to wheat root protoplasts. Imposition of anoxia resulted in an overall decrease in TMRM fluorescence. In normoxic protoplasts no significant changes were detected. The onset of anoxia was clearly detected by increased cytoplasmic Ca²⁺ – clone 106/54/0; 3. Salix matsudana – clone 68/53/1; 2. Salix alba – clone 106/54/0; 3. Salix matsudana – clone SM 4041; and 4. Salix nigra – clone 0408) were exposed to elevated concentrations of Cd, Ni and Pb in hydroponic solutions

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Four clones of Salix (1. Salix alba – clone 68/53/1; 2. Salix alba – clone 106/54/0; 3. Salix matsudana – clone SM 4041; and 4. Salix nigra – clone 0408) were exposed to elevated concentrations of Cd, Ni and Pb-EDTA in water culture solution. The aim was to determine the phytoextraction potential of the investigated willow genotypes. Translocation ratio to upper plant parts was very low for all applied heavy metals and therefore, the metal uptake was restricted to the roots. Signs of metal toxicity to the plants regarding applied metal concentration of 10⁻⁴ M was significant, especially for Cd and Ni. Toxicity of Pb was much lower, since the translocation of Pb to the green plant parts was very low, and therefore shoots and leaves were protected from the toxic effect of Pb. The ability of clones to participate in the fine tuning of Ca²⁺ cyt. We suggest that mitochondrial Ca²⁺ transport is one of the main regulatory mechanisms for maintenance of Ca²⁺ homeostasis under stress.

Pollution and climate change have become important subjects. Ozone is one of the performers involved in both. It is responsible for 8% of the greenhouse effect and is a toxic secondary pollutant. Its tropospheric concentration has increased in the last century and peak concentrations on sunny days are frequent. This study deals with the effects of ozone on poplar leaves. Poplar trees were submitted to 120 ppb of ozone for one month. Growth was barely affected, yet visual symptoms like necroses and chlorosis appeared on mature leaves. Pigment quantification showed a decrease in chlorophyll a, b and lutein, explaining the chlorosis. Leaf loss was increased by the treatment but not leaf formation. Differential 2DE revealed changes in primary carbon metabolism and in some other mechanisms. Rubisco activase was negatively affected very early, followed by a decrease in the abundance of enzymes of the Calvin cycle and associated to the electron transport chain of the chloroplast, revealing a strong impact of ozone on photosynthesis. Opposite effects were observed in carbon catabolism, where one enzyme of glycolysis and mitochondrial respiration each increased in abundance simultaneously to changes in photosynthesis. An increased abundance of enzymes and proteins involved in detoxification and protein folding was also detected. Many of these effects could be linked to an increased senescence, but only 30% of the proteins significantly different in ozone stress are common to leaf ageing.

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extract and translocate Cd, Ni and Pb differed depending on the quantity of metal content in the nutrient solution. Phytoextraction of metals was clone- or genotype-specific. Therefore selection of specific clones (genotypes) rather than species, based on environmental data on each contaminated site, should be performed. The ability of investigated clones to accumulate Cd in leaves (S. alba = 47.6 ± 6 µg g⁻¹ of dry weight; S. nigra = 507.5 µg g⁻¹) is to our knowledge, the highest so far recorded compared to other hydroponic trials in literature. Preference for Cd influenced root growth was determined. This clone-specific response could be a part of a mechanism for Cd resistance.

P09-025 Effect of red and blue light on acclimation of Chlamydomonas reinhardtii to CO₂-limiting conditions
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Effects of red (RL) and blue (BL) light on acclimation of the microalga *Chlamydomonas reinhardtii* to carbon dioxide limitation were studied. The algal cells grown at 5% carbon dioxide and under white light had a relatively low extracellular carbonic anhydrase (CAex) activity, low affinity for dissolved inorganic carbon (Ci), and low CO₂-limited rate of photosynthesis. Exposure to ordinary air under RL or BL (each was 150 μmol m⁻² s⁻¹) caused acclimation of these cells to the carbon dioxide limitation. The acclimation was manifested in a significant increase in the CO₂-limited rate of photosynthesis, the affinity for Ci, and the CAex activity with no difference between RL- and BL-cells. The acclimation completed after 5–7 h of the cell air exposure to either light. In addition to the carbon dioxide acclimation, the alga exhibited photosynthetic adaptation to light quality under RL- or BL-air conditions. As is evident from RL- and BL-dependent chlorophylls changes, this process started 4 h later than the acclimation to limited carbon dioxide, meaning that the low CO₂-induced changes were exhibited by the alga with no disturbance by the spectrum induced changes in the photosynthetic apparatus. Based on the similarity of the low CO₂-induced changes under RL and BL, we concluded that RL and BL had the same effects on acclimation of *C. reinhardtii* to carbon dioxide limitation.

P09-026 Biological diversity of plant ozone sensitivity
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Short and high pulses of the atmospheric pollutant ozone cause the formation of reactive oxygen species (ROS) in the apoplastic space of leaves. Numerous similarities between the plant responses to ozone and pathogens suggest that ozone triggers hypersensitive response-like programmed cell death (PCD). The model plant *Arabidopsis thaliana* has been instrumental for studying mechanisms regulating plant ozone sensitivity. For example mutants, like vtc1 and rcd1, screened on the basis of their ozone sensitivity, have helped to understand the role of ascorbic acid and to identify proteins involved in plant hormonal signaling during oxidative stress. The genetic variation that exists among naturally occurring populations of *Arabidopsis* is so far largely untapped source of genetic information in ozone research. Many *Arabidopsis* ecotypes have been collected from wild populations growing throughout the world and phenotypic variation among ecotypes should reflect also in the genetic variation that is important for adaptation to specific conditions. We have screened a large collection of *Arabidopsis* ecotypes for ozone sensitivity and they display a large range of ozone induced damage, ranging from extremely tolerant to hypersensitive ecotypes. The mechanisms behind ozone sensitivity of different ecotypes seems to be largely determined by stomatal regulation of ozone uptake.

P09-027 Degradation-effects of shading on individual leaves
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It is generally assumed that shaded plants show greener than light-grown plants. However, when light-grown plants are shaded they undergo a Shade Avoidance Response which increases the length of their petioles while slowly degrading their photosystems, causing them to become less green. Similarly but different, when individual leaves are shaded, their leaf morphology doesn’t change, but their photosystems are adjusted and degraded relatively fast. Whereas the adjustment might be ascribed to Photosynthetic Acclimation (PA), the degradation shows a resemblance to Dark-Induced Senescence (DIS), a degradation-process in which the liberated nutrients are relocated to the better-lit parts of the plant. To study this, we use a model system in which individual leaves are shaded or darkened with envelopes, allowing sink-source interactions to be maintained by keeping the leaves attached to the plant. By changing the transmitted light-intensity and -quality with the envelopes we try to determine when and to what extent the degradation process is initiated and what the important factors are. Our studies show that the extent of the degradation in individually shaded leaves is dependent on the light intensity and influenced by phytochrome A, but independent of the R/FR ratio at these intensities.

P09-028 Could grass pea be used in phytoremediation systems for lead?
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Grass pea (*Lathyrus sativus* L.) is an under-utilised crop with unique adaptation features to abiotic stresses, such as drought, poor soil conditions and waterlogging. Its capacity to grow in the presence of heavy metals, such as lead in the soil solution is uncharacterised to date. In the present study, young grass pea plants were grown in modified Hoagland medium for 96 h in the presence of Pb(NO₃)₂ (0.5 mM), along with control plants. Two lines, B and R, originating from Bangladesh and India, respectively, were considered. In addition to tolerance indexes, transcript accumulation for two endopro
teinases (cysteine and aspartic proteases) and a heat shock protein (HSP70), considered here as molecular stress indicator, were studied. The amounts of absorbed lead and calcium contents in the roots were determined by ICP-OES. Results showed that all plants survived despite a reduction in biomass production. Moreover, both grass pea varieties were able to accumulate lead into their roots, with higher amounts for B plants than for R plants. In addition, HSP70 transcription accumulation was more important in B roots. Lead-exposed plants showed a six-fold reduction in root calcium contents compared to control plants. Together, these results suggest that both L. sativus L. are tolerant to lead and to calcium deficiency and able to store large amounts of lead in its root tissues under our experimental conditions. Therefore, they could be included in bioremediation systems for lead.

P09-029 Zymography as a tool in revealing the heterogeneity of serine protease response to drought in common bean (Phaseolus vulgaris L)
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Total or limited protein breakdown, which may be programmed or uncontrolled, has an important function in the complex response of plants to abiotic stress. The diversity of plant proteolytic enzymes complicates the study of their role in this process. Serine proteases (SPs) have only rarely been reported in this context. Our aim has been to test the prediction that they are involved in the response of common bean (Phaseolus vulgaris L.) to drought. We developed a procedure, involving zymography with fluorescent substrates, which has revealed the heterogeneity of SPs. Zymography combined with ion exchange chromatography has enabled us to detect and quantitate relative proteolytic activities in bean leaves. Levels of several proteases changed in different ways under water deficit. The majority have been identified as SPs, based on their inhibition by specific inhibitors. Three are serine endopeptidases with different substrate and inhibitor specificities. Three others are aminopeptidases with substrate preferences against L-alanine-p-nitroanilide, L-leucine-p-nitroanilide and L-phenylalanyl-p-nitroanilide. The third kind of aminopeptidase has not been described in plants. These SPs have been identified to a level that allows further biochemical characterization and study of their gene expression. Our results point to there being a number of roles for SPs in the plant response to water stress, which can range from enhanced protein turnover to limited proteolysis at specific sites.

P09-030 Expression patterns of stress related proteins in gametic embryogenesis of Quercus suber L.
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Cork-oak (Quercus suber L.) is an important forest tree of Mediterranean ecosystems. Cork production from cork-oak supports an industry relevance from the southern Europe countries. A protocol for the production of doubled-haploids of cork oak has been developed through anther embryogenesis. Bueno et al (1997) achieved the induction of gametic embryogenesis by combining a stress treatment (starvation with heat shock) applied to anthers cultured in a simple agar medium without growth regulators. Those microspores leave the gametophytic pathway and react shifting their development to the sporophytic pathway by means of which haploid embryos are obtained. Later on, those embryos develop into haploid plants that can be converted into doubled-haploids. The proteome analysis of gametic Q. suber in vitro culture derived embryos was conducted using DIGE and MALDI-MS/MS, reporting for the first time proteomic data on this species. Specially increased levels of actin were reported, actin is involved in pollen development and gametic embryos were induced in immature pollen grains. Furthermore, diverse expression patterns for stress related proteins have been detected in these gametic Q. suber L. embryos, being stress the key for this embryogenesis induction method. Also other proteins involved in a variety of cellular processes have been analyzed, most of which had neither been previously associated with embryo development nor identified in the genus Quercus.

P09-031 Ozone stress on woody plants, detected by the chlorophyll a fluorescence transient (FT)
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This paper re-analyzes fluorescence data from open-top chamber (OTC) experiments, by different research groups, in order to individuate some general features of ozone stress on woody plants. The experiments were carried out by Swiss and Italian research groups at the experimental research facilities of the Lattecaldio (Switzerland) and Curno (Italy) forest nurseries. Chl a fluorescence transients of intact leaves were measured by means of direct fluorescence (applying the so-called JIP-test) at different times during the seasons on several tree species seedlings. Ambient ozone concentrations lead to the closure of reaction centres (RC), which function as dissipater centers. All the parameters connected to dissipation were also increased. The quantum yield efficiency (Fv/Fm) demonstrated only little sensitivity. The response was not proportional to ozone exposition and/or fluxes. During the first part of the season, leaves were very resilient and photosynthesis could be transiently stimulated by ozone. Only towards the end of the growing season, efficiency and performance parameters showed a sudden drop. The comparison of the shape of FT normalized per F0 and FM and per F0 and FJ shows evident peaks at the steps K, J and I. K indicates the reduced efficiency in the water splitting system. J indicates a QA accumulation in the single turnover region. I-peak seems to be more specifically connected to ozone stress due to the inactivation of Rubisco.
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P09-032 Contribution of proline and glycine betaine to the osmotic adjustment in durum wheat under salinity

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Soil salinity, resulting from natural processes or from crop irrigation with brackish water, occurs in many arid and semi-arid regions of the world. It is one of the major environmental constraints to crop productivity in the Mediterranean region. To prevent ion toxicity, plants sequester salt ions in the cell vacuole and accumulate compatible solutes to osmotically balance the other subcellular compartments. Proline and glycine betaine are the main nitrogen-containing osmolytes found in durum wheat under salt stress. Their accumulation is ontogenetically controlled but not synchronous: proline contributes early, at the onset of the stress, while glycine betaine contributes mainly when the stress is prolonged. At high nitrate proline accounts for more than 39% to the osmotic adjustement of old leaves. Its N-dependent accumulation may offer an important advantage, as it can be rapidly broken down upon relief of stress to provide sufficient energy, carbon and nitrogen from the older leaves to younger tissues. Whereas the contribution of glycine betaine is higher in young leaves and independent of nitrogen nutrition.

P09-033 Cadmium effects on some metabolic aspects and oxidative stress in Amaranthus lividus L. plants

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Stress responses of leaf (1) chlorophylls, carotenoids, protein and sugar contents; (2) antioxidant enzyme activities and lipid peroxidation; (3) metal stress defense through proline production in young plants of Amaranthus lividus exposed to 0, 0.5, 2.5, 50, 50 mM Cd for 96 h were studied. Chl a decreased, Chl b increased significantly and carotenoids did not change; the chlorophyll/carotenoids ratio increased significantly; protein increase was significant at the highest Cd concentration. Sugar content increased significantly at 5 mM. Lipid peroxidation increased significantly at 0.5, but showed a significant decrease at 25 mM. Proline content showed a significant increase at 50 mM. Fluctuations in antioxidant enzyme activities were observed. It seems that Amaranthus lividus plants are less affected by Cd concentrations; a Cd sequestering mechanism could be operating to prevent the deleterious Cd effects. Amaranthus lividus could be a potential species for Cd phytoextraction in contaminated soils, based on its high Cd accumulating ability in shoots tolerance to oxidative stress and compatibility with mechanized cultivation techniques.

P09-034 Involvement of antioxidants in photoprotection of lichens

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The aim of this study was to evaluate the effect of high light on antioxidants content in different lichen species. Several lichen species were collected in Norway (Peltigera canina, Lobaria pulmonaria, Lobaria scrobiculara) and in Czech Republic (Hypogymnia physodes, Lasallia pustulata). Lichen thalli were hydrated and exposed to three light treatments differing in intensity and length. Contents of photosynthetic pigments (including xanthophylls), glutathione (both oxidized and reduced form) and tocopherol were analyzed in samples before and after each treatment. Comparison of selected species shows different antioxidants content in relation to different light habitat (light/shade adapted species) and different photobiont (green/blue algae). These results, combined with chlorophyll fluorescence measurements, give better insight in the photoprotection of lichens against excess light.

P09-035 Identification of transcription factors that regulate CBF3 expression in rice

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Rice is one of the major food crops worldwide and is considered as cold sensitive due to incapability of cold acclimation. Cold stress in plants is a complex phenomenon that adversely affects the physiological, agronomical and quality traits in rice. It has been shown in different plants species that the family of transcriptional factors CBF/DREB1 play a key role in cold acclimatization. CBF1 and CBF3 gene expression is highly induced by low temperatures and their overexpression in various plants confers cold tolerance. In the present investigation, we are focussing on identification and characterization of novel transcription factors (TFs) that regulate the expression of OsCBF3 using the Yeast-one Hybrid (Y1H) system and a cold induced rice cDNA expression library. For this, we have partitioned the promoter region of OsCBF3 in four different overlapping fragments namely CBF3-1, CBF3-2, CBF3-3 and CBF3-4 to construct the bait strains. Since the leaky expression of CBF3-1 bait strain was not possible to eliminate with 3-AT up to 50 mM, we have divided the CBF3-1 fragment into CBF3-1A and CBF3-1B and the leaky expression is being investigated. The Y1H screening for the identification of new TFs binding to the OsCBF3 promoter is under way. Presently, we have already identified two putative TFs and are expecting to isolate some more. TF – promoter interaction will then be validated by both re-arrangement of the bait strains and gel shift assay and the results discussed.

P09-036 CO2-media and hypoxia effect on activity and enzyme characteristics of plant antioxidant system

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Activity of catalase (CAT), ascorbate peroxidase (APOX), NADH-peroxidase (NADH-POX), glutathione peroxidase (GPOX), common peroxidase (POX) under hypoxia (3–24 h) and CO2-media in intolerant (wheat, pea) and moderate (maize, soy) plants was studied. Enzyme
activity was defined spectrophotometrically (de Marco, 1996), content of hydrogen peroxide by peroxidase method (Yimees, 1998). Accumulation of hydrogen peroxide was observed in wheat and pea seedlings under hypoxia during all analysis. CO₂-media increased hydrogen peroxide content three-fold in wheat cells, 1.5-fold in pea cells. In more tolerant soy and maize in CO₂-media the accumulation of hydrogen peroxide was observed at exposition end. During first hours its content in soy cells was lower than in aerated plants on 10–20%. Correlation between plant tolerance to hypoxia and activity of CAT, POX, APOX, NADH-POX in analyzed plants was detected. CAT activity was significantly rising in first 3–6 h of hypoxia. Under hypoxia prolongation the role in ROS detoxication was transferred to peroxidase group of enzymes. Activity of POX in legumes and of POX, APOX, NADH-POX in cereals was rising. Enzymes properties were also changing under oxygen deficit. Km and Vmax of CAT and APX in pea and soy were falling. Short term exposition to CO₂-media induced significant activity and enzyme properties changes. Obtained data shows important role of carbon dioxide in adaptation of different plant groups to hypoxic stress on antioxidant system level.

**P09-037** The use of relaxation kinetics of chlorophyll a fluorescence to detect cold stress for *Chicorium intybus* L. (industrial chicory)

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The effect of cold stress on the light reactions of photosynthesis of young chicory plants was analysed by measuring chlorophyll fluorescence induction and relaxation curves. Measurements were done either continuously (experiment 1) or with point measurements at key moments (experiment 2). In experiment 1, VL49 (inbred line with slow early vigour) and Hera (variety with fast early vigour) were tested at 10 and 2°C. At 10°C, non-photochemical quenching (NPQ) was low and remained the same for both (VL49 and Hera). At 2°C, average NPQ for VL49 and Hera increased with a factor 10 and was 13% higher for VL49 compared to Hera. Furthermore, at the end of the relaxation NPQs (slow NPQ) was 87% higher for VL49 compared to Hera, indicating more damage or slower relaxation. In experiment 2, VL49, Hera and Eva (variety with intermediate early vigour) were tested at 2°C and 220 and 400 μmol quanta m⁻² s⁻¹. At 220 μmol quanta m⁻² s⁻¹, no differences in NPQ values between VL49, Hera and Eva were observed. At 400 μmol quanta m⁻² s⁻¹, NPQ was on average 21% higher for VL49 and Eva compared to Hera. At 220 and 400 μmol quanta m⁻² s⁻¹, NPQs was the same for Hera for both light intensities, indicating no damage of photosystem II. In contrast, NPQs of VL49 and Eva increased 5 and 30%, respectively, with increasing light intensity. These results show the possibility to (1) use relaxation kinetics to detect cold stress; and (2) use point measurements to measure more samples in less time.

**P09-038** The impact of drought stress on the expression of selected genes in tobacco plants

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Drought response was compared in tobacco plants with modulated content of plant hormones cytokinins (CKs), over-expressing transzeatin O-glucosyltransferase (*ZOG1*) gene from *Phaseolus lunatus* either under constitutive (35S promoter (uniform CK elevation) or under senescence-inducible (*SAG12*) promoter, and the correspond- ing wild-type. Dynamics of the expression profile of four selected genes was followed in the individual leaves and in roots during the drought stress progression and recovery. Monitored genes were related to the plant response to water stress (dehydrin *NIRD108*), senescence (activity of *SAG12* promoter determined as *SAG12::ZOG1* expression), degradation of osmoprotectant proline (gene coding for proline dehydrogenase - *cig1*) and regulation of the stability of chloroplast transcriptome (chloroplast endoribonuclease: *CSP41*). The expression of dehydrin gene quickly increased in the whole plant after water supply cessation and quickly decreased after rehydration. The *cig1* expression exhibited fast decrease at drought, but only gradual elevation after re-watering. *SAG12* promoter activity depended strongly on the leaf position, being stimulated starting from the lower leaves. After rehydration *SAG12* activity fell down very quickly. Opposite profile was observed in case of *CSP41*, expression of which was higher in upper leaves, being diminished during the stress.

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**P09-039** Peroxidase isoenzymes pattern and total activity in tubers of potato cultivars differing in dehydration tolerance

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Potato as compared to other crops is very sensitive to soil drought conditions. Even very short period of water shortage has negative effect on consumptive and technological properties of tubers. Fluctuations in water supply of potato plants may cause irregular distribution of different metabolites in tubers. Peroxidase seems to be involved in diverse physiological processes and plant defence mechanisms including responses to environmental stresses. Peroxidase is also suggested to be an enzyme responsible for tuberisation of potato plants. Therefore, the question arises whether 10 day soil drought applying in tuberation phase of potato development affects activity and pattern of isoenzymes of peroxidase. Thus, activities of peroxidase in slices of potato tubers of two cultivars Tajfun and Cokin which had been grown in pots with optimal water supply and with water shortage at tuberisation phase have been investigated. It was shown that Tajfun is cultivar with higher dehydration tolerance than Cokin cultivar. The higher dehydration tolerance of Tajfun cultivar was accompanied by the higher total peroxidase activity in tubers of plants growing thorough experimental period in soil with optimal water supply. The total activity of peroxidase decreased with increasing water deficit at tuberisation phase independently of plant dehydration tolerance level. The electrophoretic pattern of peroxidase isoforms depends both on genotype and on sensitivity to water supply of potato plants.

**P09-040** Photosynthesis and water relations in Brazilian sugarcane

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The expansion of sugarcane (Saccharum officinarum L.) in Brazil has been boosted by the growing demand for bio-fuels all over the world and requires the occupation of new agricultural areas, principally in regions with unfavorable agro-climatic conditions. Understanding of physiological behavior under particular environmental conditions is fundamental to plant breeding programs aimed at selecting the genotypes more resistant to drought stress. The objective of the work was to determine how photosynthetic activity and transpiration can be influenced by the stomatal. Twenty-eight genotypes of Brazilian sugarcane variety or genotypes were studied in field conditions at several water status. It was noted that the stomatal conductance had a much greater control over photosynthesis than over transpiration. Even under high stomatal conductance, photosynthesis maintained a high correlation to it, suggesting that the selection of genotypes for greater stomatal conductance should be the most productive. Under drought stress, the variety RB92579 maintained a leaf water potential lower than the other varieties studied and had better efficiency in water intake, thus maintaining a good stomatal conductance, with a higher level of transpiration and photosynthesis than the others under drought stress. These results suggest that this variety has a tolerance mechanism to endure rather than avoid drought.

P09-041 Effects of exogenously applied ascorbic Acid on red cabbage cotyledons subjected to copper excess
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Ascorbic acid (AsA) is one of the most important and abundantly occurring water soluble antioxidants in plants. In order to assess whether exogenous application of AsA through the growing medium could modulate the antioxidant activities of red cabbage cotyledons in copper tolerance, a hydroponic experiment was conducted under greenhouse conditions. Seedlings subjected to 0 and 100 μM CuSO4 solution were supplemented with 0 and 100 mg L−1 AsA for 10 days. Changes in the levels of several important parameters associated with oxidative stress, lipid peroxidation and antioxidant enzymes were measured. The effect of treatment with 100 mg L−1 AsA alleviated the inhibitory effects of Cu on antioxidant enzyme activities. The level of the Cu-induced accumulation of active oxygen species, peroxidase activity and lipid peroxidation in seedlings treated with AsA were lower than in untreated seedlings. In addition, exogenous application of AsA increased endogenous level of AsA which had a protective effect on growth, pigment content and enzyme activities of red cabbage against Cu-induced oxidative stress. The data suggest that exogenous application of AsA may protect cells against oxidative damage and Cu toxicity. Key words: Ascorbic acid, red cabbage, copper, antioxidant activities.

P09-042 Dark induction of xanthophyll cycle mediated by desiccation
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The xanthophyll cycle, which comprises Violaxanthin (V), Antheraxanthin (A) and Zeaxanthin (Z) carotenoids, modulates the rate of thermal energy dissipation in most of the plants. In high light, V is de-epoxidized to Z, via the intermediate A, by the enzyme V-de-epoxidase (VDE). The activity of this protein is controlled by the light-induced acification of chloroplast lumen. Not only the high light, but also other environmental stress factors can modulate the conversion of V into Z. We studied the xanthophyll cycle, in the poikilohydric fern Asplenium ceterach L., during a desiccation/re-hydration cycle in the dark. Whole plants, collected from field, were dark desiccated and re-watered afterward. Dehydrated plants showed a strong reduction of Fv/Fm values and Z formation. When plants were rehydrated, Fv/Fm was recovered and Z content was reduced. To test whether VDE was responsible for the dark formation of Z during dehydration process, plant fronds were pre-treated with DTT (a VDE inhibitor). DTT completely inhibited V conversion into Z in desiccating fronds, but Z was accumulated as result of β-carotene hydroxylation. Furthermore, Z formation in dark was also observed in dehydrating leaves of other homeohydric vascular plants. Plants could trigger this photoprotective mechanism in dehydration conditions, for faster acclimation when appropriate conditions return.

P09-043 Metabolic and cell structural variations produced by Cd in Zea mays, Hordeum vulgare and Pisum sativum
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Experiments with two gramineae (Zea mays and Hordeum vulgare) and one legume (Pisum sativum) were performed in order to analyse the tolerance or sensitivity to Cd, based on its effect in dry matter production, Cd uptake, lipid peroxidation and cell structure in leaves, roots and nodules. Plants were subjected to concentrations of Cd (0 and 100 μM Cd). Cadmium supply inhibited biomass accumulation and retarded the development of the three plant species. Cd accumulation in roots of Z. mays and H. vulgare was higher than in the aerial parts. Surprisingly, H. vulgare removed more Cd from the nutrient solution than Z. mays. Visual symptoms of toxicity in the leaves of the Cd treated plants were observed. Moreover, MDA content was more affected in the roots of the three plants than in the shoots. However, MDA content in pea nodules was less affected. Cd treated plants suffered slight alterations at structural level. The bundle sheath cells of Cd treated maize plants showed decline in the vacuolar content. Cell walls from barley roots cells appeared deformed as a consequence of the lost of turgor produced by the vacuolar content decline. Regarding nodules from pea plants, symbiosome degeneration with occasional peribacteridal membrane disruptions can be observed. Therefore, the data presented herein suggest that the three plants have a phytoexperator potential and that morphological changes occurred after metallo alteration.
**P09-044** The role of phenols in the modulation of apoplastic peroxidases and their role in Mn toxicity and Mn tolerance in cowpea (*Vigna unguiculata* L.)

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Typical Mn toxicity symptoms in cowpea are brown depositions in the leaf cell-wall. They consist of oxidized Mn and phenols. The oxidation of Mn(II) and phenols and the production of H$_2$O$_2$ catalysed by peroxidases (POD) in the leaf apoplast are considered as key reactions leading to Mn toxicity. A kinetic study of Mn toxicity revealed that an enhanced activity of apoplastic H$_2$O$_2$-producing NADH-peroxidase is among the most sensitive responses of the leaf to supra-optimal Mn concentrations. This NADH-peroxidase requires Mn and phenols as co-factors. For the investigation of apoplastic POD isoenzymes apoplastic washing fluid (AWF) of the Mn-sensitive cv. TuV 91 and the Mn-tolerant cv TuV 1987 was separated by Blue Native-PAGE. In-gel activity staining of PODs revealed qualitative differences in isoenzyme pattern between the cultivars not only regarding constitutively expressed but also Mn toxicity-induced POD isoenzymes. Specific isoenzymes of TuV 91 were further characterized regarding pH optimum and response to phenols. Different phenols exerted enhancing and inhibitory effects on the POD activities. Identification and quantification of Mn toxicity-induced changes in the apoplastic phenol composition supported the key role of phenols in Mn sensitivity and tolerance. The results presented underline the key role of the interaction of apoplastic PODs and metabolites in the development of Mn toxicity.

**P09-045** Influence of nickel stress on nitrogen metabolism in wheat shoots

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Nickel, similarly to other microelements, at excess concentrations is toxic for most plant species. The purpose of the present work was to get better insight into the mechanisms of Ni phytotoxicity, in particular its effect on nitrogen metabolism. The activities of glutamine synthetase (GS), NADH-dependent glutamate synthase (NADH-GOGAT), ferredoxin-dependent glutamate synthase (Fd-GOGAT), NADH-dependent glutamate dehydrogenase (NADH-GDH), alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT) as well as Ni, glutamate (Glu) and proline (Pro) contents were studied in the shoots of wheat plants treated with 50 and 100 μM Ni for 4 and 7 days. Wheat shoots responded to Ni stress with a transient reduction in Glu content and accumulation of Pro. The activity of Fd-GOGAT decreased after Ni application, while that of NADH-GOGAT was significantly enhanced. The activity of GS remained unchanged, however NADH-GDH and Glu-producing AlaAT and AspAT activities considerably increased. The results indicate that exposure of wheat plants to Ni affects the activity of Fd-GOGAT, considered as the main Glu-synthesizing enzyme in green tissues. Enhancement of NADH-GOGAT, NADH-GDH, AlaAT and AspAT activities in the shoots of Ni-stressed wheat may suggest the induction of alternative ways of synthesis of Glu, which is required for production of important protective compounds such as Pro and glutathione. This work was supported by University of Łódź Grant No 506/819.

**P09-046** The effect of heat stress on phytohormone levels in grapevine


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Plant responses to environmental changes are, at least partially, mediated by plant hormones. One of the most frequent abiotic stresses is heat. The effect of elevated temperature (40°C) on the content of phytohormones (cytokinins, auxin and abscisic acid) was followed in upper, middle and lower leaves and roots of two grapevine (*Vitis vinifera* L.) cultivars (Müller Thurgau and Blue Portugal). Heat stress decreased significantly levels of physiologically active cytokinins in leaves, and to lower extent, also in roots of both grapevine cultivars. Cytokinin storage forms O-glucosides were slightly increased in stressed middle and lower leaves of Blue Portugal. Cytokin in deactivation products N-glucosides and cis-zeatin derivates were not substantially affected by elevated tempera ture. Free auxin content was maintained in leaves and increased in roots of Blue Portugal, but gradually diminished in upper leaves of Müller Thurgau. Levels of abscisic acid were decreased after 1 h heat stress which might coincide with the necessity to increase the stomata aperture. After prolonged heat stress ABA increased.

**P09-047** Mode of action of the Asahi SL biostimulator

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Biotimulator Asahi SL, internationally known as Atonik, is used in many crops for years and its positive effect was proved in practice but mode of action of this compound is not understood yet. In this work an attempt was made to evaluate the effect of Asahi SL on Arabidopsis thaliana L. plants based on selected physiological processes and changes in profile gene expression. A. thaliana were grown in growth chambers under optimal and drought stress conditions. Asahi SL was applied either as supplement to nutrient solutions or as foliar spray at several concentrations. Data for plant growth and development, biomass accumulation, efficiency of photosynthetic apparatus, water status, and membrane integrity were collected. For the profile gene expression micro-array technique was applied. Asahi SL had diverse effects, for some parameters it was unstable and not always significant, but positive effect was clear. Asahi treated plants (at stimulatory concentrations) were more vigorous, better developed, taller, with longer inflorescences and roots. In general, they produced more biomass, had higher photosynthetic efficiency and transpiration, with no or minor changes in
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RWC. Asahi SL often reduced the negative impact of stress, apparently via improved efficiency of photosynthetic apparatus, better water status, and less damaged membranes. Profile gene expression was also affected by Asahi SL. Acknowledgement. This study was financed by Arysta LifeScience Ltd.

P09-048 Response of Arabidopsis thaliana L. plants to platinum in growing medium

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Platinum group element, although a noble elements, might have negative impact on living organisms when occur in higher concentration and some of their oxides are allergenic or carcinogenic. Modern cars with catalyzer emit platinum into environment, which can be a source of pollution. In this work we attempt at: (1) evaluation of platinum uptake by A. thaliana plants and its distribution between roots and rosette; and (2) comparison of changes in selected physiological processes elicited by Pt ions present in growing medium. Plants were grown in continuously aerated hydroponic culture with Hoagland’s nutrient renewed weekly. Six-week-old plants were exposed during 14 days to Pt(NH3)4(NO3)2 in concentrations: 5, 50, 500, 1000, 5000, 10 000 and 20 000 µg dm⁻³, added during nutrient solution change. Measurements on gas exchange, chlorophyll content, chlorophyll a fluorescence, were performed weekly. At harvest sub-samples for relative water content, platinum accumulation and distribution were collected and fresh and dry weights were recorded. Amount of Pt taken up by plants increased along with its concentration in medium. Up to 14% of total Pt in plants was transported to rosette. Platinum at higher concentration exerted negative effects on plants manifested by lowered biomass partitioning and leaf chlorophyll fluorescence parameters. Studies on Pt uptake by plants were done in Biología Aplicada del Segura (C.E.B.A.S.), Spain.

P09-049 Hormonal changes in relation to salinity-induced leaf senescence and shoot growth impairment in tomato (Solanum lycopersicum L.)

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Accelerated leaf senescence and decreased shoot growth are the most limiting factors to plant productivity under salinity. Plant hormones are believed to be involved in both processes. However, few studies have quantified the change in major plant hormones under salinity. Tomato plants (Solanum lycopersicum L.) were cultivated for 3 weeks under high salinity (100 mM NaCl); biomass partitioning and leaf chlorophyll fluorescence parameters were studied in relation to ion accumulation and changes in five major plant hormones (ABA; Z; ZR; IAA; and ACC). Salinity accelerated senescence of tomato leaves and impaired shoot growth. In prematurely senescent leaves, ABA content increased while IAA strongly decreased with the duration of exposure to salt, while IAA accumulated in the roots. Salinity dramatically decreased the total cytokinins in leaves and their root-to-shoot transport. Accelerated leaf senescence and decreased shoot growth may be attributed to different hormonal balances. ACC was the only hormonal compound increasing in leaf tissue, coinciding with the onset of oxidative damage and the decline in chlorophyll fluorescence and prior to massive Na⁺ accumulation. (Z + ZR) and ACC contents and their ratio (Z + ZR/ACC) were the best hormonal parameters explaining the onset and progression of leaf senescence. Furthermore, decreased shoot cytokinins and auxins concentrations may better explain the shift of biomass allocation to the roots and the decrease in shoot vigour.

P09-050 Regulation of adaptive plants opportunities by means of nitrogen nutrition

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Inorganic nitrogen is a substrate for nitrogen assimilation of higher plants and also functions as a signal triggering widespread changes in gene expression that modulate metabolism and development. Soil nitrogen content fluctuates highly and plants have evolved the capacity to adapt to changes in nitrogen quantity and quality by physiological response. In present work adaptive reactions of plants that were grown up at different concentrations of NH₄NO₃ have been studied. Increasing concentrations of NH₄NO₃ resulted dose-dependent rising of chlorophyll a and b content and protein in leaves. But the highest researched concentration reduced root length and mitotic activity of soyas root cells decreased on 38%. Accumulation of lipid peroxidation product – malonic dialdehyde (MDA) in soyas leaves has been noticed at plants processed by 9 mM NH₄NO₃ and raised on 30%. The influence of different concentration of nitrogen has been estimated by changes in SQDG quantity in soyas leaves. Dose-dependent increasing of SQDG content was found out after treating plants with different concentrations of NH₄NO₃ and quantity of this sulfur-containing lipid was doubled by 9 mM NH₄NO₃. Changes in SQDG content and increasing of MDA quantity show that treatment soyas plants with NH₄NO₃ caused stress reaction. Activation of defence systems and triggering of eustress reactions by low nitrogen doses as a stress factor are discussed.

P09-051 Functional characterization of the Arabidopsis single TSPO-related protein

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Tryptophan-rich sensory proteins are membrane-bound proteins already described in bacteria and mammals, and contain the so-called TspO/MBR domain, however their function remain unclear.
In facultative photosynthetic bacteria, the outer membrane protein TspO may function as an oxygen-dependent signal generator to negatively regulate photosynthetic genes expression under oxidative growth conditions. The mammalian TSPO18 associates with the mitochondrial outer membrane and seems to be important in sterol genesis. In an in silico search for Arabidopsis genes encoding membrane proteins specifically induced by water-related stress, we identified At2g47770 coding for a TspO/MBR domain-containing protein (AtTSPO). We generated an affinity-purified antibody against AtTSPO and used this tool to show that this protein accumulates in Arabidopsis seeds, but is only detected in vegetative tissues upon water-related stress or abscisic acid (ABA) treatment. ABA-induced AtTSPO, and as in seeds, localized to Golgi stacks. This conclusion was compelled by immunocytochemistry, subcellular fractionation, and fluorescence protein tagging experiments. Constitutive expression of AtTSPO in Arabidopsis cultured cells affects the greening and functioning of the chloroplasts. Our data suggest that the angiosperm TSPO-related protein may have evolved as an ABA-regulated, Golgi-localized membrane protein, modulating chloroplast functioning under abiotic stress through a yet unknown molecular mechanism.

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**P09-052 Double inactivation of primary-like σ factors in Synechocystis sp. PCC6803: answers and questions**

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Sigma factors are exchangeable subunits of bacterial RNA polymerase, and while the core polymerase can continue the polymerization reaction in the elongation phase, a σ factor is needed for promoter recognition and initiation. Sigma factors can be classified according to their structure and function. Group 1, or primary σ factors, are essential and cannot be deleted from the genome. They are responsible for the transcription of housekeeping genes in the growth phase. Group 2 σ factors are very closely related to Group 1, but are nonessential. They are activated in response to certain environmental or developmental signals. It is assumed that different σ factors recognize slightly different promoter elements, thus resulting in the characteristic transcription profile. But there are few differences in the DNA binding amino acids, so how do the different σ factors distinguish the promoters? We have constructed single and double inactivation strains of group 2 σ factors in all possible combinations in Synechocystis and built homology models of the RNA polymerase with Group 1 and 2 σ factors. We have also studied the physiology of these inactivation strains in various environmental conditions. The results indicate that a specific sigma factor is connected to a certain stress response.

**P09-053 Stress response in Synechocystis sp. PCC 6803 σ factor inactivation strains**

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**P09-054 Drought response strategies under grain filling in wheat. Changes in photosynthesis, ABA levels and grain yield**

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**P09-055 Concentration of SO₂, NOx, and dust from power plants Kosova A and B, as a stress indicators to the lichens in the area of Kastrioti**

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P09-056 Addition of ribitol into Xanthoparmelia somloensis thallus alters photosynthetic processes in PS II of symbiotic alga at sub-zero temperature
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Ribitol and other polyols have a cryoprotective role in plants and lichens. Wetted positive effects of externally added ribitol (32, 50 mM) on primary photochemical processes at low temperature (5, 0, and −3°C) in X. somloensis. After ribitol addition, thalli segments were exposed to 300 μmol m−2 s−1 at the above temperatures for 168 h. Each 24 h, chlorophyll fluorescence parameters (Fv/Fm, effective quantum yield of PS II–Yield PS II, non-photochemical quenching–NPQ) were measured by an imaging fluorometer (HFC-010, P.S.I., CZ). Positive effect of ribitol on Fv/Fm and Yield PS II, was apparent only at −3°C. The significant effect was seen early, i.e. within the first 24 h. The difference in Fv/Fm and Yield PS II between control and 32 mM treated thalli was seen throughout the exposition period. Surprisingly, 50 mM treatment led to a decrease in Fv/Fm and Yield PS II values at −3°C, while no change was seen at 0, 5°C. Such ribitol concentration was a considerable stressor to PS II which might be documented by dramatic increase in NPQ. Supported by the GAAV KJB601630808 funding.

P09-057 Expression of Thlaspi caerulescens metallothioneins MT2 and MT3 in intraspecies crosses segregating for Zn accumulation
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To study the possible role of metallothioneins (MTs) in metal accumulation, their expression was studied in Cd and Zn hyperaccumulator Thlaspi caerulescens accessions and in intraspecies crosses segregating for Zn accumulation. The highest TcMT2a, TcMT2b and TcMT3 mRNA levels were found in the shoots of a superior metal-accumulating accession from Ganges region, with over ten-fold TcMT3 mRNA levels compared to calaminous and non-metallocolous accessions. In line with this, the F3 lines from a cross between Ganges and a calaminous accession, which harboured Ganges allele, had generally higher MT2a and MT3 expression. However, no segregation of TcMT2a or TcMT3 expression and Zn accumulation was evident in the sibling lines even though lines homozygous for calaminous MT2a allele were low-accumulators. Whole-mount immunohistochemistry, using anti-peptide TcMT2a antibody, showed that the MT2 protein is localized in root epidermis and root hairs both in T. caerulescens and in Arabidopsis, being most abundant near or at the root tip. The Arabidopsis lines transformed with TcMT2a or TcMT3 did not show increased Cd, Cu or Zn tolerance or Cd or Zn accumulation compared to the wild-type plants. These results show that TcMT2a, TcMT2b and TcMT3 are not the primary determinants of Zn accumulation. Elevated expression of these genes in the metal-adapted phenotype may reflect an increased requirement for maintaining the metal homeostasis.

P09-058 Effect of short-term low temperature stress on cucumber seedlings grown in the presence of selenium
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The objective of this study was to investigate the effect of selenium (0, 2.5, 5, 10 or 20 μM) on cucumber seedlings grown under short-term low temperature stress. Plants were grown in Hoagland nutrient solution under 14-h day length and temperature 25/20°C (day/night). About 14–16-days old seedlings were exposed to short term sub-optimal temperature (24 h 10°C/5°C and 24 h 20°C/15°C; day/night) and then transferred to 25/20°C (rewarming). Immediately after stress proline and malondialdehyde (MDA) content were determined. Seven days later the plants were examined for chlorophyll, carotenoids, proline and MDA content and harvested. Biomass of seedlings exposed to 20 μM Se decreased. Contents of photosynthetic pigments did not significantly oscillate within the range of applied Se concentrations. Se-treated plants showed an increase of proline content in leaves, once immediately after chilling and again during rewarming. Immediately after stress MDA content in roots of plants treated with 2.5–10 μM Se decreased and increased in roots and leaves of plants exposed to 20 μM Se. Seven days later MDA level in roots of plants growing in Se presence was still lower than in plants not treated with Se and did not change significantly in leaves. Although Se at concentrations 2.5–10 μM caused increase in proline content and decrease of MDA level but the resistance of seedlings to low temperature stress did not enhance.
P09-059 Phenotypical and molecular characterization of the UV-B acclimation process

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Ultraviolet-B (UV-B, 290–315 nm) radiation levels in the biosphere have increased due to the depletion of the stratospheric ozone layer. UV-B radiation has many direct and indirect effects on plants (i.e. alterations in photosynthesis, damage to DNA and proteins and morphological changes) and these may, in turn, impact on the functioning of ecosystems. However, plants have developed a range of protective responses such as the accumulation of UV-B absorbing polyphenolic compounds and the induction of DNA-repairing photolyases. Nevertheless, the molecular mechanisms behind the complex acclimation process are only poorly understood. Therefore, the effects of chronic and ecologically relevant UV-B dose-rates on Arabidopsis plants were determined by measuring radiation effects on morphology, physiology and gene expression profiles. The used dose-rates of UV-B radiation did not affect photosynthesis nor expression of known stress-responsive genes. UV-induced morphological changes in acclimated plants included decreased inflorescence height, increased numbers of flowering stems and decreased rosette diameter, accentuating that chronic UV-B treatment induces a redistribution of growth rather than a cessation. Gene expression profiling using Arabidopsis microarrays indicated possible morphogenic roles for brassinosteroids, gibberellins and auxins. It is concluded that the process of UV-B induced morphogenesis, observed in acclimated plants, is uncoupled from stress responses.

P09-060 ERD15 – a negative regulator of ABA responses modulates abiotic and biotic stress tolerance in Arabidopsis

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The phytohormone abscisic acid (ABA) has a wide range of both developmental and physiological functions as well as being the key mediator of adaptive responses to various abiotic stresses. Recent studies suggest that ABA can also modulate plant-pathogen interactions. We identified ERD15 (EARLY RESPONSIVE TO DEHYDRATION) in a subtractive screen for pathogen-induced genes in Arabidopsis and showed that it is rapidly induced in response to various abiotic and biotic stress stimuli. Functional analysis by overexpression or RNAi silencing of ERD15 in Arabidopsis and demonstrated that the gene encodes a negative regulator of several ABA-controlled processes. RNAi silencing of ERD15 resulted in plants that were hypersensitive to ABA and showed enhanced tolerance to both drought and freezing. Accordingly, plants overexpressing ERD15 were less responsive to ABA and impaired in their abiotic stress tolerance. Interestingly, these plants showed improved resistance to the plant pathogen Erwinia carotovora suggesting a role for ABA signaling in disease resistance. We have characterized the ERD15 regulon by transcriptome analysis and identified several protein phosphatase genes as potential ERD15 targets. Preliminary analysis of plants containing a T-DNA insertion in some of these protein phosphatase genes suggest that these phosphatases are involved in control of plant freezing tolerance.

P09-061 The origin of cadmium-induced ROS production: mitochondrial electron transfer versus plasma membrane NADPH oxidase

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Cd2+ is an environmental pollutant that causes increased reactive oxygen species (ROS) production. We studied two potential targets for Cd2+ in the cell, the ROS-producing NADPH oxidase in the plasma membrane and the mitochondrial electron transfer chain. ROS production was followed in isolated soybean plasma membranes, potato tuber mitochondria and in roots of intact seedlings of soybean and cucumber. The effects of Cd2+ on the kinetics of superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (OH) generation using absorption, fluorescence and spin-trapping electron paramagnetic resonance spectroscopy. In isolated plasma membranes Cd2+ inhibited O2- production. This inhibition was reversed by Ca2+ and Mg2+. In isolated mitochondrial Cd2+ increased O2- and H2O2 production. In intact roots Cd2+ stimulated H2O2 production while it inhibited O2- and "OH production in a Ca2+-reversible manner. The immediate (≤1 h) consequence of exposure to Cd2+ in vivo is the stimulation of ROS generation in the mitochondrial electron transfer chain and the inhibition of the NADPH oxidase activity in the plasma membrane. Therefore, Cd2+ can be used to distinguish between ROS originating from mitochondria or from the plasma membrane by measuring different ROS individually.

P09-062 Adverse effects of gene stacking on improving UV tolerance in Nicotiana tabacum

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Reactive oxygen species (ROS) are well recognized as elicitors and propagators of stress-related oxidative damage as well as signal molecules inducing stress-responses. Reactions initiated by ROS may also yield reactive carbonyl species (RCS) (e.g. MDA, 4-HNE), but stress conditions can directly evoke RCS as well (e.g. methylglyoxal). RCS can increase ROS initiated cellular damage further, due to their better penetration through membranes and reactions with proteins or DNA. In this way, increasing the intracellular scavenging capacity of RCS is expected to improve stress tolerance [Hideg et al. (2003), Oberschall et al. (2000)]. In the present study, transgenic Nicotiana tabacum SR1 plants that overproduce aldo-keto reductase (MrAER) [Oberschall et al. (2000), Hideg et al. (2003)] or xanthoxin reductase (AbaER) [Mano et al. (2005)] were found to have improved tolerance to supplemental ultraviolet (UV-B) irradiation as compared to the SR1 plants. When both RCS detoxifying pathways were reinforced in the same plant (gene stacking), no UV tolerance was found and double mutants proved more sensitive than...
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SR1 plants. Possible mechanisms leading to this increased sensitivity are discussed in terms of ROS, RCS and other oxidized products.


P09-063 Occurrence of growth disturbance in plants and the adaptive root elongation of Suaeda salsa under high pH conditions

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Deficiencies of P, Fe, and some other elements due to precipitation of these compounds in high pH medium, and NH4+ toxicity due to dissociation of NH3 have been well documented as factors inducing growth disturbance in plants under high pH conditions. However, little is known regarding the direct effect of the high concentration of OH– on plant growth. We examined whether growth disturbance occurs in rice plants, tomato plants, and the halophyte Suaeda salsa as a result of OH– toxicity under hydroponic conditions. Growth at pH above 10 was poorer than that at pH 6 in all three species, but no symptoms of P or Fe deficiency were observed. The root length of rice and tomato grown at pH above 9 was significantly shorter than that of those grown at pH 6. In contrast, the root length of S. salsa grown at pH 9 was longer than that of those grown at pH 6. Then, we investigated primary root elongation in tomato and S. salsa seedlings grown in the range of pH 4.5–10 under strong buffer condition to determine if the pH of the cell wall of the elongation zone was altered. The root elongation of the tomato was remarkably retarded at pH 7.5–10. In contrast, the root elongation of S. salsa was normal at pH 6.5–8 but remarkably retarded at pH 4.5–6, pH 9, and 10. These results indicate that S. salsa possesses an adaptive property with regard to root elongation under high pH conditions.

P09-064 The use of photosynthetic parameters as secondary selection traits for the assessment of maize tolerance to drought: parent-progeny analysis

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Breeding for improved drought tolerance is one of the most important tasks maize breeders are currently confronted with. To simplify procedures used in the conventional selection, various photosynthetic parameters have been proposed to serve as secondary selection traits for such assessment. However, even if drought-tolerant genotypes are selected by such screening, it is not known whether this tolerance will be transmitted to their progeny. We have examined whether the photosynthetic parameters could be used not only for the selection of drought-tolerant inbred lines of maize but also as predictors for the heritability of such tolerance. Fifteen maize inbreds and F1 hybrids were grown under two irrigation regimes: continuous water supply or water supply withheld for up to 14 days; at the beginning of drought treatment, plants were at V3 developmental stage. Water use efficiency, net photosynthetic rate, chlorophyll fluorescence parameters and content of photosynthetic pigments were analyzed together with transpiration rate, stomatal conductance, relative water content and various morphological and developmental parameters. The results of this analysis showed that while the response of photosynthetic parameters usually correlates well with the overall response of maize inbreds to drought, the response of their F1 hybrids cannot be reliably predicted from the behaviour of parents. The study was supported by grants No. 521/07/0470 of the grant agency GACR and MSM 0021620858.

P09-065 Stress tolerance of yeast expressing genes for taurine synthetic enzymes

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Taurine, 2-aminoethanesulfonic acid, is a β-amino acid and commonly found in marine molluscs or fishes, but very rare in plants. It is known to function as a compatible solute against osmotic or salt stress and as an antioxidant against oxidative stress in animal cells. At present, taurine has never been investigated about its involvement in the development of stress tolerance in yeast and plants. Our purpose is to show the use of taurine as a cellular protectant in yeast against stresses.

In the present study, we tried to isolate cDNA clones, which encode two enzymes (cysteine dioxygenase; CDO and cysteine sulfinate decarboxylase; CSD) involved in taurine synthesis, from Cyprinus carpio. The amino acid sequences of the putative proteins encoded by the isolated cDNA clones showed similarity to those of the objective two enzymes from other animals. The coding regions of the two cDNA (cdo and csd) clones were introduced into an expression vector (pESC-Trp) and the corresponding genes were expressed individually or as a fusion protein in yeast. The expression of the proteins was confirmed by Western blotting. The accumulation of taurine in yeast was confirmed by amino acid analysis. Accumulation of taurine appears to lead to improvement of freezing and oxidative stress tolerances of transformed yeast cells.

P09-066 Changes of plasma membrane bound redox components due to iron deficiency: a comparison between pea and maize

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Iron is despite its abundance hardly bioavailable. This is due to the formation of sparingly soluble oxides and hydroxides. Plants have to cope with this situation and they developed two different strategies. Pea is an iron uptake strategy I plant, which reduce the iron before uptake by a transmembrane activity. With the aid of an enhanced proton extrusion iron can be taken out of soil this way. Grasses like maize belong to the group of strategy II plants. Excretion of high-affinity chelators

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Glassworts are salt marsh halophytes, included in the Chenopodiaceae family, which are apparently leafless and have articulated and succulent stems. Two groups exist: annual glassworts, with the genus *Salicornia*, and perennial glassworts with the genera *Sarcocornia* and *Arthrocnemum* but taxonomy remains unclear. There is an increasing interest in investigations of chemotaxonomic markers to answer to ambiguities between « morphotypes » and « species » of different populations of the tribe of *Salicornieae*. Some glassworts species present tissue reddening at the end of their life cycle due to specific pigments. The aim of this study was to develop a method of purification of implicated pigments and to identify them in an annual reddening glasswort species. *Salicornia ramosissima* was sampled in situ at the ‘Port du Collet’ (Vendée, France). Water extraction of red pigments was performed from freeze-dried plants and solid-phase extraction purification was used. NMR and mass spectrometry assays identified these pigments as betalains. These nitrogenous pigments are known to be separated between two groups: betacyanins (red pigments) and betaxanthins (yellow pigments). These pigments replace anthocyanins in most of the Caryophyllales species and could be potential interested chemotaxonomic markers. This results obtained in *Salicornia ramosissima* should be enlarged to others glasswort populations to validate the possible taxonomic value of betalains.

**P09-067 Purification and identification of pigments in an annual reddening glasswort specie**

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Plants respond to many stresses by producing reactive oxygen species (ROS). The production and the action of ROS are critical to the recognition of stress and the coordination of stress responses. Gaseous ozone (O_3) induces apoplastic ROS production similarly to many biotic and abiotic stresses. As the delivery of gaseous ozone to plants does not require any hands-on manipulation, and thus reduces the induction of responses through handling, it is an excellent tool to study the effects of the apoplastic ROS. While many different studies emphasize the importance of ROS during stress response, a few important question remains: How are ROS signals perceived and transduced, how are signal transduction networks triggered and signalling activity regulated? We have identified two members of DU可以让26 (DU đức-domain of unknown function) subfamily of receptor-like protein kinases (RLKs) which are involved in ozone stress response. Ozone sensitive phenotype of knock-out plants and up-regulated gene expression of wildtype plants after ozone exposure indicates that these proteins are crucial to the stress signal transduction pathway in protecting plants against ozone induced cell death. To characterize these identified DU可以让26 RLKs in detail, we will use the corresponding over-expression lines for phenotype analysis, protein expression and localization analysis, extracellular modification analysis, complex isolation assays, protein kinase activity assays, ligand/target hunt, etc.

**P09-069 Receptor-like protein kinases in ozone stress response in Arabidopsis thaliana**

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The presence of Glucosinolate-Myrosinase system is a classical characteristic of plants of the order Capparales that includes Arabidopsis and other crucifers. The enzyme β-Thioglucosidase Glucohydrolase 1 (TGG1) hydrolyzes glucosinolates into toxic compounds, that deter herbivory. TGG1 is expressed in stomatal guard and phloem cells and in above-ground organs. To analyse TGG1 expression against methyl Jasmonate (MeJA) exposure, transgenic Arabidopsis plants carrying [β-glucuronidase (GUS) fused to 2.5 kb TGG1 promoter; pBITGG1-GUS] were used. PBITGG1-GUS plants were exposed to MeJA for three and six days, respectively. Six days MeJA treated leaves showed very weak GUS staining in older leaves. In parallel, MUG (4-methyl umbelliferone glucuronide) assays showed lower activity in MeJA treated plants. In order to observe the effect of dehydration stress, we exposed wild type (wt) and TGG1 knockout (KO) plants, which had been conditioned for growth on MS-agar plates, to dehydration under laminar flow conditions for 30 min. Transcriptional analysis of dehydrated TGG1 (KO) vs wt plants showed upregulation of Aquaporin TIP2.3, Osmotin 34, Dehydrin XEO2, and Dehydrin Rab 18 genes; reported to be abscisic acid and cold responsive. The results from MeJA experiments highlight that long term exposure of MeJA lowers TGG1 expression, while dehydration experiment shows TGG1 (KO) plants to be more stressed as compared to wt plants.

P09-071 Light and temperature dependence of cold hardness in wheat plants with different freezing tolerance

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Even in frost-tolerant species a certain period of growth at low, but non-freezing temperature is required for the development of frost hardness. Keeping plants at 20°C with high light intensity also increased the freezing tolerance. The interaction between light and temperature during the development of freezing tolerance was studied in wheat plants. The freezing survival rate, the photosynthetic electron transport processes, the lipid composition, the antioxidant activity, and the salicylic acid content were investigated during frost hardening in a winter wheat variety. The saturation level of hexadecanoic acid decreased not only in plants hardened at low temperature, but also, to a lesser extent, in plants kept under high light irradiation at normal growth temperature. The greatest induction of the enzymes glutathione reductase and ascorbate peroxidase occurred when the cold treatment was carried out in normal light, but high light intensity at normal, non-hardening temperature also increased the activity of these enzymes. The quantity of bound ortho-hydroxy-cinnamic acid increased by up to two orders of magnitude in plants that were cold hardened in normal light. Changes in the polyamine and thiol contents during cold hardening under different light and temperature conditions, and the correlation between the freezing tolerance induced by low temperature and by light in wheat plants with different levels of frost tolerance will also be discussed.

P09-072 Cd influence on LHCII aggregation

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Heavy metals, including cadmium, similarly as excessive illumination generate very reactive oxygen species causing photo-damage of the photosynthetic apparatus. Plants develop various photoprotective mechanisms operating at their molecular organization level. One of them is presumably the aggregation of the light-harvesting chlorophyll a/b protein complexes (LHCII) associated with thermal dissipation of excitation energy excess. To exactly examine this possibility, Cd-induced changes in the composition and function of LHCII complexes isolated from Secale cereale L. leaves were analyzed. The infrared absorption spectra of LHCII in the Amide I region were recorded and analysed by Gaussian deconvolution. We found of the spectral components centered in the regions of 1628 cm$^{-1}$ and 1611 cm$^{-1}$, typical for aggregated a-helices of LHCII. The aggregation level of the protein isolated from the Cd-treated plants was lower than in the control plants. Measurements of the 77 K chlorophyll a fluorescence emission spectra and the studies of monolayers compressed at argon-water interface with and without Cd ion presence showed the inhibitory effect of Cd on LHCII aggregation. It can be concluded that Cd negatively affects the photoprotection mechanism of the photosynthetic apparatus by disruption of LHCII organization.

P09-073 Imaging-based phenomics revealed enhanced drought tolerance of Arabidopsis thaliana transgenic PARP2-deficient plants

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Morphology and physiology of a plant lead to a phenotype which is the result of the interplay of the plant’s genome and environmental factors. The environmental influences render phenotypes variable. Therefore it is necessary to standardise cultivation conditions and experiments for phenotyping analyses. The Jülich Plant Phenomics Centre follows these aims of standardisation to enable comparisons of stress tolerance of different genotypes, transgenic plants, or chemically treated plants. Here, we demonstrate, how Growscreen-Fluoro, a setup enabling simultaneous screening of plant growth and potential quantum yield of photosystem II (Fv/Fm) was used for analyses of tolerance to drought- and cold-stress with A. thaliana. Both types of stress markedly reduced the plant growth rate, but only cold stress led to a decrease in the in Fv/Fm. Reduction of PARP2-levels by RNA-interference resulted in enhanced resistance of A. thaliana to abiotic stress. Using the Growscreen tools we analysed the dynamics of drought tolerance of PARP2-deficient plants. Those had a clear advantage of growth in drying soil compared to wild-type plants. While the automated system is suitable for small rosette forming plants like A. thaliana or seedlings of Nicotiana sp., screening of crop plants like Zea mays or Brassica napus is done by methods demanding more

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manual operation. Imaging and measurements of size and chlorophyll fluorescence are used to analyse the performance of crops under stress conditions.

P09-074 Evaluation of gene expression in winter barley cv. Luxor during acclimation and cold stress
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Together with the defense mechanisms, which were developed during the evolution, some plants are capable of a much faster adaptation to the current conditions. However, even this process, called acclimation, requires some time and appropriate environmental conditions to develop the maximum tolerance possible. For winter barley cold acclimation investigation we have chosen 21 days at 3°/2°C (day/night) and a 12 h photoperiod after 21 days of cultivation at 18/13°C. Samples (the second fully developed leaf and crown) were taken before being exposed to cold (control) and after 24 h, 3, 7 and 21 days of acclimation. After this the plants were exposed to −3°C for 24 h. We aim to compare expression profiles of leaves and crowns in the course of chilling and freezing as well as confronting different cultivars and thus contribute to better understanding of how winter cereals cope with low temperature at the RNA level. As a model cultivar we used winter hardy cv. Luxor. The Affymetrix chips were used to obtain expression profiles, which let us to compare expression of nearly 23,000 genes, including 2000 genes with significantly altered expression (P ≤ 0.01). Using Gene Spring software 25 clusters were identified, each cluster being characterized by a typical course. Freezing tests of each, leaves and crowns, and further physiological parameters are provided. Results and discussion will be presented. Supported by the Czech Ministry of Agriculture (0002700602).

P09-075 Inactivation of mitochondrial FtsH4 protease causes overproduction of molecular chaperones at the late stage of Arabidopsis vegetative growth
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FtsH4 is a membrane-bound mitochondrial ATP-dependent metalloprotease with the catalytic site exposed to the intermembrane space. Arabidopsis ftsH4 T-DNA insertion mutants were examined to investigate the in vivo function of FtsH4. Mutant plants did not have visible morphological abnormalities when grown under long days (LD), however, under short days (SD), ftsH4 mutant lines exhibited a set of morphological abnormalities. The most characteristic were the asymmetric shape and irregular serration of expanding leaf blades, visible only at the end of the vegetative phase. We found a correlation between leaves abnormalities and the elevated level of ROS as well as carbonylated proteins. These observations strongly indicate that ftsH4 plants suffered increased oxidative stress at the end of the vegetative phase under SD compared with wild type and ftsH4 under LD. We also noticed overproduction of molecular chaperones (prohibitin, Hsp70) in ftsH4 mutants at late stages of the vegetative growth in both LD and SD conditions. We postulate, that overproduction of molecular chaperones compensates lack of FtsH4 under LD, but not under SD in which a length of the vegetative phase is extended and leads to oxidative stress in ftsH4 mutants. Our data points to an important role of FtsH4 chaperone and/or proteolytic activity in prevention of carbonylated proteins accumulation at the late stage of Arabidopsis vegetative growth.

P09-076 Rice NAC genes enhance grain yield and stress-tolerance
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NAC protein family comprises a variety of plant proteins that are identified by the presence of a highly conserved N-terminal NAC domain. Using the 60K Rice Whole Genome Microarray, a number of genes encoding transcription factors with NAC domain were found to be up-regulated by stress treatments. Transgenic overexpression of these genes in rice plants under the control of either a constitutive or a root-specific promoter resulted in enhanced tolerance to the stresses. Interestingly, in some of the NAC transgenic plants, significant increases in yield-related parameters were observed as well. These parameters include leaf biomass, emergence vigour, thousand kernel weight, panicles, and total number of seeds. By combining results from the expression profiling between NAC overexpressors and nontransgenic controls and those from chromatin immunoprecipitation followed by promoter microarray analysis, we identified target genes that were activated by the NAC proteins. We conclude that NAC proteins activate the target genes, which not only make the transgenic plants tolerant to abiotic stresses but also increase grain yield.

P09-077 Physiological responses of Pinus canariensis to the environment in the upper limit of its distribution in Tenerife
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Environmental limitations to the distribution of forest trees become most obvious at tree line ecotones. Extremes in environmental factors such as temperature, water supply, irradiation, etc. accumulate stress situations than trees can hardly withstand. Therefore, the role of upper tree line ecotones as indicators of environmental changes is widely recognised. The alpine timberline in the Canary Islands is formed by the endemic pine species (Pinus canariensis) and dominated by a climate with summer droughts, frost conditions in winter and high radiation. An experimental plot has been established in Las Cañadas del Teide, Tenerife, Canary Islands, to monitor the
physiological performance of *P. canariensis* trees in relationship to the environmental factors. The site is at 2070 m a.s.l., has a density of 291 trees ha⁻¹, diameters at breast height range from 6 to 44 cm and maxima tree height is 15 m being the leaf area index nearly 4. Stem increment, stem temperature, sap flow, soil water content, soil water potential, soil temperature, and meteorological factors are continually recording, and leaf gas exchange, stem respiration, chlorophyll fluorescence, and other physiological parameters are periodically measured at the site. Some of the results obtained during the first year of study will be shown in this work. Thanks to the Spanish Government Project CGL2006-10210/BOS MEC, co-financed by FEDER and to Bilateral Project Austrian and Spanish Governments (HU2005-0007).

**P09-078** Transgenic rice overexpressing *OsAsr1* (ABA, stress and ripening induced) exhibits tolerant to abiotic stress  
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The ASR gene family is widespread in higher plants. ASR genes are known to up-regulate under different environmental stress conditions and during fruit ripening, ASR proteins are localized in the nucleus and their putative function is transcriptional regulation in grape. Yeast-one-hybrid experiments revealed that a grape nucleus and their putative function is transcriptional regulation during fruit ripening. ASR proteins are localized in the known to up-regulate under different environmental stress conditions. The ASR gene family is widespread in higher plants. ASR genes are *Corresponding author, e-mail: biojsjoo@nate.com*

**P09-079** Salinity effect on expression of dehydrins and ubiquitin in buckwheat leaves  
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Buckwheat (*Fagopyrum esculentum* Moench) is a dicotyledonous crop of the Polygonaceae family. In the present study we investigated dose- and time-dependent effect of salinity on relative water content, protein content, lipid peroxidation, ROS production and expression of dehydrins and ubiquitin in buckwheat leaves. Accumulation of dehydrins as well as the rate of protein ubiquitination has been viewed as the components of plant stress response. Two week old buckwheat plants were exposed to 10 mM, 25 mM, 50 mM and 100 mM NaCl during 2 and 7 days. The expression of dehydrins and ubiquitin was examined by western blot using antibodies against dehydrins (K segment) and ubiquitin, respectively. Among dehydrins, the most prominent was 36 kDa one. Its accumulation correlated to applied dose of salt. Dehydrins Mw range 39–42 kDa disappeared after 7 days of salt exposure. It was also observed that accumulation of ubiquitin-conjugates was not in correlation with free ubiquitin abundance. The role of dehydrins as well as protein ubiquitination in buckwheat response to salinity was discussed.

**P09-080** Impacts of blocking phloem transport on photosynthesis, VOC emissions, sapflow and soil carbon fluxes in Scots pine  
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The regulative role of functional sink-source balance in big trees within the growth season was studied in two experiments where 45-year-old Scots pine trees were girdled. In the first one two trees were girdled in June and two in August to reveal the effect of the location of the sink under functional imbalance. In the second experiment 20 trees were girdled in June to gain more information on the related soil processes. In this experiment four girdled trees were selected for intensive measurements. SMEAR II station nearby was selected as a control site. The changes in chlorophyll fluorescence, photosynthetic capacity, sapflow and VOC emissions were followed more than 10 weeks after the girdlings. Also needle and phloem samples were taken for analysing the carbohydrate concentration and VOC pools. Soil and stem respiration as well as soil VOC, CH₄ and N₂O fluxes were measured with manual chambers within the girdled area during the second experiment. The influence of blocking of phloem flow on carbohydrate sink of stem was analysed in relation both to growth and to carbohydrate stores, and its impact on xylem sapflow. The responses in the respiration of the root system were analysed as well. The effects of the lowered sink demand both on light and dark reactions of photosynthesis were analysed together with the emissions and pools of VOCs. Both the short and long term effects will be discussed as well as the temporal relationships between the different components.

**P09-081** Regulation of AREB phosphorylation in *Arabidopsis thaliana*  
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Under abiotic stress conditions such as drought and high salinity, ABA levels increase in plants, and ABA regulates the expression of many genes that function in plant stress tolerance. A conserved cis-element designated ABRE (ABA-responsive element), which controls ABA-responsive gene expression, has been identified in promoter regions of ABA-regulated genes. Arabidopsis cDNAs encoding bZIP-type transcription factors referred as ABRE-binding (AREB) proteins were isolated using the yeast one-hybrid screening method. Among these transcription factors, expression of AREB1, AREB2, and ABF3 was upregulated by ABA, dehydration, and high-salinity stresses in Arabidopsis plants. Overexpression of the intact AREB1 is insufficient to lead to expression of downstream genes. Post-transcriptional activation of AREB1 by phosphorylation was necessary for its maximum activation. Using in gel kinase assay, we analyzed phosphorylation of AREB peptide fragments. The AREB1 peptide was phosphorylated by SNF1-RELATED PROTEIN KINASE2 (SnRK2) under drought and high-salt stress conditions as well as ABA application. The both srk2d and srk2i mutants had greatly reduced kinase activity capable of phosphorlating AREB1 peptide under stress condition and ABA application. These results demonstrate that phosphorylation of AREB1 by SnRK2 protein kinases is necessary to expression of downstream genes under stress condition.

P09-082 Characterization of specific genes in reproductive stage under cold stress in rice
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Cold injuries to rice plants occur in each growth stage from germination to ripening. Especially, cool temperature at reproductive stage often causes an increase in the number of sterile pollen, eventually resulting in decreased rice yield. In this study, we used two rice cultivars, Hitomebore (cool-temperature susceptible) and Sasanishiki (cool temperature-susceptible) as plant materials. Hitomebore was given cool temperature (19°C) at reproductive stage and showed 80% fertility. Meanwhile, the fertility decreased to 55% in Sasanishiki. To reveal the morphological difference of anther development between Hitomebore and Sasanishiki under cool temperature, cross-sections of the anther at different developmental stages were checked. In normal development of rice anther, a degradation of the tapetum starts at young microspore stage and the tapetum vanishes completely at mature pollen stage. It is known that the tapetum provides nutrients for pollen development, and its degradation at the right time is important for functional pollen development. Cross-sections of Sasanishiki anther under cool temperature revealed that degradation of the tapetum did not occur at young microspore stage. It should suggest that cool temperature tolerance correlated with the tapetum degradation at the right time. In microarray analysis during developmental anther, many genes showed different expression levels between two cultivars (Hitomebore and Sasanishiki) under cool temperature.

P09-084 Increased levels of MeJA reduces grain yield under stress conditions by altering floral organ numbers in rice
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Jasmonic acid (JA) is involved in plant development and defense response. Transgenic rice plants that overexpress Arabidopsis jasmonic acid carboxyl methyltransferase gene (AtJMT) under the regulation Ubi1 promoter had 6 to 15-fold elevated levels of MeJA in flowers. Ubi1:AtJMT plants had altered numbers of floral organs including carpel, anther, lodicules, lemma and palea. These changes in floral organ numbers could be reproduced by treating nontransgenic (NT) plants with exogenous MeJA, demonstrating that the increased levels of MeJA after floral development. Surprisingly, the increased levels of MeJA in Ubi1:AtJMT and MeJA-treated NT plants resulted in significant loss in grain yield by lowering parameters including number of spikelets per panicle, total number of seeds, filling rate and total seed weight. Interestingly, NT plants exposed to drought conditions during development of floral meristems increased levels of MeJA by 7 to 19-fold in flowers, reducing grain yield. Levels of ABA, another stress hormone, in the drought-treated flowers were increased only by 1.5-fold. These observations suggest that MeJA plays a major role in loss of grain yield under stress conditions by mediating stress signals to alteration of floral organ numbers.

P09-085 Biochemical and cellular characterization of two homologous E3 ubiquitin ligases, AtPUB22 and AtPUB23, in Arabidopsis
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The U-box motif is a conserved domain found in the diverse isoforms of E3 Ubiquitin (Ub) ligase in eukaryotes. The AtPUB22 and AtPUB23 genes encode proteins, which contain a single U-box motif in their N-terminal in Arabidopsis. In vitro ubiquitination assays revealed that AtPUB22 and AtPUB23 possessed E3 Ub ligase activity. The AtPUB22 and AtPUB23 transcripts were rapidly induced by abiotic stresses. Transgenic Arabidopsis plants that overexpressed AtPUB22 and AtPUB23 under the control of the CaMV 35S promoter exhibited markedly longer root in comparison to wild type and atpub22atpub23 double mutant in normal conditions. Also, 35S:AtPUB22 and 35S:AtPUB23 transgenic plants showed hypersensitivity in response to drought stress. In contrast, atpub22 and atpub23 mutant plants increased the tolerance to drought stress, and an atpub22atpub23 double mutant displayed even greater tolerance. These results demonstrate that AtPUB22 and AtPUB23 function as negative regulators in the water stress.

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Plants possess a large set of the class III peroxidase (POD E.C. 1.11.1.7). Recent genomic sequence analyses have revealed that there are 73 POD genes in Arabidopsis and 138 genes in rice. The roles of diverse POD isoenzymes have been implicated in a broad range of physiological processes, including the response to various abiotic stresses. In our previous studies, 10 POD cDNAs were isolated from cell cultures of sweetpotato (Ipomoea batatas) (Mol Gen Genet 255: 382–397, 1997; 261: 941–947, 1999; Mol Gen Genet 269: 542–552, 2003), and 3 cDNAs were isolated from dehydrated-fibrous roots of sweetpotato (Biochem Mol Biol Rep, in press) via the screening of a cDNA library. In this study, to understand the physiological function of each POD isozyme in sweetpotato, their expressions were assessed to characterize functions of each POD in relation to environmental stresses such as drought, salt, chemicals and air pollution. Among them, the expressions of four acidic PODs, such as swpa1, swpa2, swpa3 and swpa4, were highly induced by several abiotic stresses, suggesting that these POD genes are inducible by various stress conditions. Interestingly, our studies indicated that the responses of the four acidic POD genes under various abiotic stresses are well-correlated with the phylogenetic tree of the 13 POD genes on the basis of amino acid sequences. The results suggested that each POD gene might be specifically involved in the evolutionary adaptation to each environmental stress.

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Water availability is an important environmental factor affecting plants. Genotypes with different drought-tolerance exist in the majority of crop species including maize. However, whether (and how) is such tolerance to drought transmitted from parents to their progeny has rarely been studied. We analyzed selected photosynthetic, growth and morphological parameters in two maize inbreds and their hybrids of F1 and F2 generation, subjected to drought/recovery treatment. Plants in the V3 developmental stage were first exposed to a 10 days period of water deficit, then re-watered and grown for another 14 days. Due to drought, the activity of Photosystems (PS) 1 and 2 decreased to about 60–70% of control values, the content of chlorophylls decreased to a lesser degree and the total carotenoids’ content in leaves of stressed plants did not change at all. The growth and development of plants subjected to drought was also slowed-down. The re-watering resulted in full recovery of the activity of both PSs, as well as in the return of a photosynthetic pigments’ content in leaves to a control level. The individual genotypes/generations differed mainly in the morphological and weight parameters.
but an alternating generation-dependent response of plants to water deficit was observed for the activity of PS2 (negative dependence on the maternal genotype of the respective generation). The study was supported by grants No. 521/07/0470 of the grant agency GACR and MSM 0021620858.

P09-089 Comparative study of cold-responsive genes in wheat
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Winter frosts significantly reduce the growth, development and yield of winter wheat varieties. The genetically determined maximum level of freezing tolerance can be achieved during the cold hardening. Cold-responsive genes affecting freezing tolerance were determined by transcript analysis of a cold-hardened chromosome 5A substitution line since this chromosome is a major regulator of this trait. Genes coding for a cold-responsive (Tacr7), a Ca2+-binding (Cab) and the Dem (deficient embryo and meristems) protein had a significantly greater expression level in the freezing-tolerant substitution line compared to the sensitive one. In case of Cab three genes having identical coding regions but different 5¢- and 3¢-UTR sequences were compared and only one of them was induced during cold treatment. The effect of various stress hormones and abiotic stresses on the expression of the selected genes was also tested in order to determine whether there are cold-specific. The Dem gene was induced only by cold, whereas the transcript level of Tacr7 gene was also higher after salicylic acid and H2O2 treatments and the expression of Cab gene was also increased by NaCl. Further experiments are planned in order to find out how these genes can improve freezing tolerance in wheat.

P09-090 Osmotic stress-induced nitric oxide (NO) in drought tolerant and sensitive wheat cultivars and its source in arabidopsis mutants
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Nitric oxide (NO) is a key signal molecule in abiotic stress responses. During this work NO was detected in 0, 50, 100, 200, 400 mOsm polyethylene glycol (PEG 6000)-treated wheat and Arabidopsis thaliana L. roots by using 4,5-diaminofluorescein-diacetate (DAF-2DA) and Zeiss Axiosvert 200M-type fluorescence microscope. In wheat roots (Triticum aestivum L. var. GK Olathom) every PEG treatment enhanced the NO fluorescence in concentration-dependent way and approached 2.5-fold enhancement in the case of 400 mOsm PEG as related to the control value. The time-dependent kinetics showed a fast (with a maximum value at 1–2 h) transient NO generation in 200 and 400 mOsm PEG treated-roots. In roots of drought tolerant wheat cultivar (Triticum aestivum L. var. Plainsman V.) the NO generation intensified as the effect of increasing PEG concentrations, while drought sensitive wheat cultivar (Triticum aestivum L. var. Cappelle Desprez) showed higher NO content in roots under control conditions, which slightly decreased under osmotic stress. Also in wild-type and Atnno1 mutant Arabidopsis thaliana L. roots could be detected the PEG induced-NO generation, but osmotic stress treated-NR-deficient nia1, nia2 mutant roots did not show NO accumulation. It suggests that NR activity is neccessary for osmotic stress-induced NO synthesis. Acknowledgement. This work was supported by the Hungarian Scientific Research Fund grant No. OTKA T 048436 and Phare CBC HU 2003/005.830.01-04.

P09-091 Effect of synthetic growth stimulators on lipids composition of plasma membranes from root of maize seedlings under salinity stress
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The previous investigation showed that treatments with patented synthetic compounds (Methyure and Ivin) promote growth of maize under salinity conditions. This investigation was aimed at assessment of the effect of Methyure and Ivin treatment on phospholipids and fatty acid composition of plasma membrane, on phospholipase D (PL D) activity in root tissues of maize seedlings under salinity stress. Seeds were treated 10–7 M Methyure and Ivin. 7-old-days seedlings were exposed at 0.1 M NaCl presence. The NaCl exposure decreased the level of phosphatidylcholine (PC) and phosphatidyethanolamines (PE) and increased the level of phosphatic acid (PA) and lysophosphatidylcholine (LPC). The Methyure treatment with salt exposition increased the level of PC and decreased the level of PE, PA and LPC. The salt exposition decreased the level of unsat. Fatty acids (18:2, 18:3) and increased the level of sat. Fatty acids (18:0), is resulting in a lower unsat/sat ratio. The treatments with the compounds increased the level of oleic acid (18:1), is resulting in increased unsat/sat ratio under salinity condition. 0.1 M NaCl decreased the activity of PL D, but increased the content of PC in the plasma membrane, in comparison to the non-salt control. The treatments with Methyure and Ivin increased the activity of PL D under salinity condition. It has been shown that Methyure treatment decreased the content of PA in the plasma membrane. The Ivin treatment had the opposite effect.

P09-092 Stress proteins as biomarkers of plants with different types of ecological strategies
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Changes of physiological characteristics of plants in unfavorable conditions take a place within forming of concrete ecological strategies. The main differences between ecological strategies are founded on efficiency of environmental resources usage, resistance to ecological stresses, relative velocity of development. Physiological mechanisms of ecological strategies formation include among other the proteins biosynthesis. We selected Brassica campestris var. ollitra and Amaranthus caudatus L. as an exponents or R-strategists. Exploters are equally responsive to abiotic and biotic stresses and their surviving is providing with essential cutting of life cycle and considerable contributation in reproduction. As patient or S-strategist we selected Rumex patienza x R. tianshanicus A. Los. Patients are steady to environmental stresses thanks to usage of...
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their own resources for adaptation. The character of stress proteins biosynthesis analyzed after short (2 h) heat (40°C) and cold (2°C) temperature stresses in 7-days seedlings by disc-electrophoresis in PAAG. Temperature stresses called the synthesis of 72 kD Hsp in B. campestris and A. caudatus, intensified synthesis of 94 kD Hsp in A. caudatus, called synthesis de novo 71 kD Hsp and intensified synthesis of 44, 50, 78 and 109 kD Hsp in Rumex seedlings. The amount of 47 kD Hsp became lower after temperature stresses in B. campestris. The stressful polypeptides has been detected, one of them can be reviewed as biomarkers.

P09-093 The effect of enhanced UV-B radiation and allelochemical stress in cucumber
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Cellular responses to a range of environmental challenges are rather similar, while the effects on plant growth and their physiology are more complex. Moreover, the response of plants to a combination of different stresses is unique and cannot be directly extrapolated from the response to each of these stresses influencing individually. Some effects can be additive or antagonistic and help us understand the potential impact of plant cross-tolerance. This report refers to the mechanism of simultaneous allelochemical and UV stresses in terms of phenylpropanoids metabolism. Cucumber genotypes (selected for their cold tolerance and resistance to biotic stresses), cultivated in a growth chamber, were exposed to UV-B (6 hours at 16 kJ m\(^{-2}\) d\(^{-1}\) – additionally to PhAR light) and to phenolic allelochemicals (2 mM ferulic acid supplied to vermiculite). The dynamics of plant growth, i.e. fresh and dry matter and leaf area, were recorded as the response to stressor. Phenylalanine ammonia lyase (PAL) – responsible for the first step of phenylpropanoids biosynthesis, i.e. the formation of cinnamic acid, was activated under ferulic acid and UV-B radiation. Both stresses applied in combination had an additive effect. In contrast, only the allelochemical substance and combined stresses influenced the 4-coumarate-CoA ligase (4CL), while UV had no effect on its activity. The activation of phenylpropanoid enzymes convinced us to evaluate RT PCR gene expression, which is still in progress.

P09-094 Low temperature and humidity as sprouting factors for some Kazakhstan soft wheat cultivars
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Preharvest sprouting is a major problem in northern and eastern regions of Kazakhstan. The main factor of sprouting damage risk in developing grain is the especial enzyme form, which called ‘malt’ α-amylose. It was shown that sensitiveness of wheat genotypes to sprouting damage is a genetic defect that may result in the accumula-
specifically recognizes phosphoproteins and Mn$_2^+$-Phos-tag, which suggesting deleterious effects on the glycolate cycle. However treatment with exogenous CIT led to GLY accumulation in those of wW subjected to drought conditions where it acts as a compatible solute and a scavenger of ROS. We investigated the functions of CIT because wW did not accumulate CIT although this response is almost general under water limitation. The Cucurbitaceae behaves as weak accumulators of PRO which could be related to abundance of CIT. This substance indeed, when exogenously supplied to Canola leaf discs, suppressed the osmo-induced PRO response of this strong PRO accumulator. Its property might be due to a beneficial effect on PRO degradation. However treatment with exogenous CIT led to GLY accumulation suggesting deleterious effects on the glycolate cycle.

P09-097 Regulation of sucrose non-fermenting 1 related protein kinases 2 (SnRK2s) during osmotic and abscisic acid signalling
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SnRK2s have been described as signal transduction factors related to osmotic stress and abscisic acid (ABA) responses in plants. In Arabidopsis cells, mannitol and NaCl activate nine SnRK2s, five of them being additionally activated by ABA (Boudsocq et al. 2004, J Biol Chem 279: 41758). Moreover, SnRK2 kinases remain activated by hyperosmotic stress in ABA-deficient and ABA-insensitive mutants, indicating that SnRK2 osmotic activation is independent of ABA (Boudsocq et al. 2007, Plant Mol Biol 63: 491). The aim of this work is to understand the in planta regulation of these kinases, using SnRK2.6, which is activated by osmotic stress and ABA and SnRK2.10, which is activated only by hyperosmolarity. One important mode of regulation of SnRK2.6 and SnRK2.10 is through phosphorylation, since a phosphatase treatment abolished their activation by osmotic stress and ABA. Using ProQ® Diamond, a dye that specifically recognizes phosphoproteins and Mn$^{2+}$-Phos-tag, which leads to mobility shift of phosphorylated proteins in SDS-PAGE gels, the phosphorylation state of each kinase was characterized. The two kinases are regulated differently, SnRK2.6 being already phosphorylated in an inactive form contrary to SnRK2.10, while both are multi-site phosphorylated when active. Current research aims at identifying the phosphorylation sites in planta of the two kinases and characterizing regulating interactors.

P09-098 Expression of rice cold-regulated AP2/ERF genes induces the transcription of stress-responsive genes in transgenic Arabidopsis
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We previously identified transcription factors regulated by cold stress in rice microarray, which included ERF family genes conserved AP2/ERF domain. The expression patterns of the isolated two genes (named OsEP1 and OsEP2) were analyzed by RNA-gel blot hybridization in response to various stress factors such as cold, drought, high salinity, Me-JA, ABA and ethephon. Low temperature treatment by placing the seedlings at 4°C dramatically upregulated expression of ERF genes in leaves, but the other factors rarely affected. To better understand the function of the isolated rice ERF genes, we constructed transgenic Arabidopsis plants by overexpressing the genes under control of the CaMV 3SS promoter. We are monitoring the stress tolerance of the transgenic Arabidopsis lines by analyzing the effect of high salt, cold and ABA on leaf disc of the transgenic lines. Both transgenic lines significantly enhanced salt tolerance than the wild type in leaf-disc assays. Microarray analysis demonstrated that transgenic Arabidopsis under unstressed conditions highly induced several stress-associated genes, including several dehydrin family proteins, cold-regulated proteins, RD22, KIN2 stress protein, and P5CS for proline synthesis. These observations suggest that the rice ERF genes may function in Arabidopsis to improve the environmental stress tolerance.

P09-099 Arabidopsis NADPH dependent thioredoxin reductase type C is an efficient electron donor of 2-Cys peroxiredoxin
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2-Cys peroxiredoxins (Prxs) play important roles in the antioxidative defense systems of plant chloroplasts. In order to determine the interaction partner for these proteins in Arabidopsis, we used a yeast two-hybrid screening procedure with a C175S-mutant of Arabidopsis 2-Cys Prx-A as bait. A cDNA encoding an NADPH-dependent thioredoxin reductase (NTR) isotype C was identified and designated ANTR-C. We demonstrated that this protein effected efficient transfer of electrons from NADPH to the 2-Cys Prxs of chloroplasts. Interaction between 2-Cys Prx-A and ANTR-C was confirmed by a pull-down experiment. ANTR-C contained N-terminal TR and C-terminal Trx domains. It exhibited both TR and Trx activities and co-localized with 2-Cys Prx-A in chloroplasts. These results suggest that ANTR-C functions as an electron donor for plastidial 2-Cys Prxs and represents the NADPH-dependent TR/Trx system in chloroplasts. [Supported by EB-NCRC & BK21 program].

P09-100 Physcomitrella patens responds to chitosan with release of a specific peroxidase
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Class III peroxidases have versatile functions in plants. While required for normal physiological processes, peroxidases also participate in various biotic and abiotic stress reactions. Generation of...
reactive oxygen species (ROS), which involves peroxidases, is one of the first events occurring in defence of seed plants. On the other hand, peroxidases are capable of detoxifying the harmful ROS. We studied Physcomitrella patens to find out whether similar defence mechanisms are present in bryophytes. Chitosan was used as an elicitor to induce possible defence responses. Chitosan application to the liquid culture of moss caused a rapid increase in peroxidase activity in the medium. Browning and cell death were observed later, mainly in the protonema and rhizoids and at the base of the gametophores. Peroxidase activity was due to a single secreted peroxidase. The protein was isolated, sequenced and the gene identified based on the genome sequence of P. patens. The gene has two copies in the moss genome. Induction of the peroxidase gene by chitosan treatment, as measured by quantitative PCR, occurred later than the observed increase in peroxidase activity in the culture medium. Both copies of the gene were removed by targeted gene replacement. The liquid cultures of the knock-out lines did not have peroxidase activity but appeared more necrotic following chitosan treatment, implying that the peroxidase may have a protective role against oxidative damage.

P09-101 Effect of heat stress on photosynthesis of Rhododendron simii Planch. (pot azalea)
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During the vegetative phase, pot azaleas are grown outdoor without any protection (April – October). Due to global change, periods with high irradiance in combination with high temperatures are more frequent. This leads to leaf burn and temporary growth cessation. Photosynthesis and chlorophyll fluorescence was measured at leaf temperatures (Tl) from 20 to 40°C. Maximum net photosynthesis (Pnmax) decreased slightly at 35°C (Pnmax = 8.58 μmol CO2 m-2 s-1) in comparison to the lower temperatures (Pnmax = 9.91–10.77 μmol CO2 m-2 s-1). At 40°C, the leaves kept on respiring CO2 whatever light level (Pnmax = -1.62 μmol CO2 m-2 s-1). Stomatal conductance was not different for the tested Tl. Consequently, CO2 intake was not limited. As relative humidity was kept constant, vapor pressure deficit increased and forced transpiration to a factor 4 higher than in controls (Pnmax = 1.62 μmol CO2 m-2 s-1). The chlorophyll fluorescence measurements showed that Fo increased and Fm decreased significantly at 40°C, resulting in a significant lower Fv/Fm (~21%). In light, the maximum efficiency of PSII photochemistry was 50% lower and NPQ was significantly increased at 40°C. At an air temperature of 40°C in combination with 1300 μmol quanta m-2 s-1 during 4 h, leaves at the top of the branch started to wilt. Leaves were able to recover at 20°C. After 1 day, some leaves showed brown spots due to excessive water loss. We can conclude that at a Tl of 40°C, PSII gets thermally damaged and leaves dehydrate rapidly.

P09-102 Chloroplast NADPH-dependent thioredoxin reductase from Chlorella vulgaris functions as an antioxidant with 2-Cys peroxiredoxin
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Chlorella vulgaris C-27 acquires freezing tolerance when exposed to low temperatures before frozen. By using CDNA subtraction method and quantitative real-time RT-PCR analysis, a chloroplastic NADPH-dependent thioredoxin reductase (NTR-C) gene has been identified as a low-temperature-inducible gene from Chlorella. In order to clarify the function of NTR-C in freezing tolerance of Chlorella, we have identified a protein cooperating with NTR-C. A mature form of NTR-C protein (mNTR-C) was purified from Chlorella culture. The protein showed thioredoxin activity in addition to NTR activity, similar to the behavior observed in NTR-Cs from higher plants. By using in vitro pull-down assay, a 21.2-kDa protein, which formed a complex with mNTR-C, was purified using mNTR-C as a carrier protein. The protein was identified as 2-Cys peroxiredoxin (2-Cys Prx) based on the N-terminal amino acid sequence. Peroxide reduction activity of the identified Prx was confirmed by reconstitution assay using both mNTR-C and Prx prepared with E. coli as His-tagged proteins. Thus, NTR-C was suggested to function in freezing tolerance of Chlorella by cooperating with 2-Cys Prx to eliminate peroxides generated under low temperatures and freeze-thaw stress. For clarifying the involvement of the antioxidant system in freezing tolerance, a yeast transformant expressing both NTR-C and Prx genes is under construction.

P09-103 Protein profiles in response to salt stress in seeds of Brassica napus
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Salt tolerant and sensitive cv. Of canola (Brassica napus L.) was grown in greenhouse conditions in presence or absence of different concentrations of NaCl. Inhibition of plant growth and modification of plant morphology are the most sensitive responses of canola plant to salt stress. The ratio of fresh weight to dry weight of plants after stress application of NaCl was strongly increased in comparison to their corresponding control. Electrophoretic analysis of total soluble protein (SDS-PAGE) profiles were carried out in order to evaluate the response of canola cultivars to salt stress. SDS-PAGE analysis has revealed that plant grown under NaCl showed induction in the synthesis of few polypeptide in seeds increasing of these proteins was greater in tolerant cv. than the sensitive. This differences reflected the biochemical adjustment of the plant to cope with the saline conditions. These results can be translated into efforts aimed to develop salt tolerant cultivars and maximiz the use of saline soils.

P09-104 Chilling resistance in Hevea brasiliensis
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The diseases caused by low temperatures affect mainly plant species from hot areas (rice, corn, tomato). The symptoms generally appear at temperatures definitely higher than 0°C and below than 12°C. For economic reasons, Hevea brasiliensis is more and more cultivated in sub optimal zones like Mato Grosso in Brazil where it undergoes these low temperatures. On these plantations, in addition to the damages caused by the lowest temperatures, harvest of the latex is stopped for at least two months during the coldest period. Therefore the selection for the resistance to cold temperatures is necessary for the extension of hevea culture. The behaviour of different clones of hevea from varied origins was analyzed using a complementary technique. The measures of chlorophyll fluorescence, gas exchange and stomatal conductance informed us about the state of the photosystem II. The measures of electrolyte leakage and the MDA content gave an evaluation of the membrane damages. These measures were carried out during a time course of 96 h at 10°C, as well as during the passage of the plants from 10 to 28°C (stage of recovery). This ecophysiological approach allowed to classify the clones according to their different sensibilities to the cold. Furthermore, this approach was useful to target key stages for further molecular analyses (cDNA-AFLP) which will be carried out.

**P09-105 Comparative investigation of polyamines under salt stress in halophyte (Mesembryanthemum crystallinum) and glycophyte (Plantago major)**

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High salt concentration induce disturbances in the ionic, osmotic, and oxidative state of the organisms. Among all terrestrial plants halophytes comprise only 2%. The remaining 98% of all species are glycophytes displaying sensitive or relatively tolerant species to salinity. Polyamines are are involved in various processes. Cellular PA content changes upon exposure to abiotic stresses. PAs occur in free, conjugated to low molecular compounds, or bound to macromolecules forms. The *M. crystallinum* L. and *P. major* L. plants were grown in water culture with modified Winter nutrient medium, 14 h of 350 mmol m⁻² s⁻¹, at 23°C/16°C. Six weeks old plants were exposed 100–400 mM NaCl. After different times of salinity growth the leaves and root were frozen and used for PAs free, soluble bound and pellet conjugated content analyses. All types of common PAs are present in all fractions in both halophyte and glycophyte plants. The changes in content, quality and fraction is different between the plants. Put seems to be the main one in *P. major* and salt treatment evidenced a decrease in free form and increase in insoluble bound fraction. In *M. crystallinum* Spd is the main component in free PA fraction and Put in the insoluble one. Interesting to note seems the Cad in leaves of *M. crystallinum*, and its increase in the soluble bound fraction after long exposure to salt. The modification of PAs content and fractionation are only partially related to PAs genes expression and salt adaptation.

**P09-106 Functional characterization of cytosolic and peroxisomal isoforms of ascorbate peroxidase in rice (Oryza sativa L.)**


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Reactive Oxygen Species (ROS) are continuously produced by aerobic metabolism. In plants, the production of ROS is drastically increased in response to biotic and abiotic stress, disturbing the normal balance of superoxide radicals, hydroxyl radicals and hydrogen peroxide in the intracellular environment. Ascorbate peroxidases (APx) catalyze the conversion of hydrogen peroxide into water using ascorbate as a specific electron donor. Previously, we identified the presence of eight APx genes in the nuclear genome of *rice*, encoding isoforms that are located in different subcellular compartments. To address the functional role of the OsAPx isoforms, we generated transgenic rice plants silenced for APx-encoding genes by RNAi strategy. The reduction of cytosolic APx function correlates with a global reduction of APx activity, which strongly impacts the whole antioxidant system regulation. APx1/2 silenced plants showed increased hydrogen peroxide accumulation under control and stress situations. Also, transgenic plants presented higher tolerance to toxic concentration of aluminum when compared to wild type plants. Taken together, the results strongly suggest that an increased intracellular hydrogen peroxide, mediated by the silencing of cytosolic OsAPx genes, modulate the antioxidant system, contributing to stress tolerance in plants. (Supported by: CNPq, CAPES, UNESCO and ICGEB).

**P09-107 Changes in photosynthesis and proline metabolism in response to drought stress in glutamine synthetase mutants from Lotus japonicus**


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Drought stress constitutes one of the most frequent problems affecting productivity and persistence of Lotus plants used for forage. We use the model legume *Lotus japonicus* to help in the analysis of the response of Lotus plants to drought stress. Previously characterized photorespiratory mutants deficient in plastidic glutamine synthetase (GS2) were included in our study. *L. japonicus* plants show alterations of chlorophyll fluorescence and thermoluminescence (TL) emissions in response to drought stress, indicative of functional and structural alterations of PSII. However no major changes of high-temperature thermoluminescence (HTL) were observed. GS2 deficiency produce a significant decrease of fluorescence of plants under active photorespiration.
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conditions, particularly in drought stress. This suggests an interconnection between PSII and photosynthetic ammonium resimulation. However, no major alterations were observed in TL and HTL bands. The mutants show a substantial decrease in proline accumulation in response to drought, indicating an active contribution of CS2 in this condition. However, only minor changes were observed in water loss and gain in younger leaves of these mutant plants, concomitant to the lower proline accumulation. Transcriptomic studies with Affy chips are in progress to further characterize the different responses to drought stress in this plant.

Acknowledgements – EU LOTASSA project FP6-517617 and MEC (Spain) BFU2003-3120 and BFU2007-6107-C02-01.

P09-108 Purification, identification and biochemical characterisation of iron reductase (FRO 1) from plasma membranes of pea (Pisum sativum L.) roots
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Iron uptake strategy I plants like pea reduce iron by a transmembrane Fe³⁺-chelate reductase activity before uptake. Initial attempts at the purification of FRO (28 kDa, 200 kDa) have been published for tomato (Bagnaresi et al. 1997; Holden et al. 1991, 1995). Meanwhile the tomato gene coding for LeFRO1 (81 kDa) was isolated and characterized (Li et al. 2004). PsFRO1 (flavocytochrome b family) was identified as a pea Fe³⁺-chelate reductase involved in iron deficiency (Waters et al. 2002). The predicted protein has a molecular mass of 81 kDa, 10 transmembrane helices, heme, FAD and NADPH binding-sites. In the present study a significant transmembrane Fe³⁺-chelate reductase activity was demonstrated in sealed and NAD(P)H-loaded apoplastic-side-out plasma membrane (PM) vesicles. The protein corresponding to this activity was partially purified from PM of iron deficient pea roots. Fe³⁺-chelate reductase activity of the purified protein was compared to that of PM isolated from roots of both iron-sufficient and iron-deficient pea. Enzyme kinetics (Km, pH-optima, and inhibitors) of Fe³⁺-chelate reductase activity will be shown. Properties like MW, pl, co-factors, etc. were investigated by in-gel staining procedures and absorbance spectra. Possible protein-protein interaction was analysed by native PAGE and Western-blot analysis.


P09-110 Jungermannia exsertifolia subsp. cordifolia: an aquatic liverwort responsive to enhanced ultraviolet radiation
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Liverworts are structurally simple plants important in the evolutionary transition from water to land. In this process, the tolerance to UV radiation might be crucial, but liverworts (as bryophytes in general) lack the structural protecting mechanisms that are present in higher plants: thick cuticles, epidermis and hairs. Thus, liverworts may develop only biochemical protections, such as UV-absorbing compounds. We have demonstrated that some hydroxycinnamic acid derivatives from the aquatic foliose liverwort Jungermannia exsertifolia subsp. cordifolia, in particular p-coumaroylmalic acid, increased when the liverwort was exposed to enhanced UV radiation, under both laboratory and field conditions. By contrast, enhanced UV radiation hardly caused any change in several physiological variables indicative of vitality, such as the maximum quantum yield of PSII (Fv/Fm). Thus, this liverwort seemed to be tolerant to UV radiation, probably due to the accumulation of UV-absorbing compounds. Given that p-coumaric acid, a UV-absorbing compound chemically similar to p-coumaroylmalic acid, has been used in higher plants as UV bioindicator, we discuss the possible use of hydroxycinnamic acid derivatives from the aquatic foliose liverwort Jungermannia exsertifolia subsp. cordifolia in the bioindication of the temporal UV changes caused by the anthropogenic ozone layer depletion.

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Aluminium has been recognized as a main toxic factor in crop production in acid lands. The rapid inhibitory effect of Al³⁺ on root elongation suggests an involvement of signal transduction pathways. The goal of the present study was to assess the impact of Al³⁺ on the players of the phospholipid signalling pathway, mainly phospholipases, in BY-2 tobacco cells. Special interest was given to diacylglycerol (DAG) which is emerging as an important lipid molecule in plants. It stands on the crossing of several signalling and metabolic pathways as well. We pre-labelled cells with fluorescent derivative of phosphatidylcholine (BODIPY-PC) and monitored fluorescently labelled BODIPY-DAG formation in response to Al³⁺. Treating cells with AlCl₃ (100 μM) decreased the formation of labelled diacylglycerol. Decrease was rapid (10 min), time and concentration dependent. Metabolic pathways that could be involved in the control of DAG generation and consumption during aluminium response were examined. Our results suggest that DAG formation is influenced by the inhibition of the PC-PLC activity. These findings provide the first evidence for the role of phospholipase C hydrolysing phosphatidylcholine in regulation of biochemical processes during aluminium response. The work was supported by the grant no. LC06034 of the Ministry of Education, Youth and Sports and no. 522/03/0340 of the Czech Science Foundation.

P09-110 Aluminium stress influences rapid decrease of diacylglycerol content generated by phospholipase C hydrolysing phosphatidylcholine in tobacco BY-2 cell
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P09-111 Effect of different sulphates levels in the nutrient solution on Ni-stressed lettuce plants
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The purpose of this work was to evaluate the effect of intensive S-SO₄ nutrition on nickel stressed lettuce plants cultivar Justyna. The experiment was conducted on Hoagland nutrient solution. The following levels of sulphur sulphate and nickel concentrations were used: 65; 130; 190 mg S dm⁻³ and 0; 0.4; 40; 80 μM Ni. Nickel at concentrations 40 and 80 μM caused significant yield decrease, lower coefficient of roots in organic biomass production, lower chlorophyll and carotenoids content as well as decrease in physiological parameters of roots (volume, total and active adsorptive surface and 1 cm² active surface). Intensive sulphate nutrition in the medium without Ni addition caused significant yield increase, higher coefficient of organic mass production but not changed photosynthetic pigments content and physiological parameters of roots. Moreover, the decrease in P, K, Ca and Mg in the leaves and increase of total sulphur and S-SO₄ in roots was observed. Application of additional sulphates levels into the medium under Ni presence increased yield and all analysed physiological parameters. In general under these conditions the decrease of analysed macroelements in leaves and increase in total and S-SO₄ in roots was stated. Under high sulphates levels Ni at concentration of 0.4 μM did not caused significant changes in Ni content, whereas high Ni doses i.e. 40 and 80 μM decreased and increased the content of this metal in leaves biomass, respectively.

P09-112 The GABA transporters AtGAT1 and AtGAT2 of Arabidopsis thaliana
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4-Aminobutyric acid (GABA) is a well characterized inhibitory neurotransmitter in the nervous system of animals. In contrast, little is known about the function of GABA in plants. Accumulation of GABA via increased synthesis is observed in response to a variety of stress conditions. However, the contribution of intracellular and intercellular transport to changes in GABA concentrations remains unclear. We characterized AtGAT1 (At1g08230) as the first high affinity GABA transporter in plants, by measuring uptake of radiolabeled GABA in Saccharomyces cerevisiae expressing AtGAT1 and by using two electrode voltage clamp experiments with Xenopus laevis oocytes (Meyer et al. 2006). The transient expression of AtGAT1/GFP fusion proteins in protoplasts revealed a localization at the plasma membrane. In A. thaliana, expression of AtGAT1 is highest in flowers and under conditions of elevated GABA concentrations. The highly homologous gene AtGAT2 (At5g41800) encodes for a transporter with a much lower affinity for GABA. AtGAT2/GFP fusion proteins are also targeted to the plasma membrane. Expression of the uidA gene under the control of the AtGAT2 promoter shows expression of AtGAT2 in the anthers of young flowers and in the vascular tissue. Further characterization of AtGAT1 and AtGAT2 will help to reveal the role of GABA transport in plants.

P09-113 Differential response of the water status of barley plants under drought or salt stress and its interaction with elevated CO₂
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The prolonged exposure of plants to elevated CO₂ produces a great variety of physiological responses being dependent on the availability of other resources, such as water. So, the analysis of interaction between elevated CO₂ and the water in the soil is essential to understand the responses of the plants towards environmental changes. Thus, the objective of this study has been to study how one barley cultivar (Iranis) answers to salinity or drought under different (CO₂). Plants subjected to water deficit both by withholding water or by its retention into NaCl ions showed similar water potential but reached by different strategies. When plants of barley were subjected to drought or to salt treatment (240 mM NaCl) during 2 weeks, the water potential registered were -1.3 and -1.2 MPa, respectively. Drought plants decreased their turgor potential by 60% while under salt stress the reduction was 40%. Besides, in drought plants the osmotic potential decreased 42%, mainly due to dehydration, while the ones subjected to salinity presented a decrease of 40% as a consequence above all to active accumulation of solutes. In both stress conditions, a positive effect of the elevated CO₂ was observed. In drought, elevated CO₂ provoked a delay in the dehydration rate and in salinity permitted a higher decrease of osmotic potential thanks to a higher active accumulation of solutes. This work has been supported in part by grants of UPV 118.310-G07/2001, MEC BFU2007-6023/BFI and UNESCO 07/02.

P09-114 An expression analysis of Ipomoea nil type 1 metallothioneine-like-gene in response to metal ion treatment
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Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins that can bind heavy metals. MT genes have been found in animals, plants and some prokaryotes. Metallothioneins have been divided into three classes. Class I includes MTs that contain 20 conserved Cys residues, class II includes MTs that contain Cys residues grouped in two terminad domains. Class III includes specific MTs which are not gene-encoded proteins but are synthesised enzymatically. In plants MTs are further classified into four types. Despite the fact that MTs have been studied for decades, the functions of MTs in plants still remain elusive. Probably metallothioneins in plants are involved in copper homeostasis and Cu resistance. Several lines of indirect evidence suggest that MT genes enhance resistance to various heavy metals when expressed in yeast cells. Also exposure of plants to heavy metals increases the expression of MT genes. It is not clear if MTs confer resistance to heavy metals in intact plant cells. Bacteria E. coli were transformed with expression vector bearing cDNA of Ipomoea nil metallothioneine-like-gene. Semi quantitative RT-PCR was used to determine whether level of I. nil type 1 metallothioneine gene mRNA changes during metal ion treatment. The results suggest that metallothioneine genes are responsible to metal ion detoxification. In
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the future it may be possible to use transgenic plants with overexpression of metallothioneine-like-gene for fitoremediation.

P09-115 Proteins localized in detergent-resistant plasma membrane in Arabidopsis during cold acclimation
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Arabidopsis increases freezing tolerance upon exposure to low, non-freezing temperatures, which is known as cold acclimation (CA). CA results in changes in protein and lipid compositions in the plasma membrane (PM) and the changes ultimately increase the PM cryostability at freezing temperatures. Recently, various membrane proteins associated with membrane trafficking and signal transduction are found to be localized in specific regions in the PM, which are called as microdomains and can be isolated as detergent-resistant plasma membrane fractions (DRM). To investigate the role of DRM-associated proteins in CA, we identified cold-responsive DRM-associated proteins in Arabidopsis. Two-dimensional differential gel electrophoresis showed that one-third of the DRM-associated proteins quantitatively changed during CA. Mass spectrometric analyses revealed that P-type H+ ATPase, aquaporin and endocytosis-related proteins increased and tubulin, actin and V-type H+ ATPase subunits decreased in DRM during CA. Real-time PCR analysis indicated that many cold-induced proteins in DRM are not regulated at levels of transcription, but cold-decreased proteins were largely down-regulated at transcript levels. Immunofluorescence analysis revealed that DRM-associated protein distribution changed within the PM during CA. These results indicate that CA is accompanied by changes in protein population in DRM fractions as well as protein distribution in the PM.

P09-116 Stem respiration in two different pine forests, Pinus canariensis in Tenerife and Pinus cembra in the Austrian Alps
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Gas exchange knowledge of forest trees is important for the understanding of the response of forests to predicted future changes in atmospheric CO2 and climate. A comparative study of stem respiration in two different pine forests at high elevation is shown in this work. An Austrian site established in an open, approximately 95-year-old Pinus cembra stand at 195 m a.s.l. on Mt. Patscherkofel, South of Innsbruck, with mean annual temperature of 2.4°C and a Spanish site in a pine forest of Pinus canariensis, situated in Morro de Isarda (Tenerife), at 1630 m a.s.l. with mean annual temperature 12.6°C. Main meteorological factors as well as stem temperature were continually recorded and the stems CO2 efflux of adult field grown trees was measured periodically during an entire year. The annual respiration rates of individual sample trees were combined and partitioned into maintenance and growth components. Total area-based CO2 efflux was 123 and 623 g C per m2 and year in P. canariensis trees at Tenerife and in P. cembra trees at timberline in Austria, respectively. Annual maintenance respiration however, did not differ significantly between both sites and accounted for 70 and 65% of the annual total stem respiration in P. canariensis and P. cembra, respectively. Thanks to the Spanish and Canarian Governments (Projects CGL2006-10210/BOS MEC and PIT042005/070) co-financed by FEDER and to Bilateral Project Austrian and Spanish Governments (HU2005-0007).

P09-117 Heavy metals concentration in mosses and there impact on the lichen value diversity in the Pristina region
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Some heavy metals emitted into the air from sources such as industries and power stations are mainly spread locally around the emission source. Their accumulation in plants can cause limitation on growth and distribution of plant population. Concentration of heavy metals was determined in mosses, as they are very suitable as accumulation bioindicators, whereas the lichen value diversity was determined based on the methodology of European Guideline for Mapping Lichen Diversity as an Indicator for Environmental Stress (Asta et al. 2002). Concentrations of heavy metals are measured in the species Brachythecium rutabulum (Hedw.) and Homalotheecium lutescens (Hedw.). Heavy metals in small doses in the lichens have a stimulant effects as necessary on the plant’s physiological processes, as well the enzymes activators, while in high doses are toxic. Based on the measurements it is concluded that from heavy metals, zinc has the highest concentration while the lowest cadmium. Cobalt, lead and zinc have greater positive correlation on the lichens value diversity. Cadmium and nickel have lower impacts in lichens value diversity, whereas copper has higher negative impact on lichens value diversity which was a limiting factor in the lichens. Lead concentration was much lower that zinc concentration, but have same correlation which shows the high toxicity of lead compared to zinc in the lichens.

P09-118 Effects of glutathione to cadmium transport and accumulation in oilseed rape plants (Brassica napus L.)
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Cadmium (Cd) is a toxic heavy metal which is harmful to our health. To reduce Cd accumulation in crop plants, it is necessary to elucidate mechanisms of Cd transport and accumulation. However, these mechanisms are not fully understood so far. Glutathione (GSH) is a major low weight thiol tripeptide, involved in many aspects of metabolism. In our previous work, responses of GSH in sieve tubes to Cd treatment were investigated. These results suggested that GSH might be playing important roles in controlling Cd movement in

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P09-121 Genetic dissection of hormonal responses in the roots of Arabidopsis thaliana grown under continuous mechanical impedance
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We investigated the role of ethylene and auxin in regulating the growth and morphology of roots during mechanical impedance by developing a new growing system and using the model plant Arabidopsis thaliana. The Arabidopsis seedlings grown horizontally on a dialysis membrane-covered agar plate encountered adequate mechanical impedance as the roots showed characteristic ethylene phenotypes; a 2-fold reduction in root growth, increase in root diameter, decrease in cell elongation and ectopic root hair formation. The root phenotype characterization of various mutants having altered response to ethylene biosynthesis or signaling, the effect of ethylene inhibitors on mechanically impeded roots and transcription profiling of the ethylene responsive genes led us to conclude that enhanced ethylene response plays a primary role in changing root morphology and development during mechanical impedance. Further, the differential sensitivity of horizontally and vertically grown roots toward exogenous ethylene suggested that ethylene signaling, rather than ethylene production plays a critical role in enhancing the ethylene response. We subsequently demonstrated that the enhanced ethylene response also affects the auxin response in root. Taken together, our results provide a new insight into the role of ethylene in changing root morphology during mechanical impedance.

P09-122 Identification and characterization of Cor413im proteins as novel components of the chloroplast inner envelope
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Plastids are surrounded by two membrane layers, the outer and inner envelope membranes, which have various transport and metabolic activities. A number of envelope membrane proteins have been identified by biochemical approach and assigned to specific functions. Despite those efforts, the chloroplast envelope membrane is expected to contain a number of as yet unidentified
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Proteins that may affect specific aspects of plant growth and development. In this report, we identify and characterize a novel class of inner envelope membrane proteins, designated as Cor413 inner envelope membrane group (Cor413im), by genetic and biochemical approach. Both in vivo and in vitro studies indicate that Cor413im proteins are targeted to the chloroplast envelope. Biochemical analyses of Cor413im demonstrate that it is an integral membrane protein in the inner envelope of chloroplasts. To determine in vivo roles of Cor413im proteins, we isolate T-DNA knockout lines of COR413IM1 and COR413IM2 genes. Analyses of these mutants reveal that disruption of COR413IM genes affects the freezing tolerance of Arabidopsis leaves. Based on these data, we propose that Cor413im are novel components of the chloroplast inner envelope and may affect the freezing tolerance of Arabidopsis.

P09-123 Combined action of increasing temperature and tropospheric ozone on birch (Betula pendula) and aspen (Populus tremula) and ectomycorrhiza (Arabidopsis). E. Oksanen, S. Komppa, G. Brader, P. Mullineaux, and J. Wingsle.

Northern trees are experiencing dramatic changes in their environment due to global warming, increasing greenhouse gases and changes in precipitation. An open-field experiment was established in University of Kuopio, Finland, to study impacts of combined action of warming and ozone on silver birch (Betula pendula) and European aspen (Populus tremula). Our exposure system consists of four elevated-ozone and four control plots. Each plot is divided into two IR-heated sub-plots and two ambient-temperature sub-plots. There were four genotypes for both species planted in the sub-plots. There were four genotypes for both species planted in the sub-plots. Each plot is divided into two IR-heated sub-plots and two ambient-temperature sub-plots. There were four genotypes for both species planted in soil-submerged pots. The exposure started in June 2007 and will be continued until October 2008. Exposure plants are being measured for growth, phenological parameters, gas exchange profiles, fluorescence, respiration, volatile organic compounds (VOCs), soil respiration, mycorrhizal infections, extramatrical mycelium, antioxidants, reducing potential, and changes in leaf metabolites and gene expression. C allocation will be studied with stable isotope (13C-CO2) labelling methods. Preliminary results suggest that rising temperature increases the growth, photosynthesis and VOC emissions from leaves in both species, whereas stomatal conductance and antioxidative capacity are decreasing. Elevated ozone impairs leaf growth in birch and photosynthesis in both species. There seems to be a large variation among the genotypes in their responses to warming and ozone with complicated interactions.

P09-124 Multiple paths to ROS sensitivity in ozone hypersensitive Arabidopsis mutants

P09-125 Synthetic preparation methyure can increase plant salt tolerance

T. A. Palladina and I. M. Kurylenko

Salinity which accelerated by global climate warming is a serious threat for agriculture and demands to increase salt tolerance of crop plants. Although a radical resolution of this problem consisted in transgenic form construction but there is an alternative way- bioactive preparations. Preliminary testing of growth regulators under salinity conditions realized by us on corn seedlings exposed on salinized media showed a preference of Methyure and elucidated some properties of its antistress mechanism. Further examination of its protective effect during whole vegetation have been carried out on maize plants grown in Vagner pots on a hard or middle NaCl levels and in field experiments on slightly salinized soils. Treating with Methyure was realized by seed soaking and plant sprinkling. It prevented growth detain of plants induced by salinity whereas the protective effect was more displayed in roots. Methyure decreased Na+ and Mg2+ consumption by roots and their translocation to overground organs but not influenced K+ transport. Seed soaking in Methyure prevented plant death caused by 0.1 M NaCl during

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transition to generative period and permitted them to form kernels with seeds. Additional plant sprinkling before panicle appearance considerably improved results. In field experiments a double treatment with Methyure increased corn crop on 15%. Methyure can be recommended for agriculture under stress condition caused by salinity and probably other negative factors.

P09-126  Effects of nitrate ions supply on total phenolic compounds content of hydroponically grown red beet (Beta vulgaris L.)
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In the present work we have studied the effect of nitrate ions supplied into nutrient solution on the accumulation of total phenolics in red beet (Beta vulgaris L.). Red beet plants were hydroponically grown in a watered perlite root medium with nutrient solutions containing different concentrations of nitrate ions supplied as nitrogen source i.e. 86, 173, 260, 350, 560, and 876 ppm. The concentration of total phenolics in the plant extracts was determined according to the methanol-extraction method and was expressed as Gallic Acid Equivalents (GAE). We found that the decrease of nitrate ions supplied by nutrient solution under 350 ppm, dramatically increased the concentration of total phenolics in plant leaves, petiols, and roots, while at high nitrate concentrations, no significant effect was detected. Total phenolics content at mg/g dry weight was from 6.8 to 12.6 at 876 ppm nitrate and 13.4 to 36.6 at 86 ppm nitrate in plant material (i.e. leaves, petiols, and roots). The highest amount of phenols was found in roots and petiols while the lowest was found in leaves. In addition, biomass production, total water content and leaf physiological characteristics (net photosynthetic rate, transpiration rate and stomatal conductance) also decreased under low nitrate concentrations. These data are consistent with the inverse relationship between growth and production of carbon based on secondary metabolites and predict carbon/nutrient balance (CNB) hypothesis.

P09-127  Increases in polyamine contents induce a stress tolerance by reduction of ROS accumulation in knock-out mutant of lysine decarboxylase of rice
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We choose the rice of lysine decarboxylase knock-out mutant line for further study of antioxidant mechanism through polyamine (PA) biosynthesis in plants. In spite of detection in lysine biosynthetic domain in this mutant line, the more accumulation of other PA, putrescine(PUT), spermidine(SPD), and spermine(SPM), were accompanied under oxidative stress conditions with more tolerance in the aspects of chlorosis and ion leakage of leaves than in wild-type. The production of ROS showed significantly lower level after both treatments of salt and acidic stress in knock-out mutants than wild-type. Also, exogenously applied PAs such as spd and spm, which are degraded by amine oxidase with a byproduct of H2O2, inhibited significantly ROS production in both treatments with and without salt stress. Even though the occurrence of the oxidative tolerance in this knock-out mutant, the activities of ROS scavenging enzymes, catalase and cytosolic APX, were not significantly changed. It was quantified by qRT-PCR that exogenous PAs induced more amounts of transcripts for NADPH oxidase, RbohD and RbohF, in wild-type. These results implied the direct effects of PA as free radical scavengers or antioxidant on ROS production in response to abiotic stresses was more functional than the effects of PA-derived H2O2. Therefore, it may be suggested that PAs have physiological roles for minimizing cell damage or sustaining cell growth by moderating ROS contents in response to abiotic stresses.

P09-128  Inhibition of ethylene biosynthesis or perception in antisense transgenic tobacco plants reduced ROS accumulation in response to abiotic stresses
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In transgenic tobacco leaves manipulated with antisense expression of ACC synthase (ACS) or Ein3 gene, the production of ROS was more significantly reduced in response to abiotic stress, compared to WT. The treatment of an intermediate of ethylene biosynthesis, ACC, increased remarkably ROS production, but that of an inhibitor of ACS, AVG, almost fully prevented ROS production in WT. The treatment of an action inhibitor of ethylene, NBD, was also ROS accumulation. We already reported that transgenic tobacco plants with antisense ACS gene showed a significant tolerance against high salt treatment or oxidative stress. It was determined by qRT-PCR that the decrease in ROS accumulation in those transgenic plants after stress treatment was mainly resulted from increases in ROS-detoxifying enzymes such as MnSOD, CuZnSOD, APX, and GST within 3 h. The stress-induced effects in response to high salt treatment, H2O2, and ABA treatment on ROS production were significantly suppressed in Ein3-AS-I transgenic plants. This machinery of ethylene on ROS production during stress response will be confirmed using transgenic tobacco plants with antisense gene expression of two isoforms of NADPH oxidase, RbohD and RbohF. These results might suggest that the suppression of stress-induced ethylene production in biphasic manner and then subsequent perception, which was intimately involved in the amplification of ROS production, was required to develop stress tolerance in response to abiotic stresses.

P09-129  3i: Untranslated regions mediate stress-induced mRNA decay
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Abstracts

Many environmental stimuli including water potential, temperature extremes, and high salinity, regulate gene expression at transcriptional and post-transcriptional levels. We are taking a genomics-based approach to unravel the regulation in response to such environmental stresses in rice. Expression profiling with the 60K Rice Whole Genome Microarray revealed that transcripts of a group of genes involved in light and dark reactions are decayed much earlier than the others under stress conditions. We have shown that the stress-induced mRNA decay is a post-transcriptional event by using RNA pol II chromatin immuno-precipitation assay. To delineate functional determinants, we chose two representative genes, RbcS and Cab, and dissected them into several components. Transgenic rice plants expressing different combinations of the components were analyzed under stress conditions using the real-time qPCR method, demonstrating that 3'UTR is the major mRNA sequence determinant that mediates such stress-induced mRNA decay.

P09-130 Localization of poly(ADP-ribose) polymerase in Arabidopsis thaliana plants exposed to heavy metal stress

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Poly(ADP-ribose) polymerase (PARP) plays a primary role in the process of poly(ADPribosylation). The enzyme can contribute to both DNA repair and cell death. PARP is activated by DNA strand breaks or kinks. It transfers ADP-ribose moieties from NAD+ to specific acceptor proteins to form complex, branched chains of lengths up to 200 residues. The known acceptor proteins include many peptides that function in DNA repair and cell cycle regulation. Many chemical agents (including heavy metals like Cu) induce DNA damage. We decided to check the localization of this enzyme in the parenchyma cells of plants exposed to heavy metals. The immunolocalization of PARP was assessed in Arabidopsis thaliana grown in the nutrient solution under Cd and Cu excess (0, 5, 50 µM) for 7 days. In cells of plants treated with heavy metals a strong immunopositive reaction appeared as compared to the control. Under Cd stress the immunogold particles were localized inside the nuclei, chloroplasts, the cytoplasm and vacuoles, but a stronger reaction towards PARP was observed at 50 µM Cd. Under Cu excess the same pattern of gold particles distribution was found both at 5 and 50 µM Cu. However, at a higher Cu concentration the reaction was less intensive in the chloroplasts, but it was stronger in the cytoplasm and the nuclei than in plants exposed to 5 µM Cu. After 7 days of plant exposure to heavy metal a stronger immunopositive reaction was observed in Cd- than in Cu-treated plants.

P09-132 Assessment of lead-induced lesions and oxidative alterations on DNA in Vicia faba roots

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Lead (Pb), causes many unfavourable changes in plant cells. However, few is known about its genotoxicity. Our previous work clearly demonstrated the pivotal role of a NADPH oxidase in lead-induced oxidative burst (Pourrut et al. 2007). The aim of this study is to determinate the possible relationship between lead-induced oxidative stress and genotoxicity in Vicia faba, (Vf) root cells. In the first part of the present study, the lead genotoxicity was investigated using the micronucleus assay (MN) in Vf root tips, in presence or absence of the NADPH-oxidase enzyme inhibitor DPI or a ROS scavenger alpha-Tocopherol (Vit E). Results demonstrated that lead significantly increased MN frequency. Roots treatment with DPI at 0.1 and 1 µM totally prevents lead-induced MN generation. Same results were obtained with 100 µM of Vit E. These results strongly suggest the importance of the oxidative stress in MN generation. To further investigate lead genotoxicity mechanism, lead-induced DNA damages on Vf root cells were also estimated by Comet assay. Vf seedlings were exposed to lead (10 µM) and comet assay were performed on root cells. The use of lesion-specific endonucleases (Endo III and Fpg) or antioxidant allowed the measurement of different kinds of DNA damage. Our results demonstrated that Pb significantly increased DNA lesions and oxidative alteration on DNA. All these results highlighted the implication of ROS in lead-induced genotoxic.

P09-133 Effects of the Asahi SL biostimulator on field grown oil seed rape plants

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Abscisic acid (ABA) might be a suitable candidate for improvement of ex vitro transfer of micropropagated plantlets. It could be applied either into the substrate immediately after ex vitro transfer or into the medium for the last subculture. Content of endogenous ABA increased considerably after ABA application and it further increased in tobacco (Nicotiana tabacum L. cv. SR1) plants grown ex vitro under higher irradiance (700 vs 150 × 10–6 mol m–2 s–1) showing occurrence of stress in these plants. After ex vitro transfer, stomatal conductance and transpiration rate decreased more in 5 × 10–5 M ABA-treated plants than in control plants and so their wilting was limited. Higher contents of chlorophylls and carotenoids, and decreased degree of deepoxidation of xanthophyll cycle pigments also suggested less stress in ABA-treated plants. Changes in antioxidative enzyme activities were also observed. For in vitro hardening, much lower ABA concentration should be used as 5 × 10–5 M ABA caused considerable retardation of plantlet growth. Five and 10 × 10–6 M ABA was suitable as it decreased stomatal conductance and transpiration rate during in vitro growth and increased water use efficiency after ex vitro transfer. The authors acknowledge the financial support of the Grant Agency of the Czech Republic (No. 522/07/0227).
Oil seed rape is a very important agricultural crop, acreage of which is increasing yearly mainly due interest of its cultivation for biofuel. Frost during winter and early spring might damage plants and reduce yield of this crop. Biostimulator Asahi SL, internationally known as Atonik, is often used in many crops, including oil seed rape in order to improve/repair the status of plants. This study was aimed at assessment of the effect of Asahi SL on: (1) efficiency of photosynthetic apparatus; (2) transpiration; (3) biomass accumulation; and (4) yield.

Oil seed rape (Brassica napus L. var. oleifera) cv. Lissek was grown in the 2006/2007 season on the field of the VUULS Experimental Station at Chylicze. Routine agricultural practices, recommended for this species, were employed. In spring 2007 Asahi SL was applied as single or double spray, in concentration of 0.2 % v/v, in 300 l ha⁻¹. Plant gas exchange, chlorophyll content and chlorophyll a fluorescence were measured weekly and at harvest fresh and dry matter of above ground parts and seed yield were recorded. Asahi SL had a positive effect on oil seed rape plants manifested by (1) increased photosynthesis, (2) values of Performance Index, (3) chlorophyll content, (4) biomass accumulation and (5) seed yield. Plants treated with Asahi SL plants had also (6) higher intensity of transpiration well corresponding with lower stomatal resistance.

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P09-134 Tonoplast proton pumps and Na⁺/H⁺ exchange activity in potato cell lines and implications for salt tolerance

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Salinity is an important environmental stress reducing plant growth and productivity. Ion toxicity, osmotic stress and ion imbalance are the major constraints caused by salinity. Tonoplast enriched-vesicles isolated by a discontinuous sucrose gradient from control and 150 mM NaCl-tolerant calli lines were used as a model system to study the activity of V-ATPas and V-H⁺PPase and the involvement of Na⁺ compartmentation into the vacuole as a mechanism of salt tolerance in Solanum tuberosum. Both ATP- and PPI-dependent H⁺-transport, measured as the initial rates of ACMA fluorescence quenching, were higher in tonoplast vesicles from salt-tolerant line than in vesicles from control cells. Na⁺-induced dissipation of a pre-established PPI-dependent pH gradient was used as an experimental evidence for the involvement of a tonoplast Na⁺/H⁺ exchange system. The initial rates of Na⁺-dependent fluorescent recovery followed Michaelis-Menten kinetics and the Vₘₕₜₗ of proton dissipation was 2-fold higher in vesicles from salt-tolerant calli compared with the control cells. Both Na⁺ and Li⁺, but not K⁺, dissipated the APh. The correlation between the increase of both the H⁺ pumping through V-ATPas and V-H⁺PPase and the activity of Na⁺/H⁺ exchange system in NaCl-tolerant cell line suggests that the accumulation of Na⁺ into vacuole represents an adaptive response to high salinity in S. tuberosum.

P09-135 Expression pattern of polyamine biosynthesis genes in Plantago major L. under NaCl and UV-B irradiation

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It is currently assumed that the negative effect of the various abiotic stresses (NaCl, UV-B irradiation) is at least partially due to the generation of reactive oxygen species (ROS). Considerable evidence indicates that polyamines (PAs) are involved in wide array of plant processes, including DNA replication, transcription of genes, cell division, plant development and abiotic stress response. The Plantago major L. plants were grown for six weeks in water mol m⁻³, at 23°C–16°C. Six weeks old plants were cultivated, 14 h of 350 exposed to the range of 3–9 kJ m⁻² d⁻¹ UV-B irradiation or 100 mM NaCl. The leaves and root material were fixed in liquid nitrogen at 12, 18, 24 h. and used for PA content analysis and RT-PCR. Under NaCl treatment in P. major plants were observed stress-inducible PA accumulation (especially spermidine) and changes in expression of two spermidine synthase genes sps1 and sps2 in roots. In leaves four genes were up-regulated: sam1, S-adenosylmethionine dehydroxylase gene, sam3, S-adenosylmethionine synthase gene, spds1, spermidine synthase gene, spsm1, spermine synthase gene. Under UV-B irradiation it was shown that all genes of PA biosynthesis were up-regulated in leaves. In roots the same genes are down-regulated under these conditions. We can conclude that in P. major plants under action of both stress-factors genes which play the key role in PA biosynthesis are stress-dependent as well as PAs content.

P09-136 Defining the role of the AP2/ERF transcription factor Rap2.4 in stress responses in the model plant Arabidopsis thaliana

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Identifying the transcription factors of Arabidopsis thaliana that regulate responses to abiotic stresses is a major research goal in plant biology. The objective of this study is the molecular and functional characterisation of an AP2 transcription factor Rap2.4 (At1g78080).Northern blotting showed Rap2.4 to be weakly induced during germination and abundantly expressed in roots of mature plants. Transgenic Arabidopsis plants carrying Rap2.4 promoter::GUS reporter gene constructs were used to confirm the expression patterns. Rap2.4 transcription responds to ABA, salt, dehydration and cold stress indicating likely involvement in abiotic stress response. Rap2.4 fused with GFP was shown to be targeted to the nucleus of transiently transformed Nicotiana benthamiana mesophyll cells. A basic region consisting of 12 amino acids was identified as the nuclear targeting signal. Rap2.4 protein binds to both the dehydration-responsive DRE and the ethylene-responsive GCC box elements. Single mutations in the CCGAC core of the DRE-element

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identified the 1st and 5th C as crucial bases for binding. The binding of Rap2.4 to the DRE-element was confirmed by an in vivo transactivation assay. Rap2.4 was able to activate the expression of the GUS reporter gene driven by DRE cis-element. Rap2.4 has previously shown to mediate light and ethylene signalling and act in drought-response pathways; our results suggest Rap2.4 may be involved in other abiotic stress signalling pathways.

**P09-137  In vitro effect of methyl jasmonate as a protective agent against salinity stress on two potato cultivars**

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Explants of two potato, *Solanum tuberosum* ssp. tuberosum cultivars, Agata and Red Pontiac, were grown for 4 weeks in culture media with a range of NaCl and MJ concentrations. Salinity significantly reduced the explant development. Cultivar Agata was most sensitive, 30 mM NaCl strongly decreasing growth, which was completely inhibited by the 60 mM NaCl treatment. Cultivar Red Pontiac was considerably less sensitive to salinity; its development progressively decreased as the salinity in the culture medium increased, while root formation was not affected by NaCl concentrations up to 90 mM. Salinity increased the explant proline content. A lower salinity concentration was required to cause the maximum proline level in the most sensitive to salinity stress cv. Agata, than in the least sensitive cv. Red Pontiac, 60 vs 90 mM NaCl. For both cultivars, MJ enhanced the explant development regardless the salinity in the culture medium. The proline content of the explants exposed to intermediate salinity stress was also reduced by the MJ treatments. Concomitantly, MJ significantly increased the root formation, which is one of the plant systems to tackle stress situations. Therefore, the obtained results suggest that MJ may protect potato plants against salinity stress.

**P09-138  Role of silicon in salt tolerance of tomato: beneficial effects on fruit yield and quality**

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The main goal of this study was to determine possible beneficial effects of silicon to enhance water use efficiency, yield and fruit quality of tomato grown with moderate saline irrigation under Mediterranean greenhouse conditions. Tomato plants (*Solanum lycopersicum*) were grown with a complete nutrient solution supplied with 0 and 80 mM NaCl plus 2 mM Si as SO₄K. Plant water consumption, leaf water (ψₛ), and osmotic (ψₒ) potentials, stomatal conductance (gs), transpiration (E) and net CO₂ assimilation (ACO₂) were periodically measured. At the end of experimental period, plant fresh and dry weight, leaf area, and Si, Cl and Na content were determined. Fruit quality parameters such as lycopene and sugar content, cuticle cracking and blossom end rot were also recorded. Salinity reduced plant dry weight and number of leaves. Leaf ψₒ and ψₛ decreased with salinity but leaf turgor was significantly higher in salinised than in control plants. Increasing salinity led to both reduction in plant leaf area and reduction in gs and ACO₂. Plant water uptake was reduced with salinity and also was closely related to E and gs. Reduction of net ACO₂ was explained in higher degree by gs than by foliar Na accumulation. Si in the nutrient solution resulted in a significantly higher plant water content. The results are discussed in terms of the beneficial Si effects on fruit yield and quality.

**P09-139  The nickel-citrate complex is the major nickel species in the vacuoles of nickel-tolerant tobacco BY-2 cells**

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To illustrate the cellular distribution of nickel (Ni) and develop a Ni sequestration model, we obtained protoplasts and intact vacuoles with high purity from wild-type (WT) and Ni-tolerant (NIT) tobacco BY-2 cells. We analyzed 31 intracellular elements, including Ni, present in whole cells, protoplasts, and vacuoles. The results reveal that in the NIT cells, almost all the Ni in protoplasts was present in vacuoles. In the WT cells, 82.7% of the total Ni was present in vacuoles, i.e., approximately 17% of the total Ni was present in the cytoplasm. Thus, Ni transport into vacuoles may be more efficient in NIT cells than in WT cells. To clarify the chemical forms of Ni in vacuoles, the vacuolar concentrations of 31 elements and the pH were input to the chemical speciation program, GEOCHEM-PC. The results revealed that in the NIT cells, 96% of the total vacuolar Ni was present as Ni-citrate (1:1) and that there was no free Ni²⁺. In the WT cells, although 79% of the vacuolar Ni was complexed with citrate, the remaining Ni interacted with other weak chelators or existed as free Ni²⁺. These data suggest that both the sequestration of Ni into vacuoles and the chelation of Ni with an appropriate ligand such as citrate are crucial for the survival of plant cells in environments with high concentrations of Ni.

**P09-140  ITN1, a novel gene encoding an ankyrin protein affects the ABA-mediated ROS production and is involved in salt tolerance in Arabidopsis**

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Salt stress and ABA induce an accumulation of reactive oxygen species (ROS) in plants. ROS not only act as second messengers for the activation of salt responses, but also have deleterious effects on plant growth due to their cytotoxicity. Therefore, the timing and degree of activation of ROS-producing enzymes need to be tightly regulated under salt stress conditions. We identified a novel locus of *Arabidopsis*, designated *itn1*, whose disruption leads to increased salt tolerance. *ITN1* encodes a transmembrane protein with an ankyrin-repeat motif which has been implicated in diverse cellular processes such as signal transduction. Comparative microarray analysis between the wild type and the *itn1* mutant revealed that the induction of genes encoding the ROS-producing enzyme NADPH oxidase under salt stress conditions was suppressed in the
availability and concentration in leaf tissues significantly affected both leaf blade and petiole growth. Low to moderate soil salinity (EC 2–4 mS m\(^{-1}\)) increased both intensity of mycorrhizal infection and the frequency of mycorrhiza while it was depressed at relatively high soil salinity (5–7 mS m\(^{-1}\)). \textit{H. vulgaris} plants seemed to be adapted to low light conditions since moderately increased light intensity (30–70% of maximum photosynthetically active radiation) significantly stimulated non-photochemical quenching. It is concluded that both flooding with saline water and light intensity were among the most important environmental factors affecting photosynthetic performance and growth of \textit{H. vulgaris} through the interaction between mycorrhizal symbiosis and soil/tissue nutrient balance.

**Abstracts**

**P09-141 Influence of humic acids on Cd-bioaccumulation and toxicity on spinach**

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Cd accumulation in soils and plants is of primary environmental and agriculture concern. In the present work, we have studied the role of Humic Acids on Cd bioaccumulation and toxicity on spinach (\textit{Spinacia Oleracea} L.). Spinach plants were hydropionically grown in a watered perlite root nutrient solution in the presence of different concentrations of Cd\(^{2+}\) i.e. 1, 10, 25 and 50 ppm. For each Cd concentration three different concentrations of HA i.e. 0.5\(^{\circ}\), 2.5\(^{\circ}\) and 10\(^{\circ}\) were tested. A well characterised humic acid (HA) isolated from soil was used. In a first scenario HA was bound on perlite particles while in a second scenario HA was supplied via irrigation, after spinach plants had reached a certain growth stage. For the first scenario the presence of HA had an inhibitory effect on plant growth. 10\(^{\circ}\) HA had the more severe effect (limited plant growth, stunting and chlorosis). In contrast 0.5\(^{\circ}\) HA had a significantly lower impact. For the second scenario the presence of HA had a minor effect on plant growth and Cd accumulation was lower than first scenario. The present results reveal a complex pervasive effect of HA on both Cd uptake as well as on plant growth. This discussed in terms of ion binding properties of HA and its association with nutrients and Cd bioavailability for spinach plants.

**P09-142 Dynamics of environmental factors affect photosynthetic performance and nutritional status of Hydrocotyle vulgaris in natural conditions**

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\textit{Hydrocotyle vulgaris} L. is a clonal plant species growing in sea-affected non-tidal coastal habitats. The aim of the present study was to analyze chlorophyll a fluorescence parameters, nutritional status, and mycorrhizal infection of \textit{H. vulgaris} in different microhabitats to understand environmental factors affecting plant growth and performance. Flooding and increased soil salinity significantly affected the availability and uptake of certain soil nutrients (P, Fe etc.). P availability and concentration in leaf tissues significantly affected both leaf blade and petiole growth. Low to moderate soil salinity (EC 2–4 mS m\(^{-1}\)) increased both intensity of mycorrhizal infection and the frequency of mycorrhiza while it was depressed at relatively high soil salinity (5–7 mS m\(^{-1}\)). \textit{H. vulgaris} plants seemed to be adapted to low light conditions since moderately increased light intensity (30–70% of maximum photosynthetically active radiation) significantly stimulated non-photochemical quenching. It is concluded that both flooding with saline water and light intensity were among the most important environmental factors affecting photosynthetic performance and growth of \textit{H. vulgaris} through the interaction between mycorrhizal symbiosis and soil/tissue nutrient balance.

**P09-143 Plasma membrane fluidity is the first target of aluminum ions in plants**

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Aluminum (Al) toxicity is the main limiting factor in crop production in areas with acid soils, where it is released from soil minerals in its toxic form of Al\(^{3+}\) ions. The first symptom of Al\(^{3+}\) toxicity is the rapid root growth inhibition within minutes of exposure. In spite of decades of research, the primary cause of root growth inhibition is not known. To elucidate the first target structure of Al\(^{3+}\) in roots, we have focused on early symptoms of Al toxicity. In our experiments, root growth of \textit{Arabidopsis thaliana} (A.t.) was inhibited within first 2 min of exposure. As shown by confocal microscopy using fluorescent markers bis-o-oxonol and FM-4-64 in root cells, Al\(^{3+}\) induced plasma membrane depolarization followed by the inhibition of endocytosis. On the contrary, no prominent changes in organization of cortical cytoskeleton were detected during first 30 min of exposure. Since the most immediate effects of Al\(^{3+}\) were associated with the plasma membrane we have focused on the plasma membrane properties. Using plasma membrane isolated from both A.t. plants and BY-2 tobacco cells we have showed by spectrofluorometric measurements with laurdan that Al\(^{3+}\) induced decrease in membrane fluidity. Moreover, Al-induced A.t. root growth inhibition was reversed by membrane fluidizer benzyl alcohol supporting further the role of fluidity in Al\(^{3+}\) toxicity. Altogether, our data showed that membrane fluidity decrease is the main toxic effect of Al\(^{3+}\) leading to rapid root growth inhibition.

**P09-144 Poplar roots under mechanical stress: asymmetric alterations of proteome and lignin content**

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Abstracts

In response to mechanical stress and to improve their anchorage, plants have developed complex mechanisms to detect mechanical perturbation and to induce a suite of modifications at anatomical, physiological, biochemical, biophysical and molecular level. To begin investigate the mechanisms involved in root response to mechanical stress we analyzed the alterations occurring in a popular (Populus nigra) taproot bent to an angle 90°. We compared the proteomes and lignin content of the control (non bent) and three different regions (above the bending, bending, and below the bending) of bent taproot. Compared with the control the bent poplar root displays asymmetrical alterations in lignin content and proteome alterations. Forty-three protein spots were found to change their expression. MALDI-TOF-MS analysis indicates that among the differentially expressed proteins, several are involved in the signal transduction pathway, detoxification, metabolism and stress response. These findings may provide the basis for future investigations on the complex mechanism involved in the developmental root biology under environmental stress conditions.

P09-145 Preliminary results on the effects of cadmium on poplar clones
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Potentially poplar trees represent one vegetation option for phytostabilization and in situ decontamination of soils polluted with Cadmium (Cd). Poplar is the ideal plant for metal phytoextraction: it’s highly productive in biomass, and it assimilates and translocates to shoots a significant part of metals. Additional favorable traits are fast growth, easy propagation and a deep root system. Cd is a major environmental contaminant accumulated from industrial usage, anthropogenic activity and use of agro-chemicals such as pasturelands fertilized with Cd-rich superfosfate fertilizer. Lately a major effort has been focused on the studies of molecular, genetic and physiological basis for the mechanisms of heavy metal detoxification. Here we report studies on composition and expression of metallothioneins by semiquantitative RT-PCR carried out on cDNA synthesized from leaf and root of woody cuttings of two poplar clones (Nigra poli (Populus nigra) and Lux (Populus deltoides)) after treatments with two different cadmium concentration. As plant species and even different genotypes within the same species differ in Cd accumulation. The aim of this work is to investigate at molecular level the tree reaction to cadmium excess and evaluate if our genotypes can exhibit high Cd accumulation and/or tolerance.

P09-146 Investigating the molecular mechanism of heavy metal tolerance of Typha angustifolia- a natural inhabitant of Uranium tailings
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Typha angustifolia, commonly known as narrowleaf cattail is a metal hypertolerant wetland plant and a natural inhabitant of Uranium tailings. Estimation of metals from aerial parts of Typha collected from tailings showed that it is a selective accumulator of heavy metals capable of excluding most of the heavy metal contaminants of the soil. ICP-MS and flame atomic absorption spectrums showed that Typha is capable of accumulating Manganese at very high amounts but excludes closely related metal Iron despite its presence at very high amount in the tailings. In vitro cultured plants could tolerate 10 times more Manganese without any sign of toxicity. A fluorescence differential display approach followed by reverse northern have been taken to identify differentially expressed genes in Tailings grown Typha plants as compared to control plants to investigate the molecular mechanism of this unusual metal tolerance.

P09-147 Adaptive reactions of wheat photosynthetic membranes under phosphate starvation
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Lipid peroxide oxidation products are the main mediators of plant stress. To evaluate the genetic potential of winter wheat cultivars we modelled soil phosphate starvation and assessed the cultivars by studying non-specific responses to peroxide oxidation of lipids from plant photosynthetic membranes. The efficiency of lipid oxidation was assessed by measuring accumulation of the secondary metabolic product MDA. The outcome of the study resulted in selection of a sensitive (Sirena, the steppe ecotype) and a non-sensitive (Kievskaya ostistaya, the forest-steppe ecotype) cultivars to phosphate starvation. Despite the fact that lipid composition of photomembranes is homeostatically controlled we have found that phosphate starvation can affect lipid composition in wheat. Under the deficit of phosphate both of the cultivars showed a relative increase in the amounts of galactolipids as well as decrease in the total amount of phospholipids. Thus, the total amount of phospholipids decreased by 37.7% and 45.3% in Sirena and Kievskaya ostistaya respectively. It was also found that phosphate starvation caused increase in the amounts of nonphosphorus lipids, such as SQDG by 32.8% in non-sensitive cultivar. We propose that under phosphate starvation in tolerant wheat plants the optimal level of photosynthetic processes in chloroplast thylakoid membranes could be maintained by compensating the loss of PG with the anionic lipid SQDG in order to maintain the anionic character of the membranes.

P09-148 Identification and characterization of transcription factors that regulate the expression of CRKS in rice
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Rice, one of the most important crops in the world, is extremely affected by abiotic stress conditions. Saline environments limit rice productivity, causing several deleterious effects, such as photosynthesis inhibition and leaf premature senescence. Responses to high salinity conditions rely on the ability to perceive external signals and induce specific signal transduction pathways. Transcription factors (TFs) have been shown to play an important role modulating the expression of abiotic stress responsive-genes. CRKS is a cysteine-rich RLK (receptor-like kinase), whose expression is

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induced by pathogen infection in Arabidopsis. In rice OsCRK5 expression was shown to be induced by high salinity. The main goal of our work is to study the transcriptional regulation of the OsCRK5 expression. The TFs that directly bind to the OsCRK5 promoter are being identified through a yeast one-hybrid screening using a salt induced rice cDNA expression library. This library expresses rice cDNAs fused to a strong activation domain (GAL4-AD), which will allow the identification of transcription activators and repressors. The above mentioned screening is being performed and has already revealed several putative clones. The validation of these clones will be carried out by transformation in the yeast reporter strains and using gel-shift assays. Thereafter, functional analysis of the validated TFs will be performed to understand their biological significance in the salt stress response.

P09-149  Proline buildup by the reduction of its degradation improves barley tolerance to oxidative stress
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The role of ProDH in plants tolerance to the salt induced oxidative stress was investigated in two barley H. vulgare L. varieties namely Sahand and Makou by enzyme and gene expression assay. The ProDH activity of Sahand plants was very high in all salt regimes although they had higher proline levels compared to Makou. The ProDH gene expression patterns match up the ProDH enzyme activity. These analyses indicate that ProDH activity and consequently proline content of these plants to some extent are regulated at the transcriptional level. Makou plants accumulate proline by repressing ProDH gene under stress and this expression pattern of the ProDH gene improved tolerance to high salinity. ProDH gene of Sahand plants showed more sensitivity to proline level and it was expressed with an increase in endogenous proline level. These results suggest that under optimum growing conditions Sahand may have an active proline cycle. Active proline synthesis in chloroplasts and its catabolism in mitochondria by ProDH, enable the plant to use proline as a sink for energy to regulate redox potentials resulting in better growth under optimum conditions. But it seems that high active proline cycling in plants is usually detrimental during stress conditions. As in Makou variety, some other plants accumulate proline under stress conditions through an increase in its synthesis, concomitant with inhibition of its catabolism and so they are more tolerant under stress condition.

P09-150  Oxidative stress responses in the plant RCD1-SRO gene family: transcriptional and biochemical characterization
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The ozone insensitive rcd1-1 (radical-induced cell death1) mutant of Arabidopsis has been shown to be defective in the containment of programmed cell death and in the signalling of several plant hormones. The RCD1 protein function is yet unknown, but according to a yeast two-hybrid analysis, it may include interactions with several stress-related transcription factors. RCD1 belongs to a novel gene family with five unknown genes encoding proteins distinctively similar to RCD1 (SRO1-SRO5, SIMILAR TO RCD-ONE 1-5). Interestingly, a conserved domain of ADP-riboseylation has been assigned to all the RCD1-SRO proteins. RCD1 appears to have partially overlapping functions with at least SRO1, because rcd1-sro1 double mutant plants have a severely stunted phenotype even in control conditions. In addition to global gene expression with microarrays, we have accomplished metabolite profiling of the T-DNA insertion mutants of the RCD1-SRO gene family, rcd1-1, and Col wild type, both after stress treatments and in control condition by HPLC-MS² and GC-MS. Recent HPLC-MS² and GC-MS analysis have revealed differences between Col and rcd1-1 in the diurnal rhythm of some amino acids and their TCA cycle intermediates, respectively. The observed metabolic changes are discussed in respect to the latest results obtained from microarrays.

P09-151  Drought stress markers in apple trees (Malus domestica Borkh.)
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Numerous researchers are searching for reliable and easily measurable drought stress indicators in important crop plants. The results of the research on apple trees grown in intensive orchard or in pots under natural water conditions, but with controlled water regime, are presented in this contribution. We measured biochemical parameters: ascorbic acid, glutathione, tocopherols, chlorophylls, carotenoids, soluble carbohydrates, free amino acids and some other organic acids, and physiological parameters already known as stress indicators in apple trees: predawn and midday leaf water potential, net photosynthesis, stomatal conductance, transpiration and intercellular CO₂ concentration, in leaves of apple trees subjected to different intensities of slowly progressing drought or no drought in early summer in tree following years. Our study pointed out zeaxanthin and glutathione as the best drought stress markers in apple trees. Ascorbate and sorbitol appeared to be reliable indicators of moderate drought only. Responses of other tested biochemical parameters were not consistent enough to prove their role as drought stress markers in apple trees. Relative air humidity should be taken in consideration when physiological parameters (gs, Pn, Tr, Ci) are used as drought stress markers in apple trees. Under conditions of low RAH biochemical markers may bee better tool for determination of drought stress intensities in apple trees.

P09-152  Dolichol accumulation upon abiotic stress in plant roots
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Dolichols are linear five-carbon unit polymers occurring in almost all living cells and constitute families of prenologues. Roots of model plants Coluria geoides and Cucumis sativus accumulated mixtures of dolichols from Dol-15 to 22, with Dol-16 or Dol-17 dominating, respectively. Dolichol content was increased upon all abiotic stress conditions tested. Maximal effect was found in cucumber roots after 100 mM NaCl treatment when four-fold elevation of dolichol concentration was noted. When the growth medium was supplemented with cadmium chloride (100 μM or sorbitol (500 mM) the content of dolichol was slightly increased (approx. 140% of the control). Prolonged cultivation of Coluria roots at 7°C resulted in a significant elevation (approx. 200%) of the dolichol content. In contrast, upon mineral starvation decreased dolichol accumulation (approx. 75% and 60% level of the control for phosphate and nitrogen deficiency, respectively) was observed. Additionally, dolichol distribution in cellular subfractions of the cucumber root cells was analyzed. Increased salinity resulted in the two-fold increase of the dolichol level in plasma membranes whereas in microsomes dolichol content was only slightly increased (approx. 120% of the control).

Detection of hydrogen peroxide in intact leaves: comparison of methods

Detection of H₂O₂ in intact leaves is of great importance. Due to a number of difficulties, there is only one method that is widely used for this purpose – diaminobenzidine (DAB) staining. However, our experiments with tobacco leaves showed that DAB affects photosynthesis. In order to find less invasive and more sensitive methods for H₂O₂ detection in leaves, we have tested several commercially available fluorescent sensors with respect to their light sensitivity, toxicity, cellular localization and stability in leaves. Ampliflu Red (AR) and Amplex Ultra Red (AUR) were found to be less toxic than DAB, but their applicability is limited by their sensitivity to light as well as by their instability in a leaf tissue. A common problem with DAB, AR and AUR is that these probes rely on the activity of internal leaf peroxidases. Therefore, stress conditions that change peroxidase activity may also affect the detection of H₂O₂. Europium tetracyclene (Eu₃Tc) is a new fluorescent sensor for H₂O₂ (ex: 400 nm, em: 615 nm) which is not dependent on peroxidase. In our experiments, this probe was not sensitive to light and did not disturb photosynthesis. The most serious drawback of Eu₃Tc application is low stability of the reaction product (Eu₃Tc-H₂O₂ complex) in leaves.

Acknowledgement – I.S. thanks EMBO for financial support (fellowship ASTF 387.000-2007).

P09-153 Detection of hydrogen peroxide in intact leaves: comparison of methods

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Detection of H₂O₂ in intact leaves is of great importance. Due to a number of difficulties, there is only one method that is widely used for this purpose – diaminobenzidine (DAB) staining. However, our experiments with tobacco leaves showed that DAB affects photosynthesis. In order to find less invasive and more sensitive methods for H₂O₂ detection in leaves, we have tested several commercially available fluorescent sensors with respect to their light sensitivity, toxicity, cellular localization and stability in leaves. Ampliflu Red (AR) and Amplex Ultra Red (AUR) were found to be less toxic than DAB, but their applicability is limited by their sensitivity to light as well as by their instability in a leaf tissue. A common problem with DAB, AR and AUR is that these probes rely on the activity of internal leaf peroxidases. Therefore, stress conditions that change peroxidase activity may also affect the detection of H₂O₂. Europium tetracyclene (Eu₃Tc) is a new fluorescent sensor for H₂O₂ (ex: 400 nm, em: 615 nm) which is not dependent on peroxidase. In our experiments, this probe was not sensitive to light and did not disturb photosynthesis. The most serious drawback of Eu₃Tc application is low stability of the reaction product (Eu₃Tc-H₂O₂ complex) in leaves.

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P09-154 The photoprotective roles of colourless secondary cortical compounds in lichens – quantification of light screening with chlorophyll fluorescence

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Lichens, often have high concentrations of secondary compounds. Functions like light screening and herbivory protection have been suggested for these compounds. Lichen compounds are situated as crystals outside the hypha, and can be extracted by 100% acetone from air-dried living thalli without any detrimental effects. The foliose lichen Physcia aipolia contained 2% atranorin of dry mass. Control thalli of Physcia aipolia are pale gray both in dry and hydrated state, whereas acetone-rinsed thalli become green in the hydrated state. This colour change suggests that the photobiont layer is more exposed to light in acetone-rinsed thalli than in controls. The higher apparent ETR measured at light saturation for control thalli compared with acetone-rinsed thalli must be a result of reduced light screening due to acetone rinsing. Based on differences in apparent ETR, we calculate that the acetone-soluble compounds screen as much as 40% of incident light. However, since atranorin in solution is a colourless substance, the screening effect is presumably caused by reflectance of white extracellular atranorin crystals in the lichen cortex. This is verified by a higher reflectance in control thalli compared to thalli from which atranorin has been removed. This pattern is distinct both in dry and hydrated thalli. In conclusion, colourless lichen compounds may efficiently screen visible light, and the mycobiont plays a significant photoprotective role for symbiotic photobiont cells.

P09-155 Ethylene induced programmed epidermal cell death in rice is mediated through reactive oxygen species

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Partial submergence of rice leads to growth of adventitious roots which is preceded by programmed death of epidermal cells above roots. Epidermal programmed cell death (PCD) is induced by ethylene and mediated by reactive oxygen species (ROS). ROS accumulated in epidermal cells above roots and ethylene promoted accumulation of H₂O₂ specifically in these cells. Endogenous accumulation of ROS resulted in elevated PCD rates whereas inhibition of ROS production resulted in lowered ethylene-induced PCD rates. Through microarray analyses we identified 61 genes which were coordinately regulated by both ethylene and H₂O₂ in epidermal cells undergoing cell death. The transcriptome study revealed a positive feedback regulation loop on ethylene synthesis through up-regulation of the ACC oxidase gene OsACO1 and down-regulation of the ACC synthase inhibitor gene OsETO1. Other major groups of genes identified had predicted functions in stress adaptation and signaling.

The metallothionein OsMT2b which functions as a ROS scavenger was found to be down-regulated by ethylene and H₂O₂. Constitutive down-regulation of OsMT2b expression resulted in constitutively elevated epidermal PCD rates. The results indicate that ethylene and H₂O₂ act as mutually self-amplifying signal molecules through enhanced synthesis of ethylene and reduced scavenging of H₂O₂.
This mechanism may be useful for rapid initiation and execution of the cell death program.

P09-156  Decrease of growth and changes in protein profile observed for poplars growing under Cu-stress
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Protein profiles of four species of poplar (P. nigra, P. deltoids, P. nigra x deltoids and P. trichocarpa) growing under Cu stress were investigated in 2D electrophoresis approach. Applied concentration of Cu was 300 mg kg\(^{-1}\). Cu was added in the form of CuSO\(_4\), thus additional ‘sulphates control’ variant was also performed in order to estimate the impact of SO\(_4\)-3 ions. For analysis, the fine roots and leaves were collected from poplar cuttings which were rooted in plastic 2-L pots and grown 3 months under soil tunnel. Growth rate of shoots was monitored all time to end of growing. For all species, we observed over 33% lower rate of growth under Cu stress, and no differences for ‘sulphates control’. Additionally, we also found over 50% lower values for roots weight for all poplars growing under Cu stress in comparison to the controls. However, also roots of plants from ‘sulphates control’ were significant lower than control. Finally, we found essential changes in protein profiles of poplars growing under Cu stress, both in the fine roots as in leaves, independent from acidity of the soil. Some of them can be grouped into metabolic paths, showing their enhancing or inhibiting.

P09-157  Influence of solar UV radiation on the nitrogen metabolism in needles of scots pine (Pinus sylvestris L.)
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Needles of 20-year-old Scots pine (Pinus sylvestris L.) saplings were studied in an ultraviolet (UV) exclusion field experiment (from 2000 to 2002) in northern Finland (67°N). The chambers held filters that excluded both UV-B and UV-A, and were controlled UV-B only, transmitted all UV (control), or lacked filters (ambient). UV-B/UV-A exclusion decreased nitrate reductase (NR) activity of 1-year-old needles of Scots pines compared to the controls. The proportion of free amino acids varied in the range 1.08–1.94 % of total proteins, and was significantly higher in needles of saplings grown under UV-B/UV-A exclusion compared to the controls or UV-B exclusion. NR activity correlated with air temperature, indicating a ‘chamber effect’. The study showed that both UV irradiance and increasing temperature are significant modulators of nitrogen (N) metabolism in Scots pine needles.

P09-158  Effect of temperature drop on growth, cold resistance and chlorophyll fluorescence of young cucumber plants under different photoperiods
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Temperature drop is widely used modern horticultural technique. Different photoperiods are also used to control plant growth and development. However little attention has been paid to the combination of different photoperiods with temperature drop treatments in order to benefit plant growth and resistance. Experiments were carried out with young cucumber plants at the stage of fully expanded first leaf in growth chambers under different photoperiods (8, 12, 16 and 24 h). The experimental design included temperature drop from 20 to 12°C for 0 (control), 2 and 6 h (drop treatments) at the end of the night during 6 days. Plant cold resistance was measured by LT50-method. The measurement of Chl fluorescence parameters was carried out with MINI-PAM, Walz. Data were processed by principal component analysis. Both 2-h and 6-h temperature drop treatments influenced plant morphology by decreasing petiole length under all photoperiods, while the most pronounced effect was observed under 24-h photoperiod. Both temperature drops increased plant dry weight, however 6-h temperature drop decreased allocation of dry matter to leaves. Cold resistance was significantly higher in drop-treated plants. Drop treatments increased non-photochemical quenching and quantum yield of PSII. Possible mechanisms of plant response to temperature drop treatments under different photoperiods are to be discussed. The study was supported by the RFBR (project no 07-04-00063).

P09-159  Influence of copper ions on regeneration capacity of carrot androgenic embryos
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Copper (Cu\(^{2+}\)), at low concentrations plays an important role in plant development but in higher doses becomes toxic and can disturb basic biological processes. The purpose of the current work has been to compare the effect of Cu\(^{2+}\) on the regeneration of androgenic embryos of two carrot genotypes. Embryos of Feria and 1014 genotypes were cultured on the medium containing Cu\(^{2+}\) in concentrations: 0.1 \(\mu\)M (control), 1 \(\mu\)M, 10 \(\mu\)M, 100 \(\mu\)M and analysed after 8, 16 and 24 weeks. After 8 and 16 weeks of cultivation more significant dose-dependent growth inhibition was observed in the genotype 1014, which was associated with almost double TBARS content in comparison with Feria. In the genotype 1014 treated with 10 \(\mu\)M Cu\(^{2+}\) the significant regeneration ability and extensively increased level of free proline accompanied by relatively low TBARS content were observed after 24 weeks. However, in the genotype Feria 100 \(\mu\)M Cu\(^{2+}\) triggered large increase in proline content associated with high regeneration capacity of embryos in comparison with genotype 1014 cultivated 24 weeks on this Cu\(^{2+}\) concentration. The accumulation of free proline may be involved in a protective mechanisms against Cu\(^{2+}\) stress during regeneration of androgenetic embryos of carrot. Scientific work financed by Ministry of Sciences and Higher Education in 2006-2009 as a research project No. N305 040 31/1576.
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P09-160 Effect of grain soaking in salicylic acid on physiological changes in pea and maize plants
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It is known that soaking grain in salicylic acid (SA) prior to sowing may increase the stress tolerance of the plants, but the physiological/biochemical background of this effect has not yet been clarified. One-week-old pea (*Pisum sativum* L.) and maize (*Zea mays* L.) plants were used to monitor physiological changes after soaking in 0.1 and 0.5 mM SA. Leaves, roots and seeds were collected for analysis from maize and pea, and epicotyls from pea plants. There were no changes in the fresh and dry mass production but the germination rate increased after SA treatment. Changes in the antioxidant enzyme activities and polyamine content differed for pea and maize. The bound SA level increased in the seeds and roots of both plants but an increase in the leaves could only be observed in maize. The bound ortho-hydroxycinnamic acid (oHCA) content increased in pea plants and in maize leaves and seeds, but it was below the detection limit in the roots of maize. A very great increase in the bound oHCA level was detected in pea epicotyls. It can be concluded that the higher level of protective compounds (for example oHCA, certain polyamines) could be the reason for the increased stress tolerance after SA soaking.

P09-161 Monitoring of lipoxygenase-related plant emission for early detection of drought stress in greenhouse
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Early detection of plant stress is a key to effective plant management for crop production. Drought stress is a common abiotic stress in crop production and early detection of drought stress allows us to improve water usage efficiency and crop quality by demand-based irrigation. This study demonstrated an early detection of drought stress by monitoring lipoxygenase-related plant emission from tomato plants in greenhouse. The drought stress was induced by stopping irrigation, and then re-irrigated. To quantify the effect of the drought stress on plant, leaf water potential and leaf photosynthetic rate were measured. During air sampling for plant emission monitoring, plants were temporarily enclosed in a plastic bag and then the concentrated volatiles inside the bag were captured by purge and trap technique. The air samples were analyzed with gas chromatography-mass spectrometry. During the drought stress treatment, leaf water potential and photosynthetic rate decreased. Furthermore, slight wilting of leaves was observed at the end of the treatment. After the re-irrigation, leaf water potential and photosynthetic rate increased and the wilting symptoms disappeared. The recoverable drought stress induced lipoxygenase-related plant emission of (Z)-3-hexenal, n-hexanal and (Z)-3-hexenol and the emission stopped after the recovery. This result suggests that early detection of drought stress by monitoring lipoxygenase-related plant emission is feasible under greenhouse conditions.

P09-162 Influence of abiotic stress to plant cells in vivo and in vitro
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The problems of drought- and salt tolerance of cereals are very actual. It is known that stability to stress is shown at different levels of plants organization such as cell, organism and population. The purpose of our researches is to reveal influence of abiotic stress (drought and salt) to plant cells of cereals (wheat and barley), and also to compare the influence by stress in vivo and in vitro. We note a generality reaction of plant cells to stress. On a background of delay and termination by growth both in vivo and in vitro, we see infringements of osmotic parameters in cells. It is available dystrophic changes of cellular structures. The quantity of large vacuoles decreases. We are possible to observe the lost meristem cells that testifies to development of necrobiosis processes. Change of ionic balance in salt stress conditions was studied. It is shown, that with the termination by growth at increase of salt concentration there is an essential increase by Na⁺, decrease by K⁺, decrease in tens times of parity K to Na, and increase by Na₂^⁺. Higher activity of Superoxide Dismutase at salt-tolerant forms and smaller decrease of enzyme activity at stress is marked. The relative accumulation by free proline at a drought and salt stress also increases. We are come to light the correlation between reaction to stress by cells and organisms. It will give us adequate criteria of an estimation of stability to abiotic stress in laboratory conditions including in vitro.

P09-163 In which way do natural doses of UV-B light affect leaf growth of model and horticultural plant species?
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UV-B radiation induces a wide range of responses in plants. Plant morphology, pigment composition and leaf growth are affected by UV-light. In horticultural plants, a more compact growth with reduced leaf expansion is often desirable. As dicot plants show pronounced, endogenously triggered diel variations in leaf growth activity, analysis of the effect of UV-light on diel leaf growth cycle can shed light on mechanisms, how UV affects plant performance. Hence, non-invasive analysis of plant growth dynamics has been applied in this study to investigate, to which extent and at which time during a diel cycle (24 h) leaf growth of tobacco and broccoli is affected by UV-B-light. Experiments were performed in UV-B-exposure chambers at the HelmholtzZentrum münchen, in which the relation between UV-B and PAR corresponds to the natural light. While at low light conditions (400 µmol PAR m⁻² s⁻¹), no effect of UV-B light was obtained, high light (800 µmol PAR m⁻² s⁻¹) led to significantly decreased biomass and leaf growth. During twilight hours a tendency of higher growth rate of UV-B treated plants were observed. The results of this study were confirmed by longer-term
growth analyses in greenhouses with different cladding material: In the course of a growing season, UV-B light can only lead to more compact growth, if environmental conditions are favourable and irradiance is high.

**P09-164** Depth-related variation of biochemical markers in *Posidonia oceanica* at the Eastern coast of the Adriatic Sea

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*Posidonia oceanica* (L.) Delile is an endemic Mediterranean seagrass that forms vast meadows from the sea surface down to depths of more than 30 m. Recently, many *P. oceanica* beds are declining due to both natural and anthropogenic disturbances. Because of its wide distribution, sedentary habit, abundance and sensitivity to ecological modifications meadows of *P. oceanica* have been recommended for biomonitoring in the Mediterranean Sea. In this study lipid peroxidation and free amino acid content as well as the activity and isoenzyme pattern of peroxidases were investigated in the extracts of *Posidonia* leaves collected from different depths (5, 15, 20 and 32 m) in an unpolluted site near Lastovo Island in order to find out possible variations of the basic levels of stress biomarkers. In general, shearwaters (photosynthetically inactive) had higher levels of MDA and free amino acids than blades (photosynthetically active). With the increasing sea depth MDA content decrease in both blades and sheaths while free amino acids increased. The activity of peroxidases was higher in the blades than in the sheaths although native electrophoresis revealed a common isoenzyme pattern. In both blades and sheaths the highest peroxidase activity was measured in *P. oceanica* living at 15 m while the lowest was in plants from 32 m. Our results showed depth-related variation in the levels of biochemical markers in *Posidonia oceanica* which should be taken into account in the future biomonitoring.

**P09-165** Metabolomics and expression of some phenylpropanoid structural genes in two cell lines of *D. carota* under different environmental conditions

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The cell lines R3M1 and R4G1 were selected from a non-pigmented cell culture of *Daucus carota* L. cv Flakkese for their ability to produce high yield of anthocyanins in the light and dark respectively. These two lines were subjected to mechanical stress in the light and dark to investigate the secondary metabolites accumulation (anthocyanins, hydroxycinnamic acids and hydroxybenzoic acids) and the expression of main genes involved in phenylpropanoid biosynthesis pathway (DePAL1, DePAL3, DeCHS1) under different environmental conditions. The treatment-induced metabolome modulation was detected by HPLC-DA and HPLC-MS and the contribution of the individual molecules to the treatment-induced modifications was investigated by PCA multivariate analysis. The R3M1 and R4G1 cell lines, in the investigated conditions, showed qualitative and quantitative differences in the individual anthocyanins, in the hydroxycinnamic acid derivatives and in hydroxybenzoic acid derivatives. The PCR Real time analysis shows that expression of DePAL genes in R4G1 is higher than in R3M1; moreover light and mechanical stress conditions modulate in a different manner in the two lines. The relationship between different classes of secondary metabolites and the phenylpropanoid pathway genes is under investigation through PLS multivariate analysis.

**P09-166** Poplar roots under mechanical stress: asymmetric alterations of proteome and lignin content

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In response to mechanical stress and to improve their anchorage, plants have developed complex mechanisms to detect mechanical perturbation and to induce a suite of modifications at anatomical, physiological, biochemical, biophysical and molecular level. To begin investigate the mechanisms involved in root response to mechanical stress we analyzed the alterations occurring in a poplar (*Populus nigra*) taproot bent to an angle 90°. We compared the proteomes and lignin content of the control (non bent) and three different regions (above the bending, bending, and below the bending) of bent taproot. Compared with the control the bent poplar root displays asymmetrical alterations in lignin content and proteome alterations. Forth-three protein spots were found to change their expression. MALDI-TOF-MS analysis indicates that among the differentially expressed proteins, several are involved in the signal transduction pathway, detoxification, metabolism and stress response. These findings may provide the basis for future investigations on the complex mechanism involved in the developmental root biology under environmental stress conditions.

**P09-167** Effect of temperature and UV-B exclusion on the soluble phenolics of Buckbean (*Menyanthes trifoliata*) in the subarctic

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*Menyanthes trifoliata* L. (Mt), a circumboreal perennial plant, has been used in traditional medicine e.g. as a remedy against scurvy and other diseases. It is also a forage of reindeer and elk at the subarctic. The aim of this study was to investigate the effects of UV exclusion and temperature on the soluble phenolics of Mt. The UV-B experiment was conducted in northern Finland (68°N) in an oligotrophic *Sphagnum* maral fen (2002–2007). It was arranged in a randomized block design consisting of wooden frames covered...
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with plastic filters: (1) UV-B exclusion (polyester filter); (2) control (cellulose acetate filter); and (3) ambient plots. Leaves of Mt were sampled in 2006 and 2007 for the measurements of soluble phenolics after methanol extraction and HPLC analysis. Three groups of compounds were identified: ascorbic acid (AsA) derivatives, chlorogenic acid derivatives, and flavonoids. Analysis of control samples showed that a small variation in the temperature did not affect the total content of soluble phenolics but may increase the proportion of flavonoids. Similarly, UV-B exclusion did not affect the total content of soluble phenolics in Mt leaves but modified its composition. Although the amplitude of the effect varied according to the sampling date, UV-B exclusion induced a significant increase in AsA derivatives with a decrease in the proportion of flavonoids. Chlorogenic acid derivatives were not significantly affected by the treatments.

P09-168 Growth and primary photosynthetic production of green algal lichen symbionts of the genus Trebouxia: optima and limits of temperature and irradiance

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Lichens are symbiotic organisms surviving in extreme conditions of temperature and irradiance. A photobiont, considered as key element in sensitivity of whole organism to environmental extremes, belongs mostly to genus Trebouxia. In the study, three lichen photobiont species with different chloroplast morphology (erici, gigantea and irregularis) were investigated to characterize interspecific differences in physiological processes of growth and primary photosynthesis. Within experiments, techniques of cultivation of algae in crossed gradients of temperature and irradiance were used. Growth differences and production of colonies were analyzed by photo grammatic image analysis, determination of chlorophylls and carotenoids contents and by weighing of algal colonies. Primary photosynthetic processes at the level of photosystem II were analysed by the parameters of chlorophyll fluorescence in vivo (Fv/Fm, NPQ). An optimal parameter of image analysis for characterization of growth of algal colonies was found. Supported by the GACR 206/07/P394 funding.

P09-169 Comparison of the drought and/or heat stress responses in tobacco plants over-producing proline and the corresponding wild-type

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Responses to abiotic stresses are in plants, at least partially, mediated by phytohormones. The impact of drought, heat and their combination was compared in tobacco plants over-expressing gene for proline 5-carboxylate synthetase (P5CS, the key enzyme of proline biosynthesis) from Vigna aconitifolia and non-transformed plants. Both stresses were connected with the decrease of the bioactive cytokinin (CK) levels, more profound in case of severe water deficit (RWC decrease by ca 30%) than during heat stress (HS). Transgenic plants, which were more drought stress tolerant (exhibiting prolonged chlorophyll retention), had relatively higher levels of bioactive CKs, as well as of protective xanthophyll cycle pigments. The activity of the main CK degrading enzyme, cytokinin oxidase/dehydrogenase, decreased at drought in roots, while at HS in the whole plant. High elevation of abscisic acid at drought and its decrease at HS coincided with the corresponding regulation of stomata aperture. The level of free auxin decreased (in relation to stress strength) in upper leaves, increasing in roots and lower leaves. HS and drought had differential impact on polyamine content. Spermidine and spermine increased after both treatments. Putrescine decreased during HS, being elevated after prolonged water deficit. Transgenic plants had higher total polyamine content under both control and stress conditions. This work was supported by GACR project no. 206/06/1306.

P09-170 Abscisic acid levels and dehydrin expression profiles as drought-tolerance markers in wheat

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Drought stress induces cellular dehydration, by which water from within the cell migrates to outside the cell. At the cellular level, this activates different structural and biochemical changes. The phytohormone abscisic acid (ABA) has attracted much research attention as a potentially useful trait in selecting for drought tolerance in crops. ABA stimulates osmotic adjustment and induces the synthesis of protective proteins including dehydrins. The synthesis of dehydrins is a common response to drought in plants. These proteins are highly abundant in desiccation-tolerant seed embryos, and accumulate during periods of water deficit in vegetative tissues. They have been hypothesized to function by stabilizing large-scale hydrophobic interactions such as membrane structures or hydrophobic patches of proteins. Changes in abscisic acid (ABA) and in dehydrin expression profiles in wheat varieties (Triticum aestivum L.) with different drought tolerance have been investigated. Expression of three dehydrin genes (TaDHN, WHT WCR, WDHN13-Lea11 dhn) has been analyzed by one-step RT-PCR. Results suggest that initially higher ABA levels as well as higher accumulation of dehydrins transcripts could be regarded as a prerequisite for drought tolerance in wheat. These parameters could be incorporated as reliable markers in variety assessment strategies.

P09-171 A proteomics approach of overwintering in trees

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Trees growing in northern latitudes evolved the capacity to become dormant and freezing tolerant, allowing survival through extreme winter conditions. These regulated changes involve complex interactions between environmental and cellular factors, thus securing the proper timing of dormancy induction and alleviation. A key feature of the dormant state is the physical obstruction of symplastic pathways by deposits of callose (1,3-β-D-glucan) on sieve plate pores and plasmodesmata (PD). During chilling these callose deposits are enzymatically removed by 1,3-β-D-glucanase. Our aim is to elucidate the mechanisms that regulate the activity of genes encoding PD-localised callose synthase and 1,3-β-D-glucanase, as these might regulate dormancy cycling. Since both types of gene belong to multigenic families, our first task was to pinpoint which of the genes are involved in dormancy regulation. In addition to bioinformatics we use proteomics tools to identify the candidate genes. We found several proteins in Western-blot that bind with anti-1,3-β-D-glucanase antibody and that are differentially regulated in active and dormant SAMs. Corresponding protein bands were analysed by DeNovo sequencing with CAF. Currently, we identify these proteins using mascot peptide mass fingerprint searches and poplar genomic and EST databases. In follow up studies we will use molecular genetics and biochemical approaches to study environmental and cellular factors regulating the activity of these proteins.

**P09-172** Uptake and distribution of heavy metals in copper mosses from the Schwarzwand in Salzburg/Austria

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We investigate mosses from copper-rich soils in the Grossarl valley near Hüttschlag in Salzburg (Austria). From the sites of old copper mines with rock of crystalline slate containing mainly copper and iron originates water that forms two little rivers. Here grow the mosses Mielichhotonela elongata and Pohlia drummondii. Heavy metal content of plants, substrate and water was analysed by flame atomic absorption spectroscopy (F-AAS) and by induc-tively coupled plasma-mass spectrometry (ICP-MS). The distribution of heavy metals in air dried plants was investigated by Energy Dispersive X-ray microanalysis (EDX) in the Scanning EM. To understand heavy metal uptake, shoots of the mosses were taken into in vitro cultures on agar plates with heavy metal (Cu, Fe) concentrations from 0.1 μM up to 0.1 M and also analysed by EDX. The substrate of the natural habitat contains 4.100 ppm Cu and 79.000 ppm Fe. The moss plants accumulate even higher heavy metal contents in comparison to the substrate. In culture, both mosses live without visible stress reactions in concentrations up to 10 mM Cu and 1 mM Fe. First data for P. drummondii show an uptake of copper and an exclusion of iron. We conclude that, on the basis of high heavy metal accumulation in both moss species, not only M. elongata but also P. drummondii should be considered as copper mosses. Furthermore, the data from the in vitro cultures give proof of heavy metal uptake from the substrate.

**P09-173** Influence of heavy metal on secondary metabolism of *Schisandra chinensis*


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Embryogenesis is one of the most sensitive events in plant life, which could be highly affected by heavy metal concentration in their environment. *Schisandra chinensis*, a traditional Chinese medicine herb, showed very good developed defense reactions to biotic factors. Its secondary metabolites, mainly lignans with unique structure based on dibenzocyclooctadiene, showed high potential to reduce oxidative stress. So it might be a suitable model for study of abiotic stress in plants. Our work is based on effect of Pb2+ on growth of model embryogenic culture and study of levels of some aditive markers. The levels of L-cystein, reduced and oxidised glutathion and phytochelatin2 were detected by high performance liquid chromatography (HPLC) with coulochemic detection. The production of nitric oxide was detected on base of electrochemical changes induced by Pb2+ (Apollo 4000) immediately after 10, 20, 30, 60 min, 1, 2, 3, 4, 5 h and 1, 2, 3, 4, 5 days. The levels of five bioactive lignans were determined after 21 days by solid phase extraction followed by (HPLC). On base of our results the in vitro embryogenic culture is a suitable model for the study of abiotic stress reactions. Over there, these results could help to understand to elicitation processes that could increase the in vitro production of lignans.

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**P09-174** Post-grafting physiological state of tomato (*Lycopersicon esculentum* (L.) Mill.) grafts on different rootstocks

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Grafting has become an important technique for sustainable production of fruit-bearing vegetables, e.g. tomato. In horticultural science the efficiency of grafting and the combinations rootstock-scion are most commonly studied on the level of yield response and disease resistance. On the other hand, little is known on the rootstock-dependent properties of young plant, just shortly after grafting, when the plant has to overcome stress and restore long-distance transport. In this research we studied post-grafting state of tomato grafts (scion *L. esculentum* ‘Cuor di bue’ on different rootstocks, ‘PG3’, ‘Šempeter 1’ and ‘Body’). The fitness of the scion has been daily followed by using chlorophyll fluorescence. Two weeks after grafting water potential, hydraulic conductivity and transpiration were measured in order to evaluate water balance of the grafts. Gas exchange measurements were performed at different levels of
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water vapour pressure deficit (VPD). Fluorescence measurements revealed that the photosynthetic performance of the graft is not limited by the capacity/efficiency of photosynthetic apparatus of the scion. The level of the stomatal limitation of photosynthesis and transpiration under high VPD depended on the rootstock (big in 'PG3'), but could not be directly related to the water status of the grafts. Hydraulic conductivity of the grafts was also rootstock-specific. It was, however, in all cases drastically reduced when plants were exposed to salinity.

P09-175 The role of thiol peptides in cadmium tolerance
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Phytochelatin synthase (PCS) is an enzyme synthesizing heavy-metal complexing peptides: phytochelatins. Previous studies on plants overexpressing PCS genes reported contrasting phenotypes, ranging from enhanced Cd tolerance and accumulation to Cd hypersensitivity. This study compared the effects of overexpression of two phytochelatin synthase genes AtPCS1 (from A. thaliana) and CePCS1 (from C. elegans) in one model organism - tobacco. We demonstrated that, in contrast to WT and CePCS transformants, plants expressing AtPCS1 were Cd-hypersensitive although there was no substantial difference in Cd accumulation between studied lines. Plants exposed to Cd-d (5 and 25 μM) differed in the concentration of non-protein thiols. AtPCS1 expressing plants displayed a dramatic accumulation of γ-EC and strong depletion of GSH. In CePCS transformants, a smaller reduction of the level of GSH was noticed, and less pronounced change in γ-EC level. PCS activity in AtPCS1 plants was around 5-fold higher than in CePCS and WT ones. Substantial changes in thiol homeostasis in AtPCS1 expressing tobacco, due to increased PCS activity, contributed to: (1) increased oxidative stress level in the presence of Cd (indicated by elevated H2O2 production in leaves, lower GSH redox state, changes in ascorbate pool); and (2) decreased Cd detoxification capacity (reflected by lower SH:Cd ratios); which possibly explains the increase in Cd-sensitivity.

P09-176 Cd tolerance of Dianthus carthusianorum from a calamine heap
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Dianthus carthusianorum is the dominant plant species on calamine-Zn-Pb-Cd heaps in ore-mining and smelting region near Ołkusz, southern Poland. Two populations of D. carthusianorum, one from a calamine heap and the other from an unpolluted site, were compared in hydroponic experiments with respect to their tolerance to Cd. Both populations accumulated similar concentrations of Cd; however, the calamine population showed higher Cd tolerance as determined by plant growth and the root and leaf viability. Phytochelatin accumulation in plants increased with the increasing Cd concentration and was generally higher in the less tolerant population. The content of organic acids, especially malate and citrate, was not correlated with Cd concentration and was similar in both populations. The results show that phytochelatin and organic acids accumulation is not responsible for enhanced Cd tolerance of the calamine population of D. carthusianorum.

P09-177 Photosynthesis and carbohydrates of the C4 maize and sorghum grown at double-ambient CO2 and exposed to drought stress
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Drought was imposed for 12 days on 26-day old maize and sorghum grown in split-chamber controlled chambers at daytime carbon dioxide (CO2) of 360 (ambient) and 720 (high) ppm, and canopy photosynthesis (Pcanopy) and carbohydrates were determined. From 17–39 days after sowing (DAS), enhancement by high CO2 on Pcanopy averaged 5% for control maize, compared to 28% for control sorghum. The most enhancement by high CO2 on Pcanopy occurred at early plant growth (17–22 DAS), during which increases in Pcanopy were around 22% for maize and 27–95% for sorghum. As drought became severe at 28–34 DAS for maize and 30–36 DAS for sorghum, declines in Pcanopy for stress maize and sorghum were 54–87% and 29–46% at ambient CO2 and 17–31% and 1–20% at high CO2, respectively. For maize, high-CO2 control plants were 19% less in daily total evapotranspiration (ET), while stress plants at both CO2 had similar ET. For sorghum, ET was 15–17% less for both control and stress plants at high CO2. Afternoon leaf starch level at high CO2 was hardly affected in control sorghum, but was reduced in control maize. During drought, decrease in leaf starch occurred earlier and was greater at ambient CO2, and this was more evident for maize than sorghum. Total soluble sugars were hardly affected by high CO2 but were increased by drought for both plants at both CO2, and such increase was greater for sorghum. The data suggest that differences among C4 photosynthetic subtypes will be encountered as a result of future changes in CO2 and climate.

P09-178 MFT antagonizes inhibitory effects of high salt stress on seed germination
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Germination is one of the critical stages in plant development, as it determines the time point when a plant starts its new life cycle. Plant seeds perceive environment signals, such as salinity, to control the germination process to ensure their survival afterwards in a favored condition. We demonstrate here that MOTHER OF FT AND TFL1 (MFT), which encodes a phosphatidylinethanolamine binding protein in Arabidopsis, acts as a regulator of seed germination under high salt stress. mft loss-of-function mutants showed hypersensitivity to

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high salt stress in terms of germination rate. In germinating seeds, MFT expression was dramatically upregulated in response to high salt condition via an ABA-dependent pathway. Similar upregulation was also observed when germinating seeds were treated with exogenous ABA. In situ hybridization revealed that MFT was intensively upregulated in the radicle cortex upon ABA treatment. Promoter analysis further identified a key ABA response element (ABRE) at the MFT promoter, which enabled ABA to upregulate MFT. These results suggest that MFT antagonizes the inhibitory effects of high salt stress on seed germination.

**P09-179 A plant synaptotagmin is involved in freezing tolerance**

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Many plants growing in temperate and frigid zones acquire freezing tolerance during fall and early winter, sensing low temperature. This phenomenon is referred to as cold acclimation. Freezing tolerance is associated with alternation of several compounds such as phospholipids in the plasma membrane and soluble sugars in cytoplasm during cold acclimation. We have found that a plasma membrane protein, plant synaptotagmin AtSytA, changes quantitatively during cold acclimation in Arabidopsis thaliana. Mammalian synaptotagmin is thought to be a calcium sensor to regulate the fusion of plasma membrane with endomembrane system by the membrane-membrane fusion apparatus SNARE complex. Using protoplast and plasma membrane with endomembrane system by the membrane-totagmin is thought to be a calcium sensor to regulate the fusion of plasma membrane after its disruption.

**P09-180 GGMOs alleviated Cd toxicity in Thlaspi arvense**

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Galactoglucomannan oligosaccharides (GGMOs) are active factors in growth and developmental regulation and have non-specific resistance to local viral infection. However, the protective functions of GGMOs against abiotic factors have not been studied yet. Thlaspi arvense seedlings were cultivated in vitro on agar solidified MS media containing 10−7 M to 10−11 M GGMOs in combination with 2.10−4 M Cd(NO3)2, 4H2O over a period of 1 week. GGMOs stimulated root growth in comparison with the control. The highest activity of GGMOs was ascertained at the concentration of 10−10 M. Stimulation of root growth has been determined also in the presence of GGMOs and Cd(NO3)2 in comparison with Cd(NO3)2. However, in this case the most effective concentrations of GGMOs were higher (10−7 a 10−6 M), which may suggest different mechanisms of GGMOs activity in variants with and without the cadmium salt. Besides of positive impact of GGMOs on Cd treated roots, GGMOs had also protective effect on chlorophyll degradation. These results support the assumption that GGMOs may protect plants against abiotic environmental factors as well.

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**P09-181 Role of peroxidase enzyme and anthocyanin pigment in scavenging copper-induced oxidative damage in cotyledons of red and white cabbage seedlings**

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It is known that under certain conditions some transition metals like copper (Cu) can induce the activity of some antioxidant enzymes like peroxidases (PODs). PODs (EC 1.11.1.7) are a large family of ubiquitous enzymes widely distributed in plant cells, which play many important roles in plant growth, differentiation and developmental processes, are also responsible for both the scavenging of hydrogen peroxide (H2O2) by oxidation of phenolics. PODs are also known to be heavy metal stress-related enzymes. However, understanding of the antioxidative mechanisms for plant resistance to Cu toxicity is poor. In this investigation cotyledons of non-anthocyanin producing (white cabbage) and anthocyanin producing (red cabbage) seedlings were analyzed according to their pigment (chlorophyll, anthocyanin) and malonyldialdehyde (MDA) contents and POD activities that were exposed to excess Cu. A reduction of chlorophyll content in the case of copper treatment has been detected in the cotyledons of both white and red cabbage. MDA content was induced after exposure to 50 µM Cu. On the other hand, anthocyanin accumulation was enhanced by excess Cu. The increase in POD activity was higher in red cabbage than white cabbage cotyledons, which may indicate the use of co-substrates such as anthocyanin compounds by the POD enzymes. As a result, it can be hypothesized that POD enzymes and anthocyanins may play important roles in protecting plants against injury of metal-induced oxidative stress.

**P09-182 Spectroscopic characterization of compound III decay products in Zo peroxidase, a H2O2-resistant isoenzyme from radish**

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The commercial impact of peroxidases narrows by their poor stability towards one of its substrates, H2O2. Recently, a novel H2O2-resistant isoenzyme from radish (Raphanus sativus L. cv. daikon) called Zo peroxidase (ZoPrx) was identified. Generally,
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H$_2$O$_2$ sensitivity is related to the formation and fate of compound III (CIII). In labile peroxidases, CIII partitions among alternative decay pathways, while some of them take the protein back to ground state (GS) unharmed, others lead it into irreversible deactivation. In order to identify the structural determinants underlying ZoPrx resistance, we focused on the catalytic and spectroscopic properties of CIII by Electron Paramagnetic Resonance (EPR). Our results indicate that in GS ZoPrx, heme iron presents a combination of low-spin (LS) and mixed-spin (QS) states, being the later one an admixture of high-spin (HS) and intermediate-spin (IS) states. After the spontaneous decay of ZoPrx Compound III, the original QS signal observed decomposes, preserving only the HS component. The original LS signal was recovered along with a novel putative hydroxyl-iron signal. Based on these results, we suggest that ZoPrx CIII does not follow the common decay pathways but that it is converted into a single species, similar but not identical, to GS and that this species might be the source of H$_2$O$_2$ resistance.

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P09-183 The MYB-related transcription factor PHR1 influences photosynthetic parameters
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Plants have evolved a number of adaptive strategies to cope with fluctuations in phosphate supply. The MYB-related transcription factor PHR1 (At4g28610) is known to be involved in the P-starvation response (Rubio et al. 2001, Gene Dev 15: 2122–2133). Using plants that were either T-tagged knock-out phr1-mutant or transgenic plants overexpressing PHR1, we have shown that PHR1 influences P-starvation dependent changes in gene expression, carbon metabolism, phosphate accumulation and anthocyanin levels (Nilsson et al. Plant Cell Environ 30: 1499–1512). In this study we further characterise these plants. The changes might directly influence photosynthesis. To test this plants grown at 120 µE (8 h day) were transferred to high light conditions (700 µE, constant) and photosynthetic performance was measured as fluorescence parameters after 0, 4, 8 and 12 h. Furthermore the levels of selected proteins, which are involved in photosynthesis were analyzed by Western blots. We could hereby demonstrate that PHR1 has a clear effect on photosynthetic light processes, as the KO-mutant show strong and chronic photoinhibition of PSI-I-D1 and the antenna-complex during high light stress. We also observe a direct correlation between tissue P-content and quantum yields. However, no direct correlation between anthocyanin content and quantum yield observed.

P09-184 Purification, identification and biochemical characterization of plasma membrane-associated malate dehydrogenases from higher plants
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Membrane-associated malate dehydrogenases (MDH, EC 1.1.1.37) have been described for several endomembrane systems (Lüthje 2008). In the present work multiple isoenzymes of plasma membrane-associated MDH (pmdMDH) were purified by dye-affinity and ion exchange chromatography from maize (Zea mays L.) roots and leaves, cauliflower (Brassica oleracea) inflorescences and spinach (Spinacia oleracea) leaves. Properties (pl, MW) and enzyme kinetics (Km, pH-optima and inhibitors) of the partially purified proteins showed significant differences in comparison to cytosolic isoenzymes. The maize protein was identified by peptide mass analysis and showed a high sequence similarity to cytosolic MDH. Transmembrane domains were not indicated by sequence analysis. Specific staining for posttranslational modifications suggests a phosphorylation of pmdMDH. Protein-protein interaction was investigated by blue native and high resolution clear native polyacrylamide gel electrophoresis. Results suggest a dimeric structure for pmdMDH, but in some samples a MDH-containing protein complex was found with a higher molecular mass. Possible functions of pmdMDH isoenzymes in growth control and protection against environmental stresses will be discussed.


P09-185 Analysis of microRNA diversity in nodules and salt-stressed roots of the legume model Medicago truncatula
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In plants, a large diversity of small RNAs have been identified, comprising the microRNAs (miRNAs) and short-interfering RNAs (siRNAs). MiRNAs direct the cleavage of target miRNAs, often encoding transcription factors involved in differentiation and growth. Certain miRNAs are involved in adaptation to abiotic constraints. In the legume model Medicago truncatula, two miRNAs, miR166 and miR169, have recently been shown to be involved in nodule and root development.

To investigate the diversity of small RNAs acting during nodulation and in salt stress responses in Medicago truncatula roots, large scale sequencing of three small RNA populations from mature nodules and root apexes treated or not by NaCl 100 mM was performed using 454 technology. Removal of rRNA/riRNA sequences yielded 82 519 small RNAs from 18 to 25 nt. Around 28 000 displayed a miRNA-precursor like secondary structure at one predicted genomic locus. Alignment with the miRNA database, miRBASE, revealed more than 17 000 sequences corresponding to conserved miRNA families. The remaining ones could correspond to legume-specific ones. Comparison of miRNA frequencies between nodule and root apex identify conserved miRNAs potentially induced during nodulation (e.g. miR157 and miR167) or enriched in root apexes (such as miR170 and miR390). Several small RNAs (e.g. miR169 and miR398) accumulated differentially in response to salt stress. Many other sequences may be new legume-specific miRNAs regulated during these processes.

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P09-186 Short temperature drops reduce shoot elongation by enhancing gibberellin inactivation, but do not enhance cold tolerance

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To successfully transplant agricultural species in the spring, prior hardening is of great significance. Low, non-freezing temperature increases cold tolerance in many species. Also, diurnal temperature drops have been suggested to improve cold tolerance. Furthermore, pre-treatment with lower day than night temperature prior to hardening has been reported to enhance cold resistance in winter rape. We investigated the effect of temperature drops on cold resistance of different species. In contrast to a period of continuous low temperature, short diurnal temperature drops did not enhance cold tolerance in Arabidopsis, swede, white cabbage or pea, compared to control plants. Exposure to low temperature of 6°C for 6 days increased cold tolerance by 2–3°C compared to plants exposed to diurnal temperature drops or control plants. Pre-treatment with diurnal temperature drops in the entire growth period prior to hardening with constant low temperature did not give any additional hardening in swede and pea. In conclusion, by freeze testing of whole plants under controlled conditions we have found no evidence supporting the hypothesis that diurnal temperature drops improve cold tolerance. However, temperature drops in the light period reduce plants size and thus is a tool to produce compact, robust plants. This is associated with decreased levels of gibberellin as a consequence of increased expression of a GAs-oxidase gene.

P09-187 Knockout mutants of Physcomitrella metacaspase genes are altered in responses to abiotic and biotic stress

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In plants, programmed cell death (PCD) with apoptotic characteristics can be observed during the hypersensitive response and after abiotic stress. In animals, caspasases are key components of the apoptotic machinery, and although caspase-like activities have been detected in plants, no orthologous sequences have been found in their genomes. Metacaspases belong to a family of predicted caspase-related proteases present in yeast, fungi and plants. Recent studies suggested a role for metacaspases in different forms of cell death, although a direct involvement on PCD is unclear.

Metacaspases are classified as type I and type II based on their structure. The genome of Physcomitrella patens contains two genes for type I and four genes for type II metacaspases. We did functional studies of two Physcomitrella genes, PpMCA-1 and PpMCA-2, encoding proteins predicted to be localized in chloroplasts or in the nucleus, respectively. Northern analysis indicated that these genes were constitutively expressed but upregulated in response to pathogen and salt stress. Their contribution to cell death induced by various factors was analyzed by targeted gene disruption. The phenotype of the resulting knockouts was evaluated under stress conditions such as high salinity, oxidative stress and pathogen infection. We showed that metacaspase mutants were altered in their response to biotic and abiotic stress factors.

P09-188 Genetic divergence between populations results in the generation of species or subspecies. Hybrids between species (sometimes also between subspecies) often show incompatibility reflected in problems in reproduction. We have made reciprocal crosses between subspecies of Arabidopsis lyrata which is widely used as a model in plant population and ecological genetics. When the F1 hybrid of A. l. petraea and A. l. lyrata with maternally inherited cytoplasm from A. l. petraea is backcrossed using A. l. lyrata as a pollen donor, about half of the progeny express a novel sterile anther phenotype. A similar phenotype is also found in one fourth of the F2 individuals having the same subspecies petraea cytoplasm. In addition other developmental failures are observed in some of the plants which are able to produce pollen. We have explored the phenotypes using both light microscopy and field emission scanning electron microscopy (FESEM). We are also genetically mapping the genes causing the anther and pollen development problems.

P10-012 Effects of genes VRN and PPD of wheat (Triticum Aestivum L.) and EE of soybean (Glycinemax L./Merr) on carbohydrates, phytohormones and N2-fixation

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Flowering time of wheat is controlled by Vrn and Ppd loci, and the same of soybean is controlled by genes EE. We hypothesized that these genes determine biochemical processes that influence development rates. The purpose was to ascertain potential effects of the genes on carbohydrate (CH) metabolism, hormonal status and N2-fixation activity. Vrn 1-3 near-isogenic lines (NILs) (mono-dominant), Ppd 1-3 and EE 1-3 NILs were the subjects. CH accumulation in the leaves of late heading lines was higher during the day than in the early heading lines Vrn 11 and Vrn 33. It was the reason for both more intensive storage and slow day-reflux of CHs from leaves of the line Vrn 22, than those of the lines Vrn 11 and Vrn 33. The lines Vrn 11 and Vrn 33 have higher content of IAA and GA and lower one of ABA, than the line Vrn 22 has. Associative N2-fixation activity of Vrn 11 and Vrn 33 was higher, than that of Vrn 22. The study of genes Ppd effects under different day-length shows that the genes of Ppd 3 locus exert greatest influence on storage and reflux of water-soluble CHs and fructans, activity of invertase and...
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P10-013 Carotenoids changing in germinating cabbage seeds under influence of accelerated aging and brassinosteroids
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Carotenoids (Car) occur in seeds mostly in storage organs. The mutant seeds with elevated amount of Car showed the delay in seed germination and abnormal seedlings growth. That was attributed to competition between Car and GA for precursor and to over-producing ABA via violoxanthin pathway. Brassinosteroids (BRs) together with GA are needed to overcome inhibition effect of ABA and to stimulate seed germination. In this study Brassica oleracea L. seeds were treated with epibrassinolide (Eb) or homobrassinolide (Hb) and then aged by incubation at 85% of RH and 40°C for 3 days. Aged seeds showed a lower germination performance but BRs treatment reduced the rate of seed deterioration. Chlorophylls (Chl), carotenes (Cr) and xanthophylls (Xn) were analyzed in radicles and cotyledons of germinating seeds by modified spectrophotometric method. Content of Chl a, Chl b, Cr and Xn was 31.8, 28.9, 1.5 and 11.6 microgram per gram in less-mature seeds and 1.8, 0.5, 0.6 and 4.9 in more-mature seeds. According to HPLC analysis the basic Car were xanthophyll lutein and beta-carotene. During inhibition of seeds content of Chl a, Chl b and beta-carotene raised but lutein significantly decreased. Aging of seeds resulted to accumulation of lutein as in dry seeds and in seeds imibied for 48 h. That effect intensified under influence of Eb and Hb. The possible role of xanthophylls in maintenance of seed quality at unfavourable conditions of storage is discussed.

P10-014 Using sulfur-oxidizing rhizobacteria for better crop production in canola, soybean and corn
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Using beneficial bacteria like plant growth promoting rhizobacteria (PGPR) to enhance crop production is gaining attention worldwide. However, information on successful application of sulfur (S)-oxidizing PGPR on agricultural crops is limited. Certain agricultural crops like canola (Brassica napus), soybean (Glycine max) and corn (Zea mays) have fairly high S demand. Crop growth and production can be reduced if their S-demand is not met via fertilization or any other means. Attempts have been made to utilize S-oxidizing PGPR as biological seed treatment in canola, soybean and corn to enhance their performance and yield. Positive and consistent results especially from numerous field trials showed that biological seed treatment with naturally occurring S-oxidizing PGPR could be used as a unique tool for better crop production. This microbial technology tried to investigate and evaluate the feasibility of potential use of S-oxidizing rhizobacteria as commercial crop inoculant to enhance crop production. Hence, the development of three commercial inoculant products, BioBoost for canola (a.i. Delftia acidovorans), SoySuperb for soybean (a.i. Delftia acidovorans and Bradyrhizobium japonicum) and CornBoost for corn (a.i. Achromobacter piechaudii) will be discussed.

P10-015 Effect of CO₂ enrichment during grain filling on C and N allocation in two cultivars of spring barley (Hordeum vulgare L.)
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We investigated the effect of CO₂ during the generative phase on dry matter, accumulation and translocation of C and N in spring barley. Two cultivars of spring barley (Barke, modern cultivar and HOR 3550, old cultivar) were grown in pots with 6.5 kg quartz sand containing optimal macro- and micronutrients (eight plants per pot). At 33 days after sowing (stem elongation) the plants were exposed to ambient (360 ppm) and elevated CO₂ concentration (720 ppm) in growth chambers. N nutrition (975 mg per pot) was split into 3 applications. At anthesis ¹⁵N-labelled fertilizer (325 mg N per pot as ¹⁵NH₄NO₃ with 10% ¹⁵Nex) was supplied as solution to the pots. Plant samples of Barke were taken at anthesis, grain filling and ripening. HOR 3550 plants were sampled at grain filling. Dry matter and amounts of C, N and ¹⁵N in roots, stem, upper two leaves, remaining leaves, ears were determined. ¹⁵N abundance in samples was analysed by using a continuous-flow isotope ratio spectrometer coupled with a C/N analyser. In comparison to HOR 3550 Barke showed a higher accumulation of C and N in ears, higher chlorophyll contents in the flag leaf and better N use efficiency at 360 and 720 ppm CO₂. In the generative phase Barke remobilised N mainly from the stem. Less N was remobilised at 720 ppm CO₂, supply the ear. Surprisingly, HOR 3550 and not Barke could increase C and N accumulation enormously under elevated CO₂. Apparently, old cultivars still have high accumulation resources.

P10-016 Functional characterization of B-type MADS box transcription factors as regulators of floral organ identity in Gerbera hybrida
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MADS box transcription factors are main components in the ABCDE-model of flower development that describes how organ identities are determined. The ABCDE-model is based on analysis of mutants from Arabidopsis and Antirrhinum. However, studies conducted in diverse plant species have shown interesting diversification of this model. For example, most core eudicot species have three B-function genes belonging to Pp, euAP3- and TM6-lineages while both Arabidopsis and Antirrhinum have lost their TM6-type gene. In contrast to the classical B-function genes that define petal and stamen identity, the function of TM6-type genes in Solanaceae-species has specialized in determining stamen but not petal identity. Gerbera hybrida is a member of the large sunflower family (Asteraceae),
which is characterized by composite inflorescences consisting of morphologically different types of flowers. We have studied the function of the three Gerbera B-type MADS-box genes: the Pf-type gene GGL01, the euAP3-type GDEF2 and the TM6-type GDEF1. Expression analysis and transgenic phenotypes show that GGL01 and GDEF2 mediate the conventional B-function. The pattern of GDEF1 expression deviates from the expression of conventional B-type genes, suggesting a more specialized function. Comparison of phenotypes of the transgenic Gerbera lines with reduced expression of GDEF1 and GDEF2 also suggests functional diversification.

P10-017 MADS-box genes controlling reproductive development in legumes: gene duplication and functional divergence in Medicago truncatula

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Floral ontogeny, organ arrangement and symmetry in legumes present important differences from plant model systems (A. thaliana or A. majus) and the genetic control of these processes requires further characterization. Our goal is to isolate and genetically characterize the MADS-box gene family involved in the regulation of floral development in Medicago truncatula. Using the conserved MADS-box isoforms isolated from different genes as an overall probe in the screening of a floral cDNA library, we have isolated four B-function genes involved in the specification of petal and stamen identity: MtPI, MtAP3 (paleoAP3), MtNMH7(euAP3) and MtNGL9, and two C-function genes involved in the specification of stamen and carpel identity: MtAGa and MtAGb. The expression patterns of these genes have been studied by in situ hybridization analysis. MtNMH7 and MtNGL9 are the orthologs of MsNMH7 and MsNGL9 of Medicago sativa. Both MtPI and MtNGL9 proteins lack the PI motif described as essential for the functionality of AtPI in Arabidopsis. Functional analysis of these genes in transgenic Medicago plants to produce loss-of-function mutants (RNAi, VIGS) is currently in progress. We have already generated a MtPI loss-of-function mutant showing flowers with sepaloid petals and carpelloid stamens. Our results suggest that duplications in B and C-class MADS-box genes, followed by functional divergence during evolution, contributed to the formation of the specific floral structures of angiosperms.

P10-018 Ethylene modulates pistil and ovule senescence in Arabidopsis thaliana

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It is known that ethylene controls fruit ripening and senescence but only a few studies involve ethylene in pistil senescence. Senescence is the defect program in mature pistils but can be overcome by fertilization of the ovules that induces fruit set. Auxins and gibberellins (GAs) play an inductive role and alternatively ethylene may play a role inducing senescence. Orzáez and Granell (Plant J 11: 137, 1997) showed that in pea ethylene accelerates, while inhibits the ethylene action delay, the loss of sensitivity of the pistils to GA treatment. In Arabidopsis we have found that ovule senescence is one of the first steps in pistil senescence and is modulated by ethylene according to the following observations: (1) the analysis of the expression of GUS under the control of a senescence specific promoter shows that around 2 days post anthesis (dpa) the signal is observed in the stigma and then progressively extended from the basal to the apical ovules and maintained up to 6 dpa. (2) no GUS signal is shown between 6 and 12 dpa when signal is again observed in septum and then in valves, (3) the loss of sensitivity of the pistils to GAs is correlated with the extension of GUS signal in ovules, (4) there is an advancement of the loss of capacity to fruit set in response to GAs and on the appearance of GUS signal in ovules in the ctr1-1 mutant, (5) ein2-5 mutant shows a delay on the loss of sensitivity of the pistils to GA treatment and on the appearance of GUS signal in ovules.

P10-019 Peroxidase isoforms involved in pod shattering mechanism in Arabidopsis thaliana

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Brassica plants normally disperse their seeds by a seed-shattering mechanism. Although this mechanism is advantageous in nature, unsynchronized pod shatter constitutes one of the biggest problems in terms of seed loss for canola farmers. Since the genetic pathway of this mechanism is conserved between Arabidopsis and Brassica, studies concerning the control of seed dispersal in Arabidopsis should be generally applicable to oilseed crop. Plant peroxidases are known to be involved in a broad range of physiological processes all throughout the plant life cycle. Here, we show for the first time that peroxidases are involved in pod shattering in Arabidopsis thaliana and that several peroxidase genes are under the control of known crucial transcription factors such as SHP1, SHP2, IND, ALC and FUL. We further identified one peroxidase gene AtPrx17 (A25g22420) involved in the lignification of the enb cells of the endocarp. The heavy lignification of enb cells is believed to be responsible for the generation of the tensions associated with the explosive shattering. A careful analysis of AtPrx17 expression and its regulation mainly by AGL15 and gibberellic acid is reported. This study reveals the crucial role of peroxidases during fruit development and more particularly dehiscence. The genetic engineering of peroxidase genes may therefore assist breeding efforts for discovery of shatter resistant oilseed crop varieties.

P10-020 Two CONSTANS-LIKE1 genes in long- and short-day Solanum plants

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Day length controls floral transition in many plant species, with CONSTANS and its CONSTANS-LIKE1 (COL1) orthologues as a key gene in the photoperiodic pathway in arabidopsis, rice, and several other plant species. The CONSTANS protein comprises two B-box-type zinc fingers, CCT domain, and the variable middle region (MR), which corresponds to the exon 2 of COL1 in Solanum species. Solanum COL1 proteins are over 85% identical.

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within the genus and about 50% similar to the arabisopsis prototype. Comparative analysis of COL1 clones from long-day (LD) *S. tuberosum* ssp. *tuberosum* (tubersum potato) and short-day (SD) *S. demissum*, *S. stoloniferum* and *S. tuberosum* ssp. *andigena* (andigena potato) discerned two variants, which differed in the exon 2 structure, particularly, in the numbers of AAC/AAT and CAA/CAG repeats coding for polyN and polyQ tracts in MR. The length of tandem and cryptic polyN and polyQ motifs in MR may affect protein conformation and in this way modify the stability and binding activity of this transcription factor. Both COL1 variants were found in each individual plant in all *Solanum* genotypes, independently of their photoperiodic behaviour. The temporal expression profiles of two COL1 genes dramatically differed under SD or LD. Presumably two genes have dissimilar although interdependent functions. The presence of both COL1 variants in each *Solanum* genome suggests that the evolution of two COL1 genes preceded the divergence of *Solanum* species.

**P10-021** *STY1* Acts as a transcriptional activator regulating local auxin biosynthesis

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The *SHI/STY* gene family consists of nine active members in Arabidopsis. We have previously reported that mutations in *STY1*, and related genes, affect style morphogenesis and apical-basal patterning of the gynoecium, that *STY1* activates transcription of the flavin monooxygenase-encoding gene *THREAD/YUCCA4*, involved in auxin biosynthesis and that changes in expression of *STY1* leads to altered auxin homeostasis. Now we can show that the *STY1* regulated activation of *YUC4* is independent of protein intermediates and that *STY1* interacts with a short sequence proximal to a TATA-box in the *YUC4* promoter. Consequently expression of a *STY1*-SRDX fusion protein represses expression of *YUC4* and phenocopies *SHI/STY* multiple loss-of-function mutants, suggesting several *SHI/STY* family members to participate in activating transcription of *YUC4*. Thus, our data point out the *SHI/STY* family as essential transcriptional regulators of auxin biosynthesis.

**P10-022** Characterization of the MADS-box gene *AGL63* and its possible function in concert with *ABS/TT16*

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B genes confer stamen and petal identity of angiosperms flowers. The sister clade of the B genes termed Bisster (Bs) is conserved throughout seed plant evolution. Bs genes are mainly transcribed in female reproductive organs. The Arabidopsis Bs (abs) gene knock-out mutants show an altered seed pigmentation and endothelium malformation. This mild phenotype of the abs mutant led us to the hypothesis that other genes might act redundantly to *ABS*. *AGL63* appears to be a truncated paralog of *ABS* lacking the C-terminal domain and a part of the K domain but shows high similarity to *ABS* in the remaining protein parts. Expression analysis of *AGL63* revealed a weak expression in buds, flowers, siliques, roots, cauleine and rossette leaves, whereas *ABS* is expressed exclusively in buds, flowers and siliques. Plants, which constitutively over express *AGL63* exhibit an earlier flowering phenotype and an altered inflorescence structure. Additionally, homeotic conversions of sepals into gynoecium-like structures occur, the petals are lost completely in a large fraction of the plants, and disintegrated woful structure has been observed. Gel retardation assays shows that *AGL63* can form homodimers and heterodimers with *ABS*. Surprisingly, *AGL63* does not form heterodimers with SEPALLATA3 (SEP3), which is known to be a common partner for floral transcription factors. Our preliminary data suggest *AGL63* is a functional gene with a rather general function.

**P10-023** Functional characterization of *OCL1*, an epidermis-specific HD-ZIP IV transcription factor, by identification and characterization of its target genes

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Epidermis differentiation is a key step in plant embryogenesis and a condition for a normal development of the plant embryo. HD-ZIP IV transcription factors including FWA, GL2, ATML1 or PDF2 in Arabidopsis and OCL 1–5 (Outer Cell Layer) in maize, seem to play crucial roles in the differentiation and maintenance of the epidermal cell fate. In maize, OCL1 is specifically expressed in the epidermis of embryo, endosperm and young organ primordia. Plants over-expressing OCL1 (OCL1-OE) have a pleiotropic phenotype. To identify direct or indirect target genes of OCL1, transcriptome of 18 day old plantlets OCL1-OE was compared to that of wild-type plantlets using the maize 70mer micro-array. Of 35 candidate genes, 12 were confirmed as being up- or down-regulated by Q-RT-PCR. Expression patterns of the 12 genes in the maize plant were established by Q-RT-PCR and/or in situ hybridization. Bioanalysis cDNA sequences revealed that several target genes encode proteins involved in lipid metabolism, defense or cuticle biosynthesis. Whenever available, promoter sequences were scanned for the presence of L1-box, an 8 bp motif which has been identified as the cis-element of HD-ZIP IV in Arabidopsis. The promoters of three genes with L1 box were cloned and fused to a GUS reporter gene to check for trans-activation by OCL1 by transient expression in maize kernels. The promoters of a lipid transfer protein and an ABC transporter but not a TPR domain protein seem to be direct targets of OCL1.

**P10-024** Functional characterisation of pollen-specific transcription factor AtBZIP34

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Haploid male gametophyte, the male partner in sexual reproduction of flowering plants, plays a key role in plant fertility and crop production. Our ability to control and guide this process represents an effective tool for crop breeding and genetic optimization such as reduction of allergen content for which pollen is an important source. We have a very limited understanding of the regulatory mechanisms that have evolved to specify the gametophytic developmental program and ensure its flawless progress. To unravel such mechanisms, it is necessary to identify transcription factors that are part of the regulatory network. We have focused on the bZIP family of TFs. These factors play critical roles in plants, animals and other kingdoms. Here we report precise functional characterization of male gametophytic AthZIP34 transcription factor. It involves description of phenotypic defects, demonstration of co-segregation of mutation in AthZIP34 gene with the observed phenotype, pollen tubes growth test and expression patterns of corresponding gene.

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P10-025 Programmed cell death in reproductive female tissues of kiwifruit

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Kiwifruit (Actinidia deliciosa) is a dioecious species. Stigmatic receptivity, which relies on papillar integrity, is high at anthesis and the next 4 days, decreasing by the fifth day and is nil 2 days later. In this period an abundant secretion is present all along the pistillar tract both in pollinated and unpollinated flowers. Our work aims to determine if the described process is an example of programmed cell death in reproductive tissues, and which is its relationship with pollination. Several features associated to programmed cell death were studied: DNA fragmentation and degradation by TUNEL staining and DNA laddering, as well as chromatin condensation by DAPI staining. The histological studies allowed us to detect progressive changes in the stigmatic tissues of flowers pollinated at anthesis. By the first 3 days post anthesis, nuclei appeared spherical with dispersed chromatin. After the third day chromatin tended to be slightly condensed and after the fifth day the secretory region of stigmata showed an extensive degeneration. Most of the nuclei became tubular in shape, with highly condensed chromatin. We also noticed a remarkable reduction in the number of nuclei. These histological features could be also observed in unpollinated stigmata, although in a not so evident fashion. Agarose gel electrophoresis revealed low molecular weight DNA signals (180-bp) in samples of stigmata harvested 5–7 days after pollination, but not in stigmata from unpollinated flowers.

P10-027 ‘Bold start’ auxin localization and polar auxin transport during somatic embryogenesis in Norway spruce ‘Bold end’

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Several lines of evidence implicate the plant hormone auxin (indole-3-acetic acid, IAA) to be a key signal molecule in providing positional information within the embryo, and that in angiosperms, polar auxin transport (PAT) is of particular importance during the transition of embryos from globular stage into heart stage. The PAT system appears to be controlled by the influx carrier AUX1/LAX and the efflux carrier PIN-FORMED (PIN). Experiments with various PAT inhibitors, such as 1-N-naphthylphthalamic acid (NPA), indicate the polar auxin movement to be a fundamental mechanism for initiating and maintaining the central axis in developing embryos. We have investigated the importance of PAT for proper embryo development in ‘Italics start’ Picea abies ‘Italics end’, by applying NPA to our in ‘Italics start’ vitro culture system ‘Italics end’ of somatic embryos. Embryos were after various treatments analyzed, both morphological and anatomical, and IAA was localized by immunochemistry. These experiments revealed the transition stage of embryos being most sensitive to PAT-disturbance. Pin-formed and cup-shaped embryos were produced, similar to that seen in angiosperms. Early staged embryos stained heavily in the assay for auxin, while embryos stained less as they matured. Germination experiments with the most severely disturbed pin-formed embryos showed them to increase in length for a while but they lacked roots and never formed shoots.

P10-026 Evolution of self-compatibility in apricot

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Gametophytic self-incompatibility of apricot (Prunus armeniaca) is governed by the highly variable S-locus. In China, the centre of origin, apricot is self-incompatible, but most European cultivars are known to be self-compatible. Self-compatibility (SC) in apricot is caused by a pollen-part mutation within the S₈-haplotype. This study identifies the first known progenitor allele of a naturally occurring self-compatibility allele in Prunus. The S₂- and S₈-ribonuclease showed identical intron and cDNA sequences and equal levels of RNase activity. A controlled pollination cross functionally confirmed that S₂ is a pollen-part mutated form of S₈ (hence, can be labelled as S₈*). More SNPs were identified in the S₈-RNase than in the S₈*, and these could be used for monitoring apricot dissemination routes between East and West Europe. The first intron of the Prunus S₈-RNase was identified to be a phase one intron indicating its recent evolutionary origin compared to the phase zero second intron. Our results helped to elucidate the putative origin and dissemination of the S₈-haplotype induced self-compatibility in apricot. This work was funded by the NKTH-OTKA K68921 grant.

P10-028 Binding to methylated histones is essential for LHP1 function during Arabidopsis development

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Stable gene repression is achieved through strong chromatin compartmentation mediated by the interaction of non-histone proteins with
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modified histone tails. HETEROCHROMATIN PROTEIN 1 (HP1) co-localizes with heterochromatin in mammals and flies and is guided there by recognition of H3K9me2 and H3K9me3. The plant HP1 homologue LIKE HETEROCHROMATIN PROTEIN 1 TERMINAL FLOWER 2 (LHP1/TFL2) has a very similar protein structure and is involved in gene silencing. However, instead of exhibiting a heterochromatic localization, LHP1 binds to euchromatic regions of the genome that are marked by H3K27me3, even though it also binds to di- and trimethylated H3K9 in vitro. Here, we show that disruption of the chromo domain abolishes H3K27me3 recognition, releases gene silencing and causes similar phenotypic alterations as transcriptional lhp1 null mutants. Therefore, binding to H3K27me3 is essential for protein function.

**P10-030 Sex differentiation in plants and phytohormones**

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Sex differentiation in plants depends on realization of interrelated genetic and hormonal programs. Plant cells are bisexual, i.e., they are capable of producing both genders. In plants, the characters of a certain sex are determined not only by a certain karyotype, but also by a certain gene (or genes) located in one of the autosomes. The gene informs the cells about triggering the biosynthesis of specific RNA as well as female or male protein. This protein may form a complex with phytohormones, which affects nondifferentiated flower primordia, and the development will proceed according to a certain sex pattern. For instance, the presence of a specific protein we detected and phytohormone cytokinin typical of female individuals suggests that the complex they produce will induce the expression of female characters. Apparently, this is the reason why primordia of pistillate flowers develop from the subepidermal cell layer when the plants are treated with cytokinins before the start of differentiation of the flower meristem. However, if the sex differentiation of flower meristems has already started, application of exogenous hormonal preparations will not alter the genetically predetermined direction of sex formation. This principle apparently governs the effect of gibberellins that induce masculinization of plants. Since the pathway of gibberellin and animal hormone production is the same, it is only natural to classify gibberellins with the male sex hormones of plants.

**P10-031 Endosperm growth in Sorghum bicolor: endoreduplication, cell size and number of cells**

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We analyzed the growth of Sorghum bicolor endosperm during the endoreduplication-associated growth phase. Nuclear endopolyploidy was measured in situ in median longitudinal caryopsis sections using image densitometry with the interphase-peak method. Endopolyploid nuclei with DNA content 12C were observed already 5 DAP (days after pollination). The highest nuclear DNA amount measured was 96C and was first observed 10 DAP. In further development the number of highly endopolyploid nuclei progressively increased. They were located in the central part of the endosperm with the highest amount of starch. No starch was detected in the basal part of the endosperm where the highest endopolyploidy level was 24C. Non-endopolyploid nuclei (3C and 6C) were located primarily in the peripheral layer of the endosperm that functions as a meristem tissue. Endoreduplication was positively correlated with endosperm cell volume. The total number of cells in the sorghum endosperm was calculated using the 3-D model developed for the maize endosperm, and the number of cells during the observed period between 5 and 16 DAP was fitted with the Richards’ growth function. The peak of absolute growth rate was at 9.9 DAP, afterwards the growth rate abruptly falls towards zero. The mean cell doubling time (MCDT) was highest (40 h) in the period between 8 and 10 DAP, the period that is mitotically most intense.

**P10-032 Ethylene controls gametophytic-sporophytic interactions in progametic phase of fertilization**

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Using fertile and sterile lines of petunia, we compared the structure of developing anthers and male gametophytes and evaluated the changes in the content of ACC, the ethylene precursor, and ethylene production by developing anthers. In fertile line, microscopic development was accompanied by increase in the ACC content,
whereas the increase in ethylene production peaked at the stage of vacuolated microspores. In sterile line, ACC accumulation and increase of ethylene production commenced at the stage of mother cells of microspores and proceeded at the stage of meiosis. It is necessary to mention that the greatest ethylene production by anthers of sterile line (accompanied by the degeneration of tapetum and death of male gametophyte) exceeded 5–6 times the highest ethylene production by anthers of fertile line (accompanied by the degeneration of tapetum and middle layers of anther wall). The data obtained allow us to conclude that ethylene is involved in the processes of the PCD in anther tissues. Using petunia pollen-pistil system, we showed that ACC content and ethylene production in isolated parts of pistil (stigma, style and ovary) undergo specific modulation after self-compatible and self-incompatible pollination. Basing on the results obtained an assumption was put forward that ACC-synthase is a component of signal transduction in pollen-pistil system and the ethylene, in combination with ABA and cytokinins, takes part in the gametophytic self-incompatibility.

P10-033 Egg cell specification requires VORNGLEICH
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In plants, gametes form in few-celled haploid structures, the gametophytes. The female gametophyte of higher plants integrates such diverse processes as pollen tube attraction and initiation of seed development. This is enabled by the specialized action of four different cell types. We are interested in the mechanisms that underlie the specification of the distinct cell types with a particular focus on the specification and regulation of gametic cells. In a screen for activators of egg cell identity we identified the vorngleich (vogl) mutant. Vogl gametophytes fail to express an egg cell marker. Additionally, development of cells neighboring the egg cell is affected. We present the morphological and molecular analysis of the vogl mutant together with a first functional characterization of the gene. Possible implications for mechanisms of egg cell specification will be discussed.

P10-034 Regulation of floral patterning by flowering time genes
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Floral organ development is precisely regulated by floral homeotic genes. Deregulated expression of homeotic genes in floral meristems leads to the generation of aberrant floral organs, which causes subsequent defects in gametogenesis and seed production. Here we show that the onset expression of both B and C class homeotic genes in floral meristems are redundantly regulated by three flowering time genes: SHORT VEGETATIVE PHASE (SVP), AGAMOUS-LIKE 24 (AGL24) and SUPRESSOR OF OVEREXPRESS OF CO1 (SOC1). In the triple mutants where the function of all these three MADS genes is lost, an E class gene, SEPALLATA3 (SEP3) is ectopically expressed throughout the plants including the inflorescence meristem and emerging floral primordia, where it interacts with LEAFY (LFY) to ectopically activate both B and C class genes. In such triple mutants, precociously activated floral homeotic genes result in abnormal inflorescence architecture and an early depletion of floral meristems with various defective floral organs. Our results show that tight regulation of SEP3 by three flowering time genes is a crucial step in defining the spatial and temporal expression of floral homeotic genes, thus determining floral patterning.

P10-035 Differential gene expression in defective endosperm mutants of maize
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In maize, some mutations affecting endosperm development originate the group of the so-called defective endosperm (de) mutants. Twenty mutations were isolated and ten of them were located on six chromosomal regions. Bulked segregant analysis was adopted to screen the largest possible number of primer combinations in order to find AFLP or SSR markers linked to the mutant loci, which were integrated into a high-resolution genetic map. In particular, the maize mutant del18 was mapped on chromosome 10, bin 03, coincident with the umc1962 SSR marker. del18 accumulates substantially less dry matter in the endosperm than its normal counterpart. We have confirmed that the auxin indole-3-acetic acid levels are several times lower in del18 endosperms respect to the wild type. In this study we performed experiments using oligonucleotide microarrays to determine differential gene expression between the mutant del18 and its wild type B37. mRNAs from four different stages of development - 7, 14, 21 and 28 days after pollination - were used to perform the hybridizations. After normalization and statistical analysis of data groups, differentially expressed genes were detected. Some of them are involved in the auxin metabolic pathway. These preliminary results will be validated by using RT-qPCR.

P10-036 Stress-induced somatic embryogenesis in pumpkin (C. pepo L.)
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Proembryo production in pumpkin can be induced by low concentrations of NH₄Cl as a sole source of nitrogen, or by 2,4-D. Subsequent addition of nitrogen in the first case, and removal of auxin in the second, stimulate more advanced stages of embryo development. Proembryogenic cells from the two systems showed differences in growth parameters and patterns of callose deposition. The latter disappeared when the tissue was transferred onto a medium that enables late embryo development. The activities of soluble peroxidase (EC 1.11.1.7), esterase and phenylalanine ammonia-lyase, enzymes shown to be markers of stress response and embryo development, were the highest in tissue grown in NH₄+-only medium which reflects highly proembryogenic state of the culture. Enzymatic activities decreased after buffering of the medium with MES, and additionally decreased after re-supply of different forms of nitrogen or in medium with 2,4-D. The effect of L-glutamine on
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Somatic embryogenesis was stronger than that of nitrate. The role of stress during early somatic embryogenesis in pumpkin will be discussed.

P10-037 Identification of flowering related cDNAs from Fragaria vesca using suppression subtractive hybridization and EST sequencing
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Arabidopsis thaliana is widely used as a model in plant molecular biological research. However, being a long day plant the possibilities to study other day length response types using Arabidopsis are limited. Rosaceae is a large plant family with several economically important, both woody and herbaceous species such as rose, apple and strawberry. We have studied the control of flowering in strawberry using wild strawberry (Fragaria vesca) as a diploid model plant. Diploid strawberry is widely distributed around the world and different types of flowering responses have been identified. Some everbearing (EB) forms have been shown to differ from the short day (SD) type by only one gene. We used long day grown apical bud samples of SD wild type and EB genotype (F. vesca 'Baron Solemacher') seedlings for construction of cDNA libraries. Suppression subtractive hybridization and EST sequencing of the corresponding libraries was done to enrich either flowering repressing or activating genes. Annotation of the sequenced ESTs from both libraries revealed several flowering related gene homologs belonging to the various flowering pathways in Arabidopsis. For functional studies, some of the most interesting genes have been transformed in F. vesca Hawaii-4 line using over-expression and/or RNAi-GFP constructs. In addition, we have grown a crossing progeny of the SD type and five EB types to explore the genetic basis of different flowering habits.

P10-038 Carpel development in Eschscholzia californica – a basal eudicot point of view
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Flowering plants are characterized by the presence of male and female reproductive organs, stamens and carpels respectively. The carpels serve as mechanical inbreeding barrier, protect the ovules from herbivory, and develop into fruits after fertilization of the ovules. The regulators of the carpel development belong to different families of transcription factors. There are some candidate orthologs of known Arabidopsis carpel development genes in schscolzia californica which might be involved in the carpel formation. For instance, the ortholog of the Arabidopsis YABBY transcription factor CRABS CLAW (CRC) and O. sativa DROOPING LEAF(DL) genes. The CRC gene is required for carpel polarity and carpel development as well as for nectary formation in Arabidopsis. Its rice ortholog is known to be involved in specifying carpel organ identity but it shares an additional function in regulation of floral meristem activity and in leaf midrib formation. It seems that the Eschscholzia CRC ortholog (EcCRC) is involved in the carpel formation and in the establishment of the carpel polarity like the Arabidopsis CRC. The EcCRC is expressed abaxially along the carpels from very early stages of gynoecium development. But unlike the Arabidopsis CRC, the EcCRC shows expression in the base of the gynoecium. We suggest that the EcCRC is required not only for carpel development but also seems to be involved in the floral meristem determinacy.

P10-039 Genetic approaches towards understanding flowering regulation in the sympodial species tomato Solanum esculentum L.
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The genetic control of flowering in the sympodial species tomato (Solanum esculentum L.) is still poorly understood compared with the monopodial species Arabidopsis thaliana. Since a direct extension of knowledge about flowering regulation from Arabidopsis to sympodial plants is not possible due to the fundamental differences between both growth systems, we decided to investigate the mechanism of flowering in tomato. In this plant, mutants affected in their flowering responses include uniiflora (uf) which produces solitary, normal, fertile flowers instead of inflorescences and flowers later than the wild type in both the initial and the sympodial segments. We produced double and triple mutants using, as a common parent, the uf mutant to elucidate the potential interaction between the UF gene and other genes controlling flowering in tomato. The uniiflora compound inflorescence double mutant flowered only after 1 year of growth in glasshouse. The uniiflora.blind:self pruning triple mutant produced solitary flowers and rarely initiated axillary buds. In some extreme cases, growth of the triple mutant was arrested after initiation of a single terminal flower because plants failed to produce any axillary and sympodial axes. These and all other double and triple mutants investigated were late flowering and initiated solitary flowers like the uf mutant. These results suggest that UF is epistatic to various genes in regulating morphogenesis of the reproductive structure of tomato.

P10-040 Identification of a heat shock transcription factor affecting male gametophyte development
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The haploid male gametophyte generation represents a vital role in fertility and plant life cycle. Despite a long-term research on the field of plant sexual reproduction, the knowledge of transcription factors playing role in male gametophyte development is still very limited. Exploiting microarray technologies and bioinformatic
analyses, we selected 39 genes encoding putative transcriptional factors expressed specifically during male gametophyte development. To prove the importance of selected transcription factors in pollen development, we performed phenotype screening of T-DNA insertion lines for aborted or defective pollen grains by both light and UV microscopy. Several structural abnormalities were found showing a significant impact of knocked out transcription factor genes on cellular processes. Subsequently we focused on a heat shock transcription factor since the members of transcription factor family are known to be involved in stress response and developmental processes. In addition to pollen subcellular disorder, the reduced ability of pollen tube growth was confirmed by in vivo and in vitro experiments. Moreover, selected heat shock transcription factor caused segregation ratio distortion and significantly reduced the allele transmissibility via both male and female gametophyte.

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P10-041 The effect of brassinosteroids on selected growth, reproductive and yield parameters of three genotypes of maize grown in field conditions
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Brassinosteroids (BRs) comprise the sixth main group of plant phytohormones. Their biological activity has been studied in various plant species and they have been shown to enhance photosynthesis and stimulate plant growth, to increase yield, to protect plants against biotic and abiotic stresses or to have other positive effects on plant physiology and development. The influence of BRs on flowering has been also observed in several species, but the research in this area is still somehow limited. We have examined the effect of new androstane analogue of castasterone (AAC) and 24-epibrassinolide (E) on various morphological, growth, reproduction and yield parameters in two maize inbreds and their F1 hybrid. Foliar spray of BR solutions (compared to water as the control) was applied to plants in V3-V8 stages, which were grown in field conditions in the Czech Republic during 2004-2007. The application of BRs to plants accelerated the flowering for about 3-4 days (this applied particularly for lower BRs concentrations and for the second or third female inflorescences). In some cases, positive changes of morphological or yield parameters (e.g. plant height, number of kernels in row and total ear weight) were also observed. However, all these effects strongly depended on the genotype and on the developmental stage of BR application, as well as on the type and concentration of BR. The study was supported by grants No. KJB60110611 of the grant agency GAAV and MSM 0021620858.

P10-042 Characterization of CC type glutaredoxins from Physcomitrella patens
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Within the thioloxidin (TRX) superfamily glutaredoxins (GRXs) represent a group of small oxidoreductases with a size of 10–15 kDa in size. They are involved in a large number of cellular processes, play a crucial role in redoxregulation and response to oxidative stress. According to their active site motif GRXs are classified in three subfamilies, the CPYC, CGFS and CC type. The CC type subfamily is land plant specific and expanded dramatically during land plant evolution from two in Physcomitrella patens to over 20 in Arabidopsis thaliana. Contrarily, the number of CPYC and CGFS glutaredoxins remained similar in all investigated land plant groups. Conserved cysteins located in the active site motifs participate in disulphide reduction and thus modify target proteins posttranslationally. The AVRGRX mutants rosy1 and rosy2 reveal a floral phenotype affecting petal initiation and morphogenesis as well as other differentiation. This indicates an intriguing correlation between the formation of more complex plant organs and the expansion of CC type towards understanding the ancestral function of CC type GRXs, the two Physcomitrella GRX mutants PpGRX1 and PpGRX2 are analyzed. PpGRX expression studies and complementation data of the Arabidopsis mutants will be presented.

P10-043 SINAT5 is a positive regulator of flowering in Arabidopsis
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Suppression of FLC (Flowering Locus C) is critical for the transition from the vegetative to reproductive phase in Arabidopsis. FLC represses the expression of several genes involved in floral induction and its transcription is positively or negatively regulated by FRIGIDA or by vernalization, respectively. However, the regulatory mechanisms for the level and stability of FLC protein throughout development are unknown. Here, we show that SINAT5 colocalizes with FLC in the nuclear bodies and that its zinc finger motif interacts directly with the MADS-box domain of FLC. In addition, it is shown that SINAT5 has an E3 ubiquitin ligase activity towards FLC as a substrate, suggesting that SINAT5 participates in the regulation of flowering time through the ubiquitin-mediated proteolysis of FLC. This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2006-000-10035-0) and by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

P10-044 The effects of planting density on the trend of grain filling, grain yield and yield components of three chickpea varieties at rainfed condition
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A field experiment was conducted to evaluate effects of planting density and variety on the trend of grain filling; yield and yield
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Component to chick pea. The present research was conducted at experimental farm of Mahydasht (Kermanshah). The factorial experiment was designed based on complete randomized block design with four replications. In this experiment; the variety in three level (Ily; ILC-482 and 12-60-31) and the planting density in three level (19, 28 and 57 plant.m-2) were considered. The trend of grain filling; yield component and agronomic characteristics; as some biomass yield; harvest index; number of pods per plant. Number of grain per plant; number of node per main stem; plant height; number of branch per plant; weight of 100 grain; distance between first pod to soil and phonological stages the chick pea varieties based on photo growing degree day (PHOTO GDD) Were calculated. The result achieved showed that the maximum speed of grain filling related to density of 28 plant.m-2 and ILC-482 variety. Grain yield; number of pods per plant; number of grain per plant; weight of 100 grain; plant height and distance between first pod to soil were significantly affected by variety and density but number of branch per plant was affected by density and number of node per main stem and harvest index were affected by variety. The maximum photo growing degree day related to 12-60-31 variety and the maximum grain yield related to density of 28 Plant.m-2.

**P10-045** The receptor kinase CORYNE of Arabidopsis transmits the stem cell limiting signal CLAVATA3 independently of CLAVATA1

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Stem cells in shoot and floral meristems of Arabidopsis thaliana secrete the signalling peptide CLAVATA3 (CLV3) that restricts stem cell proliferation and promotes differentiation. The CLV3 signalling pathway is proposed to comprise the receptor kinase CLAVATA1 (CLV1) and the receptor-like protein CLAVATA2 (CLV2). We show here that the novel receptor kinase CORYNE (CRN) and CLV2 act together, and in parallel with CLV1, to perceive the CLV3 signal. Mutations in CRN cause stem cell proliferation, similar to clv1, 2 or 3 mutants. CRN has additional functions during plant development, including root meristem growth and floral organ development, that are shared with CLV2.

**P10-046** Stability of the seed dormancy inducing protein DOG1 is determined by alternative splicing

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Seed dormancy is defined as the failure of a viable seed to germinate under favourable conditions. Besides having an adaptive role in nature, dormancy control is important in crop plants. DELAY OF GERMINATION 1 (DOG1) was identified as a major determinant of natural variation for seed dormancy in Arabidopsis. DOG1 is alternatively spliced and encodes a protein of unknown function. Mutant dog1 seeds are non-dormant and DOG1 transcription levels correlate with dormancy status, suggesting that DOG1 is absolutely required for seed dormancy induction. DOG1 is expressed during seed maturation. The transcript is present in the vascular system of the embryo and accumulates in its shoot apical meristem.

The splice variants of DOG1 encode proteins with different carboxyl ends. We studied the functionality of the alternative transcripts by transgenic complementation of the dog1 mutant. Single splice variants could only induce dormancy and accumulate DOG1 protein when highly overexpressed. However, DOG1 protein can be detected at low expression levels in wild-type plants. This suggests protein degradation of the single splice variants, which can be outcompeted by high expression levels. Binding studies in yeast showed that DOG1 protein forms can bind to each other and that the amino terminal part of the protein is required for this self-binding. We propose that stable DOG1 protein requires the presence of different splice variants.

**P10-047** Molecular Phylogeny of Circadian Clock Genes (LHY/CCA1s and PRRs) in Populus, Arabidopsis and Oryza

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Circadian rhythms are generated by endogenous circadian clock systems. In the past decade; molecular genetics and systems biology have provided novel insights into a circadian clock system of plants. In Arabidopsis thaliana; the clock system is reconstructed as four transcriptional-feedback loops and consists of single Myb genes (LHY/CCA1s) and pseudo-response regulator genes (PRRs); both of which are highly conserved in Oryza sativa. However; it is still unclear how many genes are conserved in model trees; Populus spp. and; furthermore; a molecular phylogeny of these clock-related genes in angiosperms has not been determined. To answer the first question; we identified Populus LHY/CCA1s and PRRs using Blast search with information of genome sequence of Populus trichocarpa and found that Populus conserved two LHY/CCA1s and eight PRRs. Then; to answer the second question; we analyzed intron-exon structures; phylogenetic trees and chromosome syntenies of these genes among Populus; Arabidopsis and Oryza. We revealed that some genes were duplicated via whole genome duplication events and that; after these events; some of the duplicated genes were deleted from the genomes. These results indicate that precise analyses of a paralogous/orthologous relationship of each homologous gene can successfully reconstruct the evolutionary processes of the plant clock system in angiosperms.

**P10-048** Epigenetic regulation of photoperiodic flowering

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A DNA demethylating reagent; 5-azacytidine (azaC); induced flowering in Perilla frutescens; Silene armeria and Pharbitis nil which are non-vernalization requiring plants. The Southern hybridization analysis of the genomic DNA isolated from the azaC-treated P. frutescens and digested with methylation sensitive restriction enzyme with an rDNA intergenic spacer probe revealed demethylation of the genomic DNA by the azaC treatment. These findings suggest that photoperiodic flowering of these plant species is

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regulated epigenetically as is vernalization. The flowering state and DNA methylation state induced by azaC are not inherited. On the other hand, the dwarfism induced by azaC treatment in \textit{P. frutescens} inherited to the progeny. These results suggest that the expression of the photoperiodic flowering-related genes is regulated by epigenetical manner, but that of the growth-related genes is not. Genomic DNA was extracted from \textit{P. frutescens} plants grown under inductive short-day (SD) and non-inductive long-day (LD) conditions, and analyzed by the MS-AFLP. Many polymorphic bands were detected suggesting that DNA methylation state was modified by the photoperiodic treatment. Some of them were isolated and the DNA fragments were sequenced. Some fragments had CpG island-like regions. One fragment was homologous to rice gene containing Zinc finger domain. The DNA methylation in these regions may regulate the expression of the photoperiodic flowering-related genes in \textit{P. frutescens}.

**P10-049** Multimeric complexes of Gerbera hybrida MADS domain proteins

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The highly compressed inflorescences of the Asteraceae family differ from other model systems in that they bear flowers of dissimilar type, showing differences in sex, morphology and sometimes color. To study floral organ determination and identity in Asteraceae, we performed yeast two- and three-hybrid assays with MADS domain proteins of the ornamental plant \textit{Gerbera hybrida}. Reproductive roles of MADS domain proteins in plants extend from determination of floral organ type to processes such as control of meristem identity and determinacy, inflorescence architecture, and induction or inhibition of flowering. By testing protein-protein interactions, we can generate hypotheses on which complexes are relevant in floral development of Gerbera. We discovered Gerbera MADS domain protein-protein interactions that share characteristics similar to other plants, but Gerbera proteins also possessed features that are different from those in other model species. For example, Gerbera SEP-like proteins have general actors (GRCD4 and GRCD5), which provide an all-purpose E function, and more specialized proteins (GRCD1 and GRCD2), which have their specific roles. Unlike many other B function proteins, the Gerbera proteins recruit other MADS domain proteins into trimerous complexes as single proteins. Based on the interactions, we further speculate which MADS domain proteins participate in higher order protein complexes determining Gerbera carpel and stamen identity.

**P10-050** Role of proline in the reproductive phase of Arabidopsis thaliana

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Flower transition is a fundamental developmental change in plant life and is finely regulated by environmental and internal factors integrated in, at least, four different signaling pathways. We reported recently that proline is involved in the flower transition of Arabidopsis, both under long- and short-day conditions, affecting also other reproductive developmental processes, such as bolting and floral initiation. This finding, largely derived from the study of the ectopic expression of \textit{P5CS1}, and from the analysis of homozygous \textit{p5cs1} mutants, can not distinguish between the relative role of \textit{P5CS1} and \textit{P5CS2}, two closely related genes encoding the rate-limiting enzyme of proline biosynthesis in higher plants. Equally unknown is the flowering pathway proline interacts with, and the role of proline in flower transition and in reproductive development. To fill these gaps, we characterized \textit{p5cs2} Arabidopsis mutants and are currently studying, by in situ hybridization, the expression in Arabidopsis of either \textit{P5CS1} or \textit{P5CS2} genes during the reproductive phase, particularly in the SAM, before and after floral transition, in axillary buds, flowers and fruits. Genetic crosses between \textit{p5cs1} and known flowering time genes are also under way to understand the genetic and molecular relationships between proline and known flowering signaling pathways.

**P10-052** A possible involvement of salicylic acid in the stress-responding flowering

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Phytohormones, like the Bioactive hormone SA, play a crucial role in the stress response. S. Phytohormones play an important role in stress responses, and the Bioactive hormone SA plays a crucial role in the stress response. This study aimed to elucidate the role of SA in stress-induced flowering. We assessed the effects of SA on stress-induced flowering and found that SA significantly inhibited flowering in Arabidopsis. SA alone did not induce flowering under non-stress conditions. The

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**P10-051** Characterization of Gerbera hybrida CYCOIDEA-like gene family

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Gerbera hybrida is a common ornamental plant consisting of morphologically different types of flowers. One of the most prominent differences between the gerbera flower types is the change of flower symmetry across the capitulum. The specific aim of our study is to explore the genetic mechanisms that lead to differences in flower symmetry in various flower types. Studies with \textit{Antirrhinum majus} have shown that flower symmetry is regulated by TCP transcription factor called CYCOIDEA (CYC) that either enhances or represses organ growth. We have recently isolated a small gene family of \textit{CYC}-like genes from Gerbera (\textit{GhCYC}-1-8) and detailed studies with one of them (\textit{GhCYC}2) suggests that this type of transcription factors have also a key role in the flower type differentiation in Asteraceae and take part in defining the complex structure of the Asteraceae inflorescence. Phylogenetic characterization, sequence analysis and expression studies of the \textit{GhCYC} transcription factors suggest a different role for some members of this gene family. We are currently performing yeast two-hybrid screening to identify proteins interacting with \textit{GhCYCs}. Furthermore, we will perform detailed functional analysis of \textit{GhCYC} genes in Gerbera as well as analyse putative target genes regulated by \textit{GhCYCs}. The latest results of these ongoing studies will be presented.
treatment of *P. nil* with a DNA demethylating reagent, together with SA induced flowering, although the flowering response was weaker than that induced by the stress. This stress-responding flowering may be induced by SA, DNA demethylation and some other factor(s). We also found that *Perilla frutescens*, a SDP, can be induced to flower by low-light intensity stress under LD. This flowering was also inhibited by a PAL inhibitor. Thus, the metabolic pathway regulated by PAL, possibly that related to SA, may be involved in the regulation of stress-responding flowering in the both species. But, the effects of stresses on anthocyanine synthesis which is also regulated by PAL were opposite between them. Accordingly, the expressions of PAL and other gene involved in the anthocyanine synthesis were analyzed. PAL expression was suppressed under low-intensity light, and *CHALCONE SYNTHASE* (*CHS*) expression was not changed by light intensity in *P. frutescens*. These results are consistent with the fact that the color of leaves turn to green, but does not support the hypothesis that SA synthesis is promoted to induce flowering under low-intensity light.

**P10-053 MADS complexes regulate pollen maturation in Arabidopsis**

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Differences in the pollen-specific MIKC* class of MADS-domain transcription factors as major regulators of transcriptome dynamics during male reproductive cell development in *Arabidopsis thaliana*. Pollen transcript profiling of mutants deficient in different MIKC* protein complexes revealed that they control a transcriptional switch that directs pollen maturation, and that is essential for pollen competitive ability. We resolved the functional redundancy among the MIKC* proteins, and uncovered part of the underlying network by identifying the non-MIKC* MADS-box genes *AGL18* and *AGL29* as downstream regulators of a subset of the MIKC* MADS-controlled genes.

Our results provide a first, unique and compelling insight into the complexity of a transcription factor network that directs cellular differentiation during pollen maturation, a process that is essential for male reproductive fitness in flowering plants.

**P10-054 Megagametophyte tissue is functioning in pine seed during embryogenesis**

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The pine seed has been proposed to be a model system for studying the eukaryotic programmed cell death (PCD) machinery but plant seed may also function as a model system when considering the role of endogenous DNA damaging agents and environmental stresses on genome integrity. This is because from the early stages of development within the mother plant the seed experiences developmentally programmed as well as environmental stresses. In Scots pine (*Pinus sylvestris*) pollination and fertilization occur in consecutive years and the competing embryos grow and develop within the corrosion cavity of the megagametophyte, a maternally derived haploid tissue, which houses the majority of the storage reserves of the seed. During zygotic embryogenesis only the leading embryo survives while the subordinate embryos as well as the megagametophyte tissue face cell death. In this work, we studied the role of the megagametophyte tissue during the embryogenesis and show that the megagametophyte tissue was exposed to both oxidative stress (upregulation of ROS related genes) and DNA damages at the late embryogeny stage. However, no increase was found in the expression of the PCD related genes and microscopical analyses revealed that cell death occurred only in the specific areas of megagametophyte during embryo development. Thus, the megagametophyte tissue was functioning from the early embryogenesis until the imbibition phase of mature seed germination.

**P10-055 Water status and water distribution in maturing and germinating lupine seeds studied by MR imaging and NMR spectroscopy**

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Magnetic resonance imaging (MRI) was used to study water distribution in maturing and germinating lupine seeds. MRI data showed local inhomogeneities of water distribution inside the maturing seed. At the late seed filling stage the most intense signal was detected in the seed coat and the outer parts of cotyledons in the hilum area but during maturation drying the decline of MR images intensity was faster in the outer part of the seed than in the central part. During 24 h of imbibition, water was unevenly distributed within the seed, and some anatomical parts were more hydrated than others. Water entered the seed through the hilum and microyle. The embryonic axis was the first to show hydration followed by seed coat and later cotyledons. Changes in water status were characterized by NMR spectroscopy. T2 relaxation times revealed three-component water proton system in maturing lupine seeds. Three populations of protons, each with different magnetic environment causing a different relaxation rate, found during seed maturation were correlated with three fractions of water (structural, intracellular and extracellular) observed during seed germination. This study provide evidence that lupine seeds have similar state of different water components with regard to seed moisture content in two distinct physiological stages as seed maturation and germination. The unique feature of maturing lupine seeds is the presence of high 1H-NMR signal in areas corresponding to vascular bundles.

**P10-056 GNOM-mediated embryogenesis**

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Magnetic resonance imaging (MRI) was used to study water distribution in maturing and germinating lupine seeds. MRI data showed local inhomogeneities of water distribution inside the maturing seed. At the late seed filling stage the most intense signal was detected in the seed coat and the outer parts of cotyledons in the hilum area but during maturation drying the decline of MR images intensity was faster in the outer part of the seed than in the central part. During 24 h of imbibition, water was unevenly distributed within the seed, and some anatomical parts were more hydrated than others. Water entered the seed through the hilum and microyle. The embryonic axis was the first to show hydration followed by seed coat and later cotyledons. Changes in water status were characterized by NMR spectroscopy. T2 relaxation times revealed three-component water proton system in maturing lupine seeds. Three populations of protons, each with different magnetic environment causing a different relaxation rate, found during seed maturation were correlated with three fractions of water (structural, intracellular and extracellular) observed during seed germination. This study provide evidence that lupine seeds have similar state of different water components with regard to seed moisture content in two distinct physiological stages as seed maturation and germination. The unique feature of maturing lupine seeds is the presence of high 1H-NMR signal in areas corresponding to vascular bundles.
The GNOM gene is required for patterning of the Arabidopsis embryo. To identify tissue layers important for GNOM-mediated patterning of the embryo, we analysed different tissue-specific GNOM expressions in a gnom mutant background for their ability to complement the diverse gnom defects. Here we show that vascular GNOM expression rescues the apical-basal axis defect reorganizing the radial tissue layers and establishing the primary root. GNOM vascular expression is also needed to induce normal growth response along the apical-basal axis. Apical GNOM expression in the epidermis complements the apical gnom defects, namely cotyledon initiation and outgrowth reconstituting bilateral symmetry. GNOM epidermal and vascular tissue expression are also shown to reestablish epidermal and vascular polar auxin transport in the embryo, linking the organ initiation and outgrowth defects to auxin. Establishment of the root stem cell niche and radial patterning are linked, but independent radial patterning processes may also exist. In summary, primary defects in gnom, like the misspecified root stem cell niche and the radial-to-bilateral symmetry transformation defect, can be directly linked to disrupted polar auxin transport, while radial organization defects and apical fusion seem to be secondary consequences from the misspecified root pole and auxin accumulation in apical regions, respectively.

P10-057 Functional characterization of carpel development genes in E. californica through stable transformation and Virus-induced gene silencing

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Although the genetic architecture of flower development has been extensively studied in Arabidopsis, there is a need for basal eudicot species to learn more about the molecular evolution of flower development and its implication on other plant development processes. The papaveraceae member Eschscholzia californica is found to be an expedient model species because of its phylogenetic position and its amenability for stable genetic transformation. We are focusing our research on evolutionarily most important innovation of the angiosperms, ‘the carpel’ and using a candidate gene approach to analyze some of the key developmental genes. Functional characterization of the genes via Agrobacterium mediated genetic transformation proved to be the best, albeit time-consuming method. However, transient knock-down of gene expression through Virus Induced Gene Silencing (VIGS) is found to be rapid and reliable method. We have obtained robust phenotypes when EscaAG1 and EscaAG2, the Arabidopsis orthologs of AGAMOUS were silenced in poppy. Agamous plays strong role in floral meristem determinacy in Arabidopsis, in contrary to this both poppy Agamous genes are playing very smaller role in floral meristem determinacy. On the other hand, both Agamous paralogues are playing similar expression pattern in determining stamen and carpel identity, however, EcAG1 is exhibiting stronger phenotype than EcAG2.

P10-058 Plasmalemma H+-pumping ATPase in germinating petunia pollen grains is sensitive to exogenous IAA and ABA

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At present, there is known that pollen grains contain classical phytohormones and their in vitro germination is accompanied by changes in the levels of endogenous phytohormones and sensitive to exogenous phytohormones (Kovaleva et al. 2005). In addition, there are reports indicating a fundamental role of the intracellular proton gradient in driving tip growth in growing pollen tubes. Therefore, it is possible that transmembrane ion fluxes in germinating pollen grains are under the control of phytohormones. In the present work, we have found that in germinating petunia pollen grains exogenous phytohormones, such as IAA and ABA, bring about pronounced plasma membrane hyperpolarization of their cells monitored by carboxyfluorescein dye DIS-C3-(S). This hormone-induced increase in the membrane potential has been found to be completely abolished in the presence of orthovanadate, the known inhibitor of plasmalemma P-type H+-ATPase, suggesting an involvement of this H+-pump in the observed pollen grain response. This finding is in accordance with our previous results indicating that the same phytohormones exert orthovanadate-sensitive intracellular alkalization in germinating petunia pollen grains under the same experimental conditions (Andreev et al. 2007). In summary, the results obtained provide evidence that the IAA- and ABA-induced effects on germinating petunia pollen grains are due to activation by the hormones of plasmalemma H+-pumping ATPase. The work was supported by RFBR.
suggesting evolution of their roles in the plant. TDNA-insertion mutants support the conjecture that they may be involved in floral development: flowers without one of the rhomboids exhibit various aberrant features. We report here a summary of our findings.

The ureides, allantoin and allantoate, are the main nitrogen compounds produced through biological nitrogen fixation that are exported in tropical legumes. Ureides are produced through the oxidation of purines synthesized in root nodules and as a salvage pathway to remodelize nitrogen compounds in senescent tissues. Ureides accumulation in several tissues has been proposed to cause feedback inhibition of nitrogen fixation under drought stress. Despite the key role of ureides in nitrogen storage and translocation, the metabolic pathways of their synthesis and degradation have been only poorly characterized in plants. In particular there is scarce information about the genes coding for the enzymes involved in ureide degradation. Two possible branches have been proposed for allantoate degradation in nitrogen fixing plants and several reports have suggested a relationship among the enzymes of allantoate degradation and nitrogen fixation sensitivity to drought stress. We have recently identified and cloned the genes coding for the enzymes allantoate amidohydrolase and ureidoglycolate amidohydrolase from Phaseolus vulgaris. Gene expression analysis and immunological detection of the encoded proteins in plants growing under control or drought stress conditions have been used to study the regulation of ureide metabolism and its possible relationship to drought sensitivity or tolerance of nitrogen fixation in tropical legumes. Work supported by projects BIO2006-09366 and AGR01283.

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P10-060 A MATE protein involved in flavonoid transport in Arabidopsis flowers
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The biosynthesis of ASN is catalyzed by the enzyme asparagine synthetase (AS) (EC 6.3.5.4). AS protein is encoded by a small gene family in most plants. Two AS genes were identified in soybean (SAS1 and SAS2). In a recent study of the expression of AS genes in soybean plants under nitrogen stress, we cloned a class-II AS gene from soybean (SAS3). Primers were designed from an incomplete cDNA sequence and used in RACE-PCR. A 2059 bp cDNA clone of SAS3 encodes a protein of 569 amino acids with a predicted molecular weight 64.274 kDa, an isoelectric point of 6.3 and a net charge of -7.1 at pH 7.0. The SAS3 protein sequence conserves all the amino acid residues that are essential for glutamine-dependent AS. The identity of SAS3 was demonstrated by complementation of an Escherichia coli AS-deficient mutant. Northern blot analysis of SAS3 expression revealed that this gene is expressed in roots of non-nodulated soybean plants cultivated on nitrate and also in nodules of soybean plants, where symbiotic nitrogen fixation was the exclusive source of N, but showed a very weak expression in roots of nodulated plants. This expression pattern resembles that of other two AS genes in soybean. Under conditions that impair nitrogen fixation, the expression of SAS2 and SAS3 in nodule remained unaltered, while SAS1 expression was reduced. Due to the relevance of ASN in soybean metabolism, analyses of AS expression under various environmental stresses are in progress.

P10-011 Drought stress effects on ureide metabolisms in Phaseolus vulgaris
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The biosynthesis of ASN in soybean is catalyzed by the enzyme asparagine synthetase (AS) (EC 6.3.5.4). AS protein is encoded by a small gene family in most plants. Two AS genes were identified in soybean (SAS1 and SAS2). In a recent study of the expression of AS genes in soybean plants under nitrogen stress, we cloned a class-II AS gene from soybean (SAS3). Primers were designed from an incomplete cDNA sequence and used in RACE-PCR. A 2059 bp cDNA clone of SAS3 encodes a protein of 569 amino acids with a predicted molecular weight 64.274 kDa, an isoelectric point of 6.3 and a net charge of -7.1 at pH 7.0. The SAS3 protein sequence conserves all the amino acid residues that are essential for glutamine-dependent AS. The identity of SAS3 was demonstrated by complementation of an Escherichia coli AS-deficient mutant. Northern blot analysis of SAS3 expression revealed that this gene is expressed in roots of non-nodulated soybean plants cultivated on nitrate and also in nodules of soybean plants, where symbiotic nitrogen fixation was the exclusive source of N, but showed a very weak expression in roots of nodulated plants. This expression pattern resembles that of other two AS genes in soybean. Under conditions that impair nitrogen fixation, the expression of SAS2 and SAS3 in nodule remained unaltered, while SAS1 expression was reduced. Due to the relevance of ASN in soybean metabolism, analyses of AS expression under various environmental stresses are in progress.

P10-012 Cloning, molecular characterization and expression analysis of a gene coding for a class-II asparagine synthetase in soybean (Glycine max)
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The biosynthesis of ASN is catalyzed by the enzyme asparagine synthetase (AS) (EC 6.3.5.4). AS protein is encoded by a small gene family in most plants. Two AS genes were identified in soybean (SAS1 and SAS2). In a recent study of the expression of AS genes in soybean plants under nitrogen stress, we cloned a class-II AS gene from soybean (SAS3). Primers were designed from an incomplete cDNA sequence and used in RACE-PCR. A 2059 bp cDNA clone of SAS3 encodes a protein of 569 amino acids with a predicted molecular weight 64.274 kDa, an isoelectric point of 6.3 and a net charge of -7.1 at pH 7.0. The SAS3 protein sequence conserves all the amino acid residues that are essential for glutamine-dependent AS. The identity of SAS3 was demonstrated by complementation of an Escherichia coli AS-deficient mutant. Northern blot analysis of SAS3 expression revealed that this gene is expressed in roots of non-nodulated soybean plants cultivated on nitrate and also in nodules of soybean plants, where symbiotic nitrogen fixation was the exclusive source of N, but showed a very weak expression in roots of nodulated plants. This expression pattern resembles that of other two AS genes in soybean. Under conditions that impair nitrogen fixation, the expression of SAS2 and SAS3 in nodule remained unaltered, while SAS1 expression was reduced. Due to the relevance of ASN in soybean metabolism, analyses of AS expression under various environmental stresses are in progress.

P10-013 Flavonoid metabolism in Brassy Napus seed
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Data about molecular and biochemical events during seed coat development in Brassica napus are scarce although the testa contains oxidized dark pigments (proanthocyanidins, PAs) that impaired...
To clone rapeseed flavonoid genes, a candidate gene approach was based on Arabidopsis transparent testa (TT) genes. Four transcription factors (TT2, TT8, TT16 and TT29), two enzymes (BAN and TT7) and a vacuolar transporter (TT12) were recovered using Brassica genomic resources. Rapeseed cDNAs successfully restored wild-type phenotype of corresponding Arabidopsis tt mutants. Expression profiles were monitored in B. napus: BAN and TT2 were of interest because they were seed specific. Analysis of promoter activation pattern revealed that pBnTT2 and pBnBAN were restricted to the testa.

**P11-014 Post-photosynthetic fate of assimilates and plant productivity**

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Post-photosynthetic metabolism of 14C-photosynthate during seed development was studied. Response of photosynthetic metabolism was based on Arabidopsis 5-methylthioadenosine (MTA) is released which is subsequently converted to S-adenosylmethionine (SAM) serves as substrate for the synthesis of ethylene, polyamines and phytosiderophores. As by-product of ethylene, polyamines and phytosiderophores. As a by-product 5-methylthioadenosine (MTA) is released which is subsequently recycled to Met via the Met cycle, also known as Yang cycle in plants. Met cycle knock out mutants in Arabidopsis showed phenotypic differences depending on the reaction at which the Met cycle was interrupted. The Arabidopsis mtn mutant in which conversion of MTA to methylthioribose is interrupted showed delayed flowering development and infertility. In contrast, knock out of methylthioribose kinase which catalyzes the subsequent reaction did not affect plant development. These different mutant phenotypes indicate that MTA catabolism rather than Met recycling are crucial for normal plant development. In mtn seedlings grown on MTA as sulfur source MTA and SAM accumulated and growth was retarded. Growth inhibition was partially reverted by addition of the polyamines spermidine and spermine. We hypothesize that MTA inhibits polyamine biosynthesis and that reduced polyamine levels are causal for growth inhibition.

**P11-015 The Yang cycle: beyond Met recycling**

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S-adenosylmethionine (SAM) serves as substrate for the synthesis of ethylene, polyamines and phytosiderophores. As by-product 5-methylthioadenosine (MTA) is released which is subsequently recycled to Met via the Met cycle, also known as Yang cycle in

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**P11-016 Effects of the overexpression of glutamine synthetase in root nodules of Medicago truncatula on plant growth and nitrogen use efficiency**

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Glutamine synthetase (GS) is a vital enzyme for the assimilation of ammonia into amino acids in higher plants and the enhancement of its activity has the potential to increase nitrogen utilization efficiency. In legume root nodules, GS is responsible for the assimilation of the ammonium released by symbiotic nitrogen fixation.

To investigate how nodule GS activity affects plant performance, we have previously overexpressed GS1a cDNA specifically in root nodules of Medicago truncatula under the direction of a native leghemoglobin promoter. In this study we have used these plants to examine the effects of increased nodule GS activity on phenotypic development, biomass production, rhizobial nitrogen fixation and plant nitrogen use efficiency, and have used the tools of transcriptomics and metabolomics to identify the major transcript and metabolite changes associated with the altered nodule metabolism. Overall, the results indicate that M. truncatula overexpressing GS display enhanced growth phenotype as quantified by increases in biomass and seed production. Nodule GS activity was positively correlated with symbiotic nitrogen fixation activity and with plant nitrogen utilization efficiency. Our results provide further support to the notion that it may be possible to increase nitrogen use efficiency by manipulation of specific GS isoforms in transgenic crop plants. This work is supported by projects POCI/AGG/39079/2001 and FOOD-CT-2004-506223.

**P11-017 Structural features of glutamine synthetase from Medicago truncatula**

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In depth understanding of the molecular details of glutamine synthetase (GS) activity in plants is of crucial importance, given the

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enzyme’s key role in nitrogen metabolism. Although a significant effort has been devoted to understanding the mechanisms controlling GS activity in many different plant species, the structural features of plant GS have been mostly inferred from crystallographic models of the better studied bacterial enzymes. In order to unambiguously elucidate the three dimensional structure of plant GS, we decided to determine the crystallographic structure of the cytosolic GS isoform GS1a from the model legume Medicago truncatula. The fully active, N-terminal His-tagged recombinant enzyme was purified to homogeneity by immobilized metal-affinity and size exclusion chromatography. Single crystals suitable for X-ray crystallography were obtained by vapour diffusion techniques. The crystals belong to space group P21 with cell dimensions a = 99Å, b = 102Å, c = 188Å, β = 104°.

Analysis of the self-rotation function for the diffraction data, as well as single-particle reconstruction from electron microscopy micrographs of GS1a, revealed that the enzyme is a decameric protein composed by two superposed homopentameric rings, and in clear contrast with the generally accepted octameric architecture of the complex. Crystallographic structure refinement is currently underway. This work is supported by project POCI/AGR/61025/2004.

P11-018 Novel homodimeric geranyl diphosphate synthase from the orchid Phalaenopsis bellina lacking a DD(X)2-4D motif


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The cDNA of Phalaenopsis bellina GDP synthase (PbGDPS) was cloned, and its sequence corresponds to the second Asp-rich motif (SARM) but no any aspartate-rich (Asp-rich). The recombinant PbGDPS enzyme possessed a dual prenyltransferase activity, producing both GDP and farnesyl diphosphate (FDP), and a yeast two-hybrid assay and gel filtration revealed that PbGDPS was able to form a homodimer. Spatial and temporal expression analyses showed that the expression of PbGDPS was flower specific and to form a homodimer. Spatial and temporal expression analyses showed that the expression of PbGDPS was flower specific and able to form a homodimer. PbGDPS, which is more closely related to the GDPS-c clade proteins than to GDPS-a and GDPS-b proteins, and is currently the sole member of the GDPS-d clade, functions as homodimer.

P11-019 The myo-inositol oxygenase gene family in Arabidopsis thaliana – more than a bypass in cell wall biosynthesis?

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UDP-glucuronic acid, the key metabolite in the production of nucleotide sugars, is produced in the first reaction that commits a sugar to the build up of cell wall polymers and glycoproteins. It can be produced via two independent pathways, the nucleotide sugar oxidation pathway and the myo-inositol oxygenation pathway. The former is performed by the UDP-glucose-dehydrogenase (UGD) with concomitant reduction of NAD+; the latter is initiated by the enzyme myo-inositol oxygenase with molecular oxygen as a co-substrate (MIOX; Loewus et al. 1962). This unique monooxygenase reaction is performed employing an elaborate non-heme iron center. The UGD isoforms are predominantly expressed in growing tissue, while the four MIOX isoforms are mainly restricted to early developmental stages and proliferation (Kanter et al. 2005). Knockouts of the isoforms show a clear reduction of MIOX activity, but do not exhibit a phenotype under standard conditions when compared to the wildtype. Besides the role in cell wall build-up, a correlation is discussed between MIOX overexpression and an increase in ascorbic acid content (Lorence et al. 2004).


P11-020 â-Glucosidase of pea plants, intracellular localization, properties and regulation

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â-Glucosidase (EC 3.2.1.21) of pea participating in catabolism of specific isouccinimide-â-glucoside (IS-glucoside) was studied. It was extracted from different cell fractions of seedlings and defined by glucose. The properties were studied on high-purity preparations. It was shown that â-glucosidase of pea is represented by cytoplasmic Glu-I and cell-wall bound adsorbed Glu-II, taken by 0.1 M buffer, ion bounded Glu-III-1 M NaCl and covalent bounded Glu-IV molecular forms. They were differed in pH, optimum temperature, thermo stability and metal effect. All Glu forms showed high specificity to type of glucosidic bond and did not decompose raffinose, maltose, lactose, but were effective to cellobiose, IS- and methyl-â-glucosides. Km for IS-glucoside was 0.87 mM for Glu-I, 0.35 mM for Glu-II and 0.92 mM for Glu-III. Glu was not detected in seeds. Specific activity of Glu-II in 5-day old seedlings achieved 10.6 U, 18.5 U in Glu-III-1 M NaCl and 60 U in Glu-IV on mg of protein and exceeded Glu-I. It was rising 1.5 to 2-fold until 10th day than falling, it proved...
an importance of all Glu for germination. Hypoxic stress and CO₂-media affect on cytoplasmic and cell wall-bound molecular Glu forms was proved. Short hypoxic stress (6 h) of all factors increased Glu-I activity to 50%, Glu-II to 400%, Glu-III to 510%, Glu-IV to 10–30% in seedlings. At once Km and Vmax decreased especially in CO₂-media. Obtained results extend view on properties of â-glucosidases, their role in growth and under stress (hypoxia).

P11-021 Analysis of the cytosolic glycan metabolism: photosynthesis-dependent carbon fluxes into and across the glycans analyzed via Arabidopsis protoplasts

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Water soluble heteroglycans (SHG) were isolated from leaves of Arabidopsis, spinach, potato and pea as well as from potato tubers. They consist mainly of galactose, arabinose and glucose. SHG comprised both cytosolic and apoplastic glycans. The linkage pattern is very complex and revealed a high degree of branching. The interaction of the SHG with the cytosolic phosphorylase (ATPS 2/Pho 2) and the glucanotransferase (DPE2) together with results of various mutants with altered starch metabolism indicate an important role of the glycans within the plant carbohydrate metabolism, especially in the conversion of starch-derived maltose via glucose 1-phosphat into sucrose metabolism. To clearly distinguish between the cytosolic, apoplastic and starch-related processes a protoplast-based procedure was established. The investigation of the fluxes was extended by using protoplasts from various Arabidopsis mutants related to starch or nucleotide-sugar metabolism. The protoplasts were incubated with various labeled or unlabeled sugars or HCO₃⁻ in the light. Additionally pulse-chase experiments were done in which protoplasts were labeled in the light and then transferred to the dark. SHG were separated by field flow fractionation and analyzed after hydrolysis with HPAEC-PAD. The data clearly show separate fluxes into the different polymers as well as different fluxes into the various monomers of the SHG.

P11-022 Role of benzoxazinones in the activation of maize cytokinin dehydrogenase

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Cyclic hydroxamic acids 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-glc) and their degradation product 6-methoxybenzoxazolin-2-one (MBOA) were isolated from maize phloem sap as compounds stimulating degradation of cytokinin isopentenyl adenine by maize cytokinin dehydrogenase (CKX; EC 1.5.99.12) after their conversion by polyphenol oxidases or peroxidase. The resulting oxidation products function as electron acceptors in the catalytic reaction of CKX. The products of DIMBOA and DIMBOA-glc reaction with laccase and peroxidase were analyzed by UV/VIS spectrophotometry, high performance liquid chromatography and mass spectrometry. Their structure was determined using quadrupole time-of-flight (Q-TOF) mass spectrometer. Concentration of DIMBOA and DIMBOA-glc increased in phloem sap of seedlings grown in the presence of cytokinin compared to control seedlings. Cytokinin also induced CKX activity and the synthesis of total phenolic compounds in maize shoots. The data indicate a new function for DIMBOA in the metabolism of plant hormones cytokinins.

P11-023 cis-zeatin type cytokinins are biologically active and differ in their metabolism from trans-isomers

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Cytokinins (CKs) affect many indispensable processes in plants. Substantial group of natural CKs is represented by zeatins, occurring in two positional isomers, and their sugar conjugates. Trans-zeatin (tZ) was found to be bioactive unlike cis-zeatin (cZ) that has been reported to show very weak biological effects so far. However, certain plants such as oat, tobacco and maize contain predominantly cZ and its derivates.

We report that cZ as well as its 9-riboside and O-glucoside displayed biological effects on retention of chlorophyll in excised oat and maize leaves, though in higher concentrations than their tZ counterparts. No significant biological response was observed after cZ-9-glucoside treatment. Endogenous CK level was severely altered by tZ treatment in oat leaf segments incubated for 4 days in darkness but only slightly by the use of cZ. Application of radiolabelled cZ and tZ to oat leaf apices revealed similar uptake and different metabolic fate of both isomers. While [3H]tZ was rapidly O-glucosylated and then degraded by cytokinin oxidase/dehydrogenase (CKX) activity, [3H]cZ was firstly degraded by CKX and then N-glucosylated.

Overall, the results show distinct biological activities and metabolism of cZ and its conjugates compared to tZ thus indicating potential unique physiological role of cZ-type CKs in plants.

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P11-024 A protease activity co-purified with the C4 PEPC from sorghum leaves is specifically activated by a synthetic peptide from its C-terminal end

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Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) catalyses the irreversible β-carboxylation of phosphoenolpyruvate in the presence of HCO₃⁻ and a divalent cation, to yield oxaloacetate and Pi (Chollet et al. 1997). PEPC plays an essential role in the photosynthetic carbon metabolism of C4 and crassulacean acid metabolism (CAM) plants and is subject to a diel posttranslational regulation that alters its functional and regulatory properties (Chollet et al., 1997; Vidal and Echevarría 2003). This is performed by the phosphoenolpyruvate carboxylase kinase which phosphorylates the Ser located in the N-terminal domain of PEPC (Chollet et al., 1997; Vidal and Echevarría 2003). We have previously reported that a synthetic

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peptide containing the last 19 amino acids from the C-terminal end of sorghum leaves C4 PEPC (peptide C-19), specifically inhibits the in vitro phosphorylation of the enzyme by the PEPC-k (Alvarez et al. 2003). Now, we report a PEPC-protease activity from sorghum leaves that co-purifies with PEPC and is activated by preincubation with peptide C-19. Changes in the conformational state of PEPC promoted by the presence of glucose-6-P, by phosphorylation or the presence of anti-phosphorylation site antibodies (Pacquit et al. 1995) strongly reduce the proteolytic activity. This result shows by the first time that the proteolysis of PEPC could be regulated by its C-terminal end.


P11-025 Sugar signaling and redox-regulation of carbon storage in plants
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Starch is the major carbon store in plants and ADPGlc pyrophosphorylase (AGPase) is catalyzing the rate-limiting step in the pathway of starch synthesis in the plastid. In this presentation, I shall discuss recent findings showing AGPase to be subject to a novel redox-based post-translational regulation mechanism, which allows the rate of starch synthesis to be increased in response to external inputs and independently of changes in metabolite levels (Tiessen et al. Plant Cell 14: 2191–2213; Hendriks et al. Plant Physiol 133: 838–849). AGPase is rapidly redox-activated upon illumination by reduction of an intermolecular disulfide-bond between cysteines on the two small subunits of the tetrameric enzyme. This resembles the light-activation of enzymes of the Calvin cycle, where electrons are transferred from PS I to thioredoxins, which activate target enzymes by reduction of regulatory disulfides. Redox-activation of AGPase is also promoted by sugars, which act additively with light, and also on their own in darkened leaves and in non-photosynthetic tissues. Recent studies identified Snf1 related protein kinase (Tiessen et al. Plant J 35: 490–500) and trehalose-6-phosphate (Kolbe et al. PNAS 102: 11118–11123; Lund et al. Biochem J 397: 139–148) as signaling components linking redox-activation of AGPase to the availability of sugars in the cytosol. Work is in progress to elucidate these signalling pathways.

P11-026 Evidence for widespread redox regulation of starch metabolizing enzymes in Arabidopsis thaliana
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In addition to serving important structural and catalytic functions, disulfide bonds also play a role in the regulation of many enzymatic activities. This regulation occurs through the breaking and reformation of disulfide bonds in response to the redox state and has been described for many enzymes. In the chloroplast, reducing equivalents produced during photosynthesis is transferred via ferredoxin to the thioredoxins which are responsible for reducing target proteins. This system links enzyme activity to light, ensuring coordination between photosynthesis and metabolism by reductive activation of enzymes during the day. A number of redox regulated enzymes active in starch metabolism have been identified in Arabidopsis. These include two enzymes involved in starch breakdown in the dark, the beta-amylase BAM1 and the starch phosphorylating GW2, both of which are reductively activated in vitro. This apparent contradiction poses an interesting question about the precise function of these enzymes in vivo and the role of redox regulation in starch metabolism in general. We have investigated the activity of enzymes involved in metabolism of starch in Arabidopsis by manipulation of redox potentials in plant extracts and characterization of enzyme activities using native gels. Our data confirm that the chloroplastic beta-amylase BAM1 and debranching enzyme LDA are reductively activated and suggest that redox regulation is more widespread among enzymes of starch metabolism.

P11-027 Metabolic profiling of ‘Conference’ pears under oxygen stress
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Pear (Pyrus communis L. cv Conference) may be subjected to core breakdown when stored under low oxygen or elevated carbon dioxide conditions. This physiological disorder is characterized by the development of brown spots due to oxidation of phenolic compounds and, eventually, cavities in the center of the fruit. Based on metabolic profiling of brown and sound tissue using GC-EL-TOF-MS, the hypothesis that this disorder is due to an imbalance between oxidative and reductive processes at the cellular level was investigated. Brown tissue was clearly characterized by a distinctive pattern of changes which included decrease of malic and succinic acid and an increase of fumaric acid and GABA, which indicated a reduced metabolic activity at the level of the Krebs cycle and a putative block of the GABA shunt pathway.

P11-028 ABA metabolites profiling in plant tissues using UPLC/MS/MS
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The identification and quantification of abscisic acid and its metabolites in plant tissue is necessary for physiological studies of their metabolism and mode of action. Analysis of the endogenous ABA levels is extremely difficult because of its instability and the low concentration of the hormone in plants, which are generally in the ng/g fresh weight range, although concentrations can increase several-fold in mature seeds and stressed plants (Walton and Li 1995). A wide range of methods are currently employed for the quantification of ABA in plants. These include GC/MS, high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS), as well as direct methods (ELISA). Using
corrhizal interaction, while the fungi were not able to enhance the expression of endogenous Hb genes in the lines expressing bacterial haemoglobin gene of *Vitreoscilla* (vhb). We hypothesized that class-1 and truncated Hbs of hybrid aspen separately or in concert modulated NO levels in early phases of root growth and that VHb may compensate the function of endogenous Hbs. In the present work, we have constructed protein models for hybrid aspen PttHb1 and PttTrHb based on previously determined X-ray structures in order to study the ligand binding of these proteins. To unravel the functioning of plant non-symbiotic and truncated Hb genes we are also utilizing yeast (*Saccharomyces cerevisiae*) complementation tests. The yeast genome encodes a flavohemoglobin gene (*YHBI*) that has been demonstrated to be essential for survival when the cells are under nitrosative stress. We are now expressing PttHb1 and PttTrHb in the yeast *yhb1* deletion strain as cytosolic and mitochondrially targeted variants and testing for their ability to complement the nitric oxide sensitivity of the yeast strain.

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**P11-031** Fermentative induction is not mediated by pyruvate accumulation in pea roots

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To date, it is not clear what signal triggers the induction of fermentation. Several candidates have been suggested: oxygen concentration, pyruvate content, redox status and energy charge. In order to check if pyruvate was the key stimulus related to fermentative induction, it was evaluated the effect on fermentation of supplying exogenous pyruvate and it was compared to hypoxic roots. 12-day old pea plants grown in hydroponic tanks were divided in four treatments. While control treatment (C) was maintained continuously aerated, hypoxia treatment (H) was initiated by stopping air-bubbling. 8 mM pyruvate was added to the nutrient solution to a half of the control (P-C) and to a half of hypoxic plants (P-H). Root pyruvate content and fermentative and alanine aminotransferase enzymatic activities were studied during 24 h. Root pyruvate content did not differ between C, H and P-C plants. When pyruvate was fed to roots that were growing in a nutrient solution, only in non-aerated (P-H) pyruvate accumulated heavily. Indeed, after 3 h from the onset of the treatment the pyruvate content of P-H roots was 10 times C values, indicating that pyruvate could be taken by the plants. Lactate dehydrogenase and alanine amino transferase were not affected by any of the treatments. Ethanolic fermentation was induced only within 18 h of H. Fermentative induction could not be detected in P-H roots, refuting the hypothesis that fermentative induction is regulated by pyruvate concentration.

**P11-032** Metabolite profiling of ozone induced phenolic compounds in *Nicotiana tabacum* using LC-ESI-MS/MS

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Metabolite profiling of ozone induced soluble phenolic compounds in leaves of *Nicotiana tabacum* L. cv BelW3 was carried out by UPLC combined with tandem mass spectrometric (MS/MS) analysis, a 10-fold increase in throughput was obtained compared to the traditional LC/MS system. We describe a simple, reliable and rapid method of extracting and partially purifying the phytohormone ABA and its metabolites in plant tissues by fast chromatography separation-tandem mass spectrometry (UPLC/MS/MS).

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**P11-029** Redox control of ADP-glucose pyrophosphorylase in *Arabidopsis thaliana*

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ADP-glucose pyrophosphorylase (AGPase), which catalyzes the first committed step in the pathway of starch synthesis, is redox-dependent activated. To investigate the mechanism and physiological significance of the redox regulation, constructs were made to individually alter the five cysteine residues of the mature AGPase small subunit protein (APS1) to serine. These were expressed under aP11-030 Hybrid aspen haemoglobins, protein models and functional studies


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We have recently cloned and characterized the coding sequences of class-1 non-symbiotic (PttHb1) and truncated (PttTrHb) haemoglobin genes of hybrid aspen (*Populus tremula x tremuloides*). The expression studies showed that these genes are up-regulated in the roots of non-transgenic hybrid aspen lines during ectomy-
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LC-UV-ESI-MS. Twenty five metabolites have been identified that are induced in leaf samples after exposure to a high level ozone pulse (5 h, 160 ppb) compared to controls. Most metabolites accumulated in the analyzed time frame of 10 and 30 h. As ozone is known to induce the plant pathogen response, we compared the metabolite induction pattern of ozone treated leaves with those that have been treated with Pseudomonas syringae pv syringae (Pss). All ozone induced metabolites were induced in Pss treated leaves too, indicating that also the induction of aromatic secondary metabolism resembles that after pathogen attack. In a further attempt, we have identified many of the induced metabolites by LC-ESI-MS/MS fragmentation measurement. Among the induced and accumulated metabolites several hydroxycinnamoyl quinic acids and 3-p-coumaroylshikimic acid (3CoShiA) have been identified, which are discussed as lignin biosynthesis intermediates. Surprisingly there are only very few metabolite studies, which report the appearance of 3CoShiA in leaf samples of higher plants. Therefore we analysed and correlated the expression of the according synthesizing enzymes HCT and CCoAMT. Derived from our data, we discuss the induction of the identified substances being related to the cross induced plant pathogen defense rather than the ROS detoxifying system.

P11-033 Isolation of two cDNAs encoding polyketide synthases from Hypericum perforatum
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Hypericins and hyperforins are biologically active compounds of medicinal plant Hypericum perforatum L. (St. John’s wort) that is widely used for treatment of depression. It is presumed that both hypericins and hyperforins are biosynthesized via a polyketide pathway by type III plant-specific polyketide synthases (PKSs). In our studies, two previously uncharacterized cDNAs encoding for PKSs, HpPKS1 and HpPKS2, has been isolated from H. perforatum. Phylogenetic tree analysis revealed that both HpPKS1 and HpPKS2 group with other non-chalcone producing type III PKSs but they are not closely related to any of the known PKSs. HpPKS1 and HpPKS2 were found to exhibit distinct tissue-specific expression patterns in H. perforatum as analyzed with real-time PCR. The HpPKS1 was most highly expressed in flower buds and to a lower extent in stems, leaf interior parts and leaf margins. Only a weak expression of HpPKS2 was detected in roots. The expression of HpPKS2 was high in flower buds and leaf margins and low in leaf interior parts, stems and roots. The expression of the HpPKS1 correlated with the concentrations of hypericins while the expression of HpPKS2 showed correlation with the concentrations of hyperforins in H. perforatum tissues. Our results show that these PKS encoding genes are good candidate genes responsible for the biosynthesis of hypericins and hyperforins in H. perforatum.

P11-035 Pecularity of hormonal and sugar regulation of α-amylase isoenzymes in embryo and aleurone of cereal grains
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In cereal grains α-amylase carries out a key role in the starch mobilization during germination. Enzyme synthesis localized in two parts of grain, they are aleurone and scutellum, and then begin the process of its secretion to endosperm. The α-amylase synthesis induced with phytohormone GA3, another hormone ABA carries out the opposite activity on this process. In sprouting cereal grains there are more then ten individual α-amylase isoenzymes, which divided on two basic groups according their isoelectric points and some other biochemical properties and physiological destination. In the given work it was investigated the influence of GA3, ABA and sugars on the induction synthesis of different α-amylase isoazines in aleurone and scutellum of cereal grains. Summarising the above-stated and our research data it is possible to conclude that in cereal grains the synthesis of α-amylase isoform with low isoelectricpoints (group A) is strictly adjusted by endohormones level (GA3 and ABA). The mechanism of an high isoelectricpoints (group C) possible is more combined and can include double control – phytohormones and sugarmetabolites action.

P11-034 NAD metabolism and synthesis of nicotinic acid conjugates in potato plants (Solanum tuberosum)
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Pyridine nucleotides, NAD and NADP, are important cofactors in several redox reactions in organisms. Nevertheless, little is known about the biosynthesis and degradation of pyridine metabolism in plants. In the present study, we examined the general metabolic profiles of pyridine nucleotides in leaves and tubers of growing potato plants by in situ tracer experiments using 3H- and 14C-labeled compounds and by the in vitro enzyme assay. In both leaves and tubers, [3H]quinolinic acid, an intermediate of de novo pyridine nucleotide synthesis, and [14C]nicotinamide and [14C]nicotinic acid, degradation products of NAD, were converted to pyridine nucleotides. The results suggest that the pyridine nucleotide is synthesized by de novo and salvage pathways and that the pyridine nucleotide cycle operates in potato plants. Degradation of the pyridine ring was hardly observed during the experimental period for 20 h. In leaves and stems, nicotinic acid was converted to trigonelline and nicotinic acid-glucoside. In contrast, only nicotinic acid-glucoside was synthesized from nicotinic acid in tubers and stolons. These differences seem to be related to the distribution of the methyltransferase and glucosyltransferase in each organ. The results indicated that trigonelline synthesis is specific to above ground parts of potato plants. The function of trigonelline and nicotinic acid-glucoside in potato plants will be discussed.

P11-036 Utilization of 14C-glucose by flax leaves of different ages
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It is known that sucrose can be hydrolyzed into fructose and glucose in the plant apoplast. The hexoses can be transported with the transpiration water stream into different tissues but their further destiny is unknown. That is why we studied metabolism of exogenous apoplastic $\text{^{14}C}$-glucose in flax shoots in the light and in the shade. $\text{^{14}C}$-glucose was fed through the transpiration water stream for 30 min, 1 h and 2 h. The height of flax shoots was 50–60 cm. The distribution of $\text{^{14}C}$ among labeled substances in juvenile and mature flax leaves was analyzed using paper chromatography and autoradiography. Influx of labeled glucose into the shoot depended on transpiration intensity and time intervals of exposure to $\text{^{14}C}$-glucose. The influx of $\text{^{14}C}$-glucose was 2–3 times more intensive in the light than in the shade. It was found that conversion of $\text{^{14}C}$-glucose into sucrose was enhanced in the light, especially in juvenile leaves. Incorporation of $\text{^{14}C}$ into amino acids was more prominent in the shade. Light stimulated $\text{^{14}C}$-distribution into pigments and lipids. In the shade $\text{^{14}C}$ was intensely channeled into starch in the juvenile leaves. In the mature leaves, a large portion of $\text{^{14}C}$ (29%) was incorporated into soluble proteins (albumins, globulins), compared to that in the juvenile leaves (8%). The ratio of soluble/hardly soluble proteins was the same in the leaves of different ages in the light, but was higher in the mature leaves than in the juvenile ones in the shade.

**P11-037 Glutamine synthetase isoforms in wheat and barley: targets for improving nitrogen use efficiency**

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Glutamine synthetase (GS) is a key enzyme involved in assimilation of inorganic nitrogen (N) into organic. Studies in maize and rice have shown that it plays an important role in determining several aspects of N use efficiency and/or yield. Limited information is available on GS isoforms in wheat. We have therefore undertaken the cloning and characterization of GS genes in this crop. A total of ten GS gene sequences were cloned and classified according to their nucleotide and amino acids sequences in four sub-families (GS2, GS1, GSr and GSe). Expression profiles were studied in both greenhouse and field-grown plants and showed that the expression of all GS sub-families was tissue specific: GS2 mRNA was mostly present in photosynthetic tissue; GS1 transcripts were rather abundant in glumes and the flag leaf while GSr was prevalent in glumes and roots. The level of GSe mRNA was very low. In situ localisation showed that GS1 was present in the cytosol of parenchyma cell and in the perifascicular sheath cells whilst GSr predominantly occurred in vascular cells. During plant development, transcripts and polypeptides of GS2 decreased gradually while those of GS1 increased suggesting a role for GS1 in N remobilization. We are currently using reverse genetics (TILLING) and over-expression strategies to further elucidate the specific roles of the individual isoforms. The over-expression work is partly based on the so-called cisgenesis approach using a barley GS1 gene.

**P11-038 Characterization of novel nitrile specifier proteins (NSPs) involved in glucosinolate hydrolysis in Arabidopsis thaliana**

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Arabidopsis thaliana and Brassica crops such as oilseed rape (Brassica napus) contain a family of sulfur- and nitrogen-containing secondary metabolites called glucosinolates. Glucosinolate hydrolysis leads to the formation of a range of biologically active products that can have beneficial or adverse effects on human health and are involved in plant defence against pathogens and pests. Glucosinolate hydrolysis is catalyzed by thioglucosidases called myrosinases (EC 3.2.1.147) and the nature of the generated products depends on the structure of the glucosinolate in question, reaction conditions (e.g. pH) and the presence of additional proteins and cofactors (Bones and Rossiter 1996). Myrosinase activity is necessary and sufficient for the enzymatic production of isothiocyanates from all types of glucosinolates. The additional presence of the epithiospecifier protein (ESP) diverts the hydrolysis of aliphatic glucosinolates to epiphenytonitriles or nitriles (Zabala et al. 2005). We have now identified a family of nitrile specifier proteins (NSPs) in *A. thaliana* that are able to divert the hydrolysis of aromatic, indole and aliphatic glucosinolates towards nitrile production. We will present the in vitro characterization of these novel nitrile specifier proteins, the analysis of overexpression plants and discuss the role of these proteins in glucosinolate hydrolysis.


**P11-039 Site-directed mutagenesis of maize cytokinin oxidase/dehydrogenase**

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Glucosinolate hydrolysis is catalyzed by thioglucosidases called myrosinases (EC 3.2.1.147) and the nature of the generated products depends on the structure of the glucosinolate in question, reaction conditions (e.g. pH) and the presence of additional proteins and cofactors (Bones and Rossiter 1996). Myrosinase activity is necessary and sufficient for the enzymatic production of isothiocyanates from all types of glucosinolates. The additional presence of the epithiospecifier protein (ESP) diverts the hydrolysis of aliphatic glucosinolates to epiphenytonitriles or nitriles (Zabala et al. 2005). We have now identified a family of nitrile specifier proteins (NSPs) in *A. thaliana* that are able to divert the hydrolysis of aromatic, indole and aliphatic glucosinolates towards nitrile production. We will present the in vitro characterization of these novel nitrile specifier proteins, the analysis of overexpression plants and discuss the role of these proteins in glucosinolate hydrolysis.


Cytokinin oxidase/dehydrogenase (CKO/CKX) is a flavoenzyme, which irreversibly degrades the plant hormones cytokinins and thus it participates in their homeostasis. CKOs catalyze the oxidative breakdown of isopenoid cytokinins to adenine/adenosine and aldehydes. CKOs contain covalently bound FAD cofactor and show a dual functionality. Oxygen as well as quinones can re-oxidize FAD reduced during catalytic reaction. Cytokinin substrate binds at a dual functionality. Oxygen as well as quinones can re-oxidize FAD reduced during catalytic reaction. Cytokinin substrate binds at a dual functionality.
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residues pointed out the importance of D169 as a crucial catalytic residue. Mutation of residues located at the entrance or inside the active site, strongly affected the substrate specificity as well as reaction rates with various electron acceptors indicating that both substrate and electron acceptor bind at the active site. Mutant H105A containing noncovalent FAD was active and showed good activity with isopenicenyladenine. Several mutants were crystalized and X-ray data were collected up to 1.8 Å resolution. Refinement of the crystal structures is underway.

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P11-040 Reversible starch phosphorylation regulates starch metabolism in Arabidopsis thaliana
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Storage carbohydrates are of fundamental importance and represent a readily available source of energy in most living organisms. The major storage carbohydrate of plants is starch, a complex semi-crystalline glucose polymer located in plastids. It has been known for a long time that starch contains covalently linked phosphate. Yet, its biological significance was unclear. Recently, it was shown that the glucan, water dikinases phosphorylate starch and are essential for the process of starch breakdown. Phosphate introduced into starch is proposed to disrupt the granule surface and thereby making it more accessible for starch degrading enzymes. However, the metabolism of the resultant phosphoglucons is not well understood. In animals a phosphatase called Laforin releases phosphate from glycogen. Laforin-deficient mice display elevated phosphate levels in glycogen. Recent data suggest that SEX4, a Laforin-like phosphatase from Arabidopsis, can dephosphorylate soluble amylopectin in vitro. We now have evidence that SEX4 is a glucan phosphatase which dephosphorylates the granule surface in vivo. Arabidopsis mutants lacking SEX4 accumulate both starch and phosphorylated intermediates of starch breakdown. Additionally, the activity of glucan hydrolysing enzymes on isolated starch granules in vitro is increased upon simultaneous glucan phosphorylation and dephosphorylation. These data provide evidence that transient glucan phosphorylation is a critical part of starch metabolism.

P11-041 The role of PCK in remobilising nitrogen in Arabidopsis and tobacco leaves
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Nitrogen remobilisation from senescing leaves is critical for the nutritional quality of grain from crops. We are investigating the pathway responsible for nitrogen remobilisation from aging leaves. It has been proposed that a pathway involving PPDK, PCK, asparagine synthetase and glutamate dehydrogenase is upregulated during dark induced senescence in Arabidopsis (Lin and Wu 2004). We have tested whether over-expression of PCK during natural senescence affects nitrogen export from leaves. To achieve this a genomic PCK clone was fused to the SAG12 promoter (Gan and Amasino 1997). Mendelian genetics were used to confirm six independent, single insert, homozygous lines of Arabidopsis containing SAG12-PCK. Immunoblotting confirmed high expression of PCK during senescence in three of the lines and initial data suggests the hypothesis that PCK is important in nitrogen remobilisation from senescing leaves. We are also investigating alternate methods of mis-expressing PCK in leaves.

P11-042 Nitrogen and sulphur nutrition on metabolic profiling of ecotypes of Brassica rapa L. cv. Sylvestris
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Probiotic glucosinolates and nitrate content contribute to determine the food quality of brassicaceae, like broccoli. Being glucosinolates nitrogen and sulphur compounds, nitrogen and sulphur nutrition can play an important role in defining qualitative and quantitative features of such products. In this work different ecotypes of Brassica rapa L. cv. sylvestris were analysed to characterize the metabolic profiling of plants cultivated under different nitrogen and sulphur concentrations. The plants were cultivated hydroponically in floating system using adapted Hoagland solution. At harvesting samples of inflorescences and leaves (blade and stem) were collected in liquid nitrogen and used to determine the contents of pigments, total soluble protein, starch, soluble sugars, free amino acids, organic acids and nitrate as well as glucosinolates. Inflorescence, leaf blade and leaf stem differed significantly for metabolite contents. Nitrate content, in particular, was almost undetectable in the inflorescences that are the major component of the edible part. That, instead, presented higher content of carbohydrates, proteins, free amino acids and organic acids. Glucosinolate pattern differed among the different organs. The effect of different nitrogen and sulphur nutrition were also discussed. Financial support for this work was obtained by ‘Seconda Università di Napoli’, ‘Ministero dell’Università’ and ‘Ricerca Scientifica e tecnologica’ of Italy (Progetto PRIN 2006077008).

P11-043 Ascorbate metabolism during grape berry development
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Ascorbate (Asc) is a well known anti-oxidant and redox regulator within plant cells. The Asc system modulates pathways associated with fruit development including mitosis, cell elongation, the timing of flowering and processes of aging and senescence. This research, focusing on ascorbate levels across grape berry development of the Vitis vinifera cultivar Shiraz, has demonstrated that the main phase of ascorbate accumulation occurs during the growth phase of immature green berries. Our analysis of the L-galactose pathway

Physiol. Plant. 133, 2008
Glutamine synthetase (GS) is an essential enzyme in nitrogen metabolism and it is extremely important to understand the mechanisms by which it is regulated. In higher plants, GS exists as a number of isoenzymes encoded by small gene families. It is generally accepted that GS is essentially regulated at the transcriptional level and relatively little is known about the regulatory mechanisms controlling plant GS at the post-translational level. It has been shown that plant GS is regulated by phosphorylation and oxidation, and both covalent modifications affect enzyme activity and susceptibility to degradation. In this study we present evidence that GS in M. truncata is regulated post-translationally by tyrosine nitration. We have studied the effect of nitric oxide (NO) on GS activity by in vitro incubation of the enzyme with peroxynitrite. Both cytosolic and plastid GS isoenzymes were found to be susceptible to modification by NO, resulting in enzyme inactivation. The NO modified GS proteins were recognized by an anti-nitrotyrosine antibody indicating that GS is in vitro nitrated in a tyrosine residue, leading to a loss of activity. GS nitration and concomitant decrease in activity, by peroxynitrite was found to be dose-dependent. These results suggest that plant GS is inactivated by tyrosine nitration, similarly to what has been shown for the bacterial and mammalian GSs. This work is supported by projects PTDC/AGG-AAM/65024/2006.

P11-045 In vitro inactivation of plant glutamine synthetase by tyrosine nitration
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Glutamine synthetase (GS) is an essential enzyme in nitrogen metabolism and it is extremely important to understand the mechanisms by which it is regulated. In higher plants, GS exists as a number of isoenzymes encoded by small gene families. It is generally accepted that GS is essentially regulated at the transcriptional level and relatively little is known about the regulatory mechanisms controlling plant GS at the post-translational level. It has been shown that plant GS is regulated by phosphorylation and oxidation, and both covalent modifications affect enzyme activity and susceptibility to degradation. In this study we present evidence that GS in M. truncata is regulated post-translationally by tyrosine nitration. We have studied the effect of nitric oxide (NO) on GS activity by in vitro incubation of the enzyme with peroxynitrite. Both cytosolic and plastid GS isoenzymes were found to be susceptible to modification by NO, resulting in enzyme inactivation. The NO modified GS proteins were recognized by an anti-nitrotyrosine antibody indicating that GS is in vitro nitrated in a tyrosine residue, leading to a loss of activity. GS nitration and concomitant decrease in activity, by peroxynitrite was found to be dose-dependent. These results suggest that plant GS is inactivated by tyrosine nitration, similarly to what has been shown for the bacterial and mammalian GSs. This work is supported by projects PTDC/AGG-AAM/65024/2006.

P11-046 New insights into plant nucleoside transport and metabolism
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Besides macronutrients, soil contains numerous organic substances derived from decaying organisms. As a consequence of nucleic acid breakdown, nitrogen-rich nucleosides appear at concentrations up to 600 μmol/l soil. Therefore, we addressed the question whether these nucleosides can influence plant development. We used Arabidopsis seedlings grown in sterile culture under conditions of full nutrition or nitrogen starvation and supplied them with a mix of four nucleosides. These nucleosides were efficiently imported into seedlings by two members of the equilibrative nucleoside transporter (ENT) family, AtENT1 and AtENT3 (Traub et al. 2007, Plant Journal). Both are highly transcribed in roots as revealed by GUS-fusion analysis and RT-PCR and expression increases upon nitrogen limitation. Following uptake, purine and pyrimidine nucleosides were degraded to CO2 and NH4 to different degrees, or became phosphorylated to nucleoside triphosphates within the salvage pathway. Salvaged nucleosides increased the ATP/ADP ratio of seedlings and subsequently led to significant alterations in primary metabolism. The observed alterations were: (1) an increased chlorophyll content (2) increased RNA-, starch- and amino acid levels (3) elevated amounts of total nitrogen. From this we conclude that exogenously supplied nucleosides act beneficial on metabolism of Arabidopsis seedlings. Microarray data will be presented in addition, to support the observed changes in metabolism.

P11-047 Interactions between the host trees and the causal agents of Japanese oak wilt
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The complex of the ambrosia beetle, ‘Italics start’ Platypus quercivorus ‘Italics end’ (Coleoptera: Platypodidae), and the vectored pathogenic fungus, ‘Rafaellea quercivoros’, kills deciduous oak trees in Japan, Japanese Oak Wilt. Heavily attacked ‘Quercus crispula’ is often killed. But most of ‘Q serrata’ and ‘Castanea crenata’, which were attacked as the same level as that of killed ‘Q crispula’, are survive. The aggressiveness of ‘R quercivoros’ is
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not so strong that it penetrates into host from the injured cells of gallery wall tunneled in the sapwood by the ambrosia beetles. Host responses make the antifungal substances and reaction zone, and then the fungus elongation will be stopped. Chemical bases of the attack by beetles and the life or death mechanism of host trees will be discussed.

P11-048 The Arabidopsis R2R3-MYB transcription factor AtMYB60 functions as a transcriptional repressor of anthocyanin biosynthesis in lettuce (Lactuca sativa)
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The MYB transcription factors play important roles in the regulation of many secondary metabolites at the transcriptional level. We evaluated the possible roles of the Arabidopsis R2R3-MYB transcription factors in flavonoid biosynthesis because they are induced by UV-B irradiation but their associated phenotypes are largely unexplored. We isolated their genes by RACE-PCR, and performed transgenic approach and metabolite analyses in lettuce (Lactuca sativa). We found that one member of this protein family, AtMYB60, inhibits anthocyanin biosynthesis in the lettuce plant. Wild-type lettuce normally accumulates anthocyanin, predominantly cyanidin and traces of delphinidin, and develops a red pigmentation. However, the production and accumulation of anthocyanin pigments in AtMYB60-overexpressing lettuce was inhibited. Using RT-PCR analysis, we also identified the complete absence or reduction of dihydroflavonol 4-reductase (DFR) transcripts in AtMYB60-overexpressing lettuce (AtMYB60-117 and AtMYB60-112 lines). The correlation between the overexpression of AtMYB60 and the inhibition of anthocyanin accumulation suggests that the transcription factor AtMYB60 controls anthocyanin biosynthesis in the lettuce leaf. Clarification of the roles of the AtMYB60 transcription factor will facilitate further studies and provide genetic tools to better understand the regulation in plants of the genes controlled by the MYB-type transcription factors.

P11-049 The use of immunoaffinity purification and LC-MS/MS in analysis of indole-3-acetic acid and its derivatives in plants
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Indole-3-acetic acid (IAA) is an important phytohormone denoted as auxin. It is involved in a wide range of physiological processes. Although metabolism of IAA has been studied for a long time, still many important questions remain unanswered, for IAA and its derivatives easily undergo degradation during isolation from plant material and subsequent purification. Besides, many of the compounds of interest occur at very low concentrations in plants, which constitute matrix of a very complex nature. For the analysis of IAA and its derivatives, we developed an analytical protocol comprising not only modern chemico-analytical instrumentation (HPLC or UPLC coupled to tandem mass spectrometry) but a specific immunochemical purification method, as well. Sample (approximately 50 mg of fresh weight) is firstly extracted with phosphate buffer and purified by solid-phase extraction. After methylation, the immunoaffinity extraction is performed. Antibodies used for the method exhibit a wide specificity for indole compounds substituted at 3-position of indole ring. Analysis of various indole-3-acetic acid derivatives e.g. indole-3-acetonitrile (iIAN), indole-3-acetamide (IAM), indole-3-ethanol (iEt) and IAA-conjugates of Ala, Asp, Gly, Glu, Leu, Phe, Trp and Val - can be therefore easily performed. The research was supported by the Ministry of Education, Youth, and Sports of the Czech Republic grant No. MSM 6198959216.

P11-050 Biochemical characterization of barley leaves β-glucosidase that hydrolyzes piceid
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Resveratrol and resveratrol glucoside (piceid) are accumulated in response to interaction of the plant and pathogens, such as Botrytis cinerea and Colletotrichum sublineolum. The compounds have beneficial effects on human health. Resveratrol is a phytoalexin produced in various plants in response to stress, caused by UV-radiation, heavy metals, injury. It has been reported that piceid can be hydrolyzed to resveratrol by piceid-β-D-glucosidase from Aspergillus oryzae. Our observations show, for the first time, the presence of piceid-β-D-glucosidase activity in higher plants.

The piceid-β-D-glucosidase was isolated from two-week old barley leaves purified by ammonium sulphate fractionation and ion exchanged chromatography on a DEAE-Sephalac column. Two active fractions were found which recognize piceid as substrate. Fraction A did not adsorb to the ion exchanger and the fraction B did. The specific activity with respect to the piceid hydrolysis was higher in A than B. Therefore, fraction A was chosen for further studies. The more so as SDS-PAGE and gel filtration revealed its homogeneity and that the enzyme functions as a single polypeptide of 50 ± 2 kDa. The glucosidase activity had maximum activity at pH 5.5. Divalent cations were not required for activity but Ca²⁺ and Cu⁺ acted as activators. Mg²⁺ and Zn²⁺ inhibited the enzyme activity. The Km for piceid was 233 μM and the Vmax was 7.8 nmol min⁻¹ per mg protein. The substrate specificity for several glycosides were also examined.

P11-051 A genetic approach to cyanogenesis in the model legume Lotus japonicus
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Cyanogenic glucosides are defense compounds found in a large number of plant species. They protect the plant against herbivores as tissue damage will result in their breakdown and the release of toxic hydrogen cyanide gas (HCN). The presence of cyanogenic glucosides is a problem in various crops of social and economical importance such as wheat, corn, rice, sugar beet, and canola. The cyanogenic glucosides are transformed by two related enzyme activities. The first one is β-D-glucosidase that hydrolyzes the glucoside to yields the aglycone. The second enzyme is β-D-glucosidase that hydrolyzes the glucoside to yields the aglycone. The second enzyme is β-D-glucosidase that hydrolyzes the glucoside to yields the aglycone. Therefore, the enzyme has been characterized in several species but the mechanism of cyanogenesis remains unexplored.

In this project, we use Lotus japonicus, an agriculturally important model legume, as a potential source of β-D-glucosidase that hydrolyzes glucosides to yields the aglycone. The enzyme has been purified from L. japonicus by ammonium sulfate fractionation and ion exchanged chromatography on a DEAE-Sephacel column. Two active fractions were found which recognize piceid as substrate. Fraction A did not adsorb to the ion exchanger and the fraction B did. The specific activity with respect to the piceid hydrolysis was higher in A than B. Therefore, fraction A was chosen for further studies. The more so as SDS-PAGE and gel filtration revealed its homogeneity and that the enzyme functions as a single polypeptide of 50 ± 2 kDa. The glucosidase activity had maximum activity at pH 5.5. Divalent cations were not required for activity but Ca²⁺ and Cu⁺ acted as activators. Mg²⁺ and Zn²⁺ inhibited the enzyme activity. The Km for piceid was 233 μM and the Vmax was 7.8 nmol min⁻¹ per mg protein. The substrate specificity for several glycosides were also examined.
as cassava, sorghum, almonds, and forage legumes. An improved understanding of the metabolic and regulatory factors that control cyanogenic glucoside production and breakdown is required. The model legume Lotus japonicus contains cyanogenic glucosides and related hydroxynitrile glucosides derived from the amino acids valine and isoleucine. A genetic approach in Lotus was used to identify acyanogenic and reduced-cyanogenic mutants. Metabolic profiling showed the isolation of various classes of mutants in both cyanogetic glucoside production and breakdown, and will provide novel insights in the understanding and control of cyanogenesis in plants.

P11-052  Cell wall biosynthesis: heterologous expression of Arabidopsis glycosyltransferases in Nicotiana benthamiana

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Plant cell walls are composed mainly of polysaccharides, which play essential roles in growth and development, response to the environment, and interactions with symbionts and pathogens. Cell walls are the main constituent of biomass and production of biofuels from biomass requires decomposition of the polymers. Better understanding of the biosynthesis of the cell wall polysaccharides may enable development of crops with improved properties as biofuels feedstocks. Despite rather detailed information on the structure of the cell wall polysaccharides, little is known about their biosynthesis. The key enzymes are glycosyltransferases (GTs) and plants need a large number of GTs to synthesize the complex polysaccharides present in the walls. However, only a few GTs have had their activity demonstrated. In Arabidopsis thaliana, approximately 450 GT genes have been identified based on their sequence and deposited in the CAZY database (www.cazy.org). We have cloned a large number of these GTs in Gateway vectors in order to heterologously express the GTs and characterize their activity. Heterologous expression systems based on yeast or animal cell cultures have often been found to be inefficient for expression of active plant GTs. In contrast, Agrobacterium-mediated transient expression in Nicotiana benthamiana has a high success rate. The main drawback is a high endogenous background activity. Optimization of the procedure for systematic characterization of GTs will be presented.

P11-053  Ratio of metabolically connected flavonoids as a parameter different stress conditions of Achillea nobilis in the South Urals

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Distribution of a total sum of flavonoids was investigated, as well as the amount of routine and quercetine in elevated parts of Achillea nobilis, gathered in 11 cenopopulations in the South Urals, located in a gradient ‘the North - the South’. Researches were carried out by method of HPLC chromatography. The total amount of the substances registered by HPLC chromatography, in inflorescences formed 29, more over the maximum quantity of substances in inflorescences of one cenopopulation formed 18. In plant leaves these parameters formed 32 and 19, and in stalks 25 and 19 respectively. The general level of anthropogenous pressure in cenopopulations was estimated at qualitative and quantitative structure of accompanying grassy vegetation. The data of the geobotanical description of region permitted to arrange these cenopopulations in order reflecting a gradient of pasturable pressure. It is determined, that in the process of intensification of anthropogenous pressure in leaves there is the increasing of maintenance of routine with respect to quercetine, it’s direct metabolic predecessor. The described picture obviously explains protective character of the routine. Also it is ascertainment, that the total amount of the substances diagnosed by HPLC that were found in leaves and stalks, have inverse correlation \( r = -0.6, \ p = 0.02 \). Connection of structure of phenolic components with conditions of growth gives an opportunity of selection of valuable medicinal plant forms.

P11-054  Identification and quantification of mammalian-type steroids in plants by immunoaffinity extraction combined UPLC-ESI-MS/MS

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Physiological processes in plants are affected by broad spectrum of structurally various substances such as cytokinins, auxins or brassinosteroids. In comparison to these well known compounds, distribution and physiological importance of mammalian-type steroids to plants is still not generally accepted. Despite the fact that considerable amount of studies on occurrence and physiological impact of steroids on plants have been published, more accurate information about the role of these compounds in plants is still missing. Studying mammalian-type steroids in plants requires powerful analytical tool able to detect and identify trace amounts of these compounds in plant samples. Here we introduce very sensitive, rapid and versatile method for simultaneous analysis of twelve mammalian-type steroids in plant extracts by ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) combined with immunoaffinity extraction. Polyclonal antibodies used in this study were raised against 4-androsten-3-one-17-carboxymethylxime conjugated to BSA at C17. By this approach generic antibodies with high cross-reactivities mainly against 4,4-3-keto-steroids were obtained. This fact allowed usage of one IAC step for effective simultaneous extraction and thus subsequently identification and quantification of twelve steroid structures from plant samples.

P11-055  Phenolic compounds of lignin modified silver birches in the interaction with Paxillus involutus

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P11-056 Purification of 1-Aminocyclopropane-1-Carboxylic acid N-malonyltransferase from mung bean seedlings
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1-Aminocyclopropane-1-carboxylic acid (ACC) N-malonyltransferase is an enzyme in the plants’ ethylene circle. It carries out the malonylation of ACC, thus preventing the ACC from being converted into ethylene. The enzyme has not been studied thoroughly due to its low abundance. No amino acid sequence has been published so far. In this study, two purification protocols are used to purify the enzyme from mung bean seedlings. They are immunoaffinity purification with monoclonal antibody raised against the enzyme and liquid chromatography with 5 columns. A consistent result is achieved. Both show two sharp bands at the molecular weights of 26 kD and 16 kD in SDS-PAGE, indicating that the enzyme consists of two subunits. Gel filtration also determined that the enzyme’s molecular weight is around 40 kD.

P11-057 Mutants in nucleotide sugar biosynthesis for plant cell walls
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The polymers of plant cell walls are synthesized from nucleotide sugar precursors. Arabidopsis leaves are predominantly derived from UDP-glucuronic acid (UDP-GlcA), which accounts for roughly 50% of the cell wall biomass, because it is the principal precursor of galacturonic acid, xylose and arabinose residues of matrix polysaccharides in plant cell walls (Seitz et al. 2000). We study the biosynthesis of UDP-GlcA using functional genomics tools. The enzyme UDP-glucose dehydrogenase (UGD) is the major responsible reaction for the formation of UDP-GlcA and is encoded by a small gene family in Arabidopsis with four members (Klinghammer and Tenhaken 2007). An alternative pathway for the formation of UDP-GlcA is controlled by the MIOX-enzyme, which catalyses the oxygenative ring cleavage of myo-inositol into glucuronic acid (Kanter et al. 2005). We characterize the UGD- and MIOX-knockout mutants using molecular, biochemical, immunological and electron microscopy tools. Notably the two doublemutants studied so far show contrasting phenotypes indicating that the balance of pectins, hemicelluloses and cellulose is critical for normal cell differentiation. Thus the four different isoforms of UGD have distinct functions for normal development of Arabidopsis.


P11-058 Structure and function of pea seedling aminoaidehyde dehydrogenase
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Aminoaidehyde dehydrogenase (AMADH, EC 1.2.1.19) oxidizes ω-aminoaidehydes arising from polyamine degradation. Pea seedling AMADH exists in two isoforms (AMADH1 and AMADH2). Based on their amino acid sequences, the enzymes are related to betaine aldehyde dehydrogenases (BADHs, EC 1.2.1.8), which participate in plant response to osmotic stress. In this work, pea AMADH1 and AMADH2 cDNAs were expressed in E. coli and the recombinant proteins were purified to homogeneity. AMADH1 was cocryrstallized with NAD+ and then X-ray data were collected up to 2.8 Ångstroem resolution. Molecular replacement using a human aldehyde dehydrogenase confirmed that recombinant AMADH1 is a dimer. The asymmetric unit contains 12 monomers, each of 518 residues. The crystal structure is currently under refinement. Enzyme kinetics was performed with both recombinant enzymes. The obtained results show that AMADH2 has much better affinity to the best substrate 3-aminopropanal compared with AMADH1 and that the compound is also oxidized more efficiently. Interestingly, both enzymes are able to oxidize pyridine carboxaldehyde and N-pyridylmethyl derivatives of some ω-aminoaidehydes. To get insight into AMADH catalysis and specificity, site-directed mutagenesis and crystallization of AMADH2 have been proposed. Expression and purification of AMADH2 active-site mutants are in progress. Supported by the grant 522/08/0555 from the Czech Science Foundation.

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P11-059 Functional analysis of the Arabidopsis thaliana trehalose-6-phosphate phosphatase family
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Trehalose (T) functions as a reserve sugar and stress protectant in a large variety of organisms. The major pathway for T synthesis involves a trehalose-6-phosphate (T6P) synthase and a T6P-phosphatase (TTP). T synthesis has long been thought to be absent in most plants, but its significance began to dawn when ectopic expression of microbial T metabolism genes resulted in dramatic phenotypes affecting plant sugar partitioning, growth, development and stress resistance. Arabidopsis thaliana encodes a remarkably large family of putative T biosynthesis enzymes, consistent with rigid level control and important regulatory functions of T6P, emerging as a novel sugar ‘signal’ in coordinating carbon supply with plant growth, developmental signaling and morphogenesis. We are focusing at the Class III (TPPA-J) enzymes, which only have the conserved phosphatase boxes in common with the yeast TTP Tps2. The growth phenotype of the yeast tps2 mutant can be complemented by any of the 10 Class III TPP enzymes, showing clear in vivo TTP activity, in contrast to Class I (TPS1-4) and Class II (TPS5-11) enzymes. We will present detailed in vitro kinetic analysis for the Class III proteins. For a comprehensive expression analysis, we have made promoter GUS/GFP constructs for all Class III genes. Preliminary analyses reveal remarkable tissue- and developmental stage-specific expression patterns. Our results suggest important novel functions of T metabolism in plant growth and development.

P11-060 Starch metabolism and perenniality in legumes
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Perennial legume crops offer the opportunity to develop more sustainable agricultural systems because of their unique ability to perform symbiotic nitrogen fixation and their conservative growth strategy. They are of current and potential agronomic importance for forage, grain production, and the production of biofuels. Meristem activity has been suggested to be a major determinant of perenniality. The ability of the plant to store carbon as starch in their organs and remodelize it, however, is also expected to play an important role for the perennial lifestyle, especially in determining the capacity for vigorous re-growth. Almost nothing is known about the importance of starch turnover in this respect. We have used both forward and reverse genetics on an EMS-mutagenised population of the model legume Lotus japonicus to isolate mutants impaired in starch biosynthesis and degradation. We report here the identification of mutants for several key enzymes of this pathway for which further characterisation has revealed some interesting novel alleles. We are currently identifying the mutations of the yet uncharacterised mutants using a map-based cloning approach. Mapping results suggest that these mutations are likely to affect previously undiscovered proteins involved in starch metabolism. Together with natural genetic variation, these mutants are being used to elucidate the importance of starch for re-growth and uncover the pathway of starch turnover in perennial legume species.

P11-061 Distribution and characterization of peroxidases in Brassica rapa L. cv. Sylvestris
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Peroxidases and polyphenoloxidases catalyze the oxidation of phenolics, with formation of brown compounds known as melanins. They are generally involved in the browning process occurring vegetables after harvesting. This process, as part of a defence mechanism, starts in cutted zone and reaches also the other tissues during the post-harvest phase. Generally its extention in the plant tissues, is used as index of food quality loss in vegetables. In this work the distribution and characterization of peroxidases in the plant tissues has been done in broccoli from Brassica rapa L. cv. sylvestris. This could be important to increase the shelf-life of minimally processed broccoli. At the harvest and during the post harvest phase the edible plant parts were divided in inflorescences, and leaves (petiole and leaf blade). The samples were kept and powdered in liquid nitrogen and used to obtain the enzyme extracts. The peroxidase specific activities were higher in the petiole than in the inflorescence and leaf blade. The enzyme isoforms were analysed by 2D-native electrophoresis. Acid, basic, and neutral isoforms were differently distributed in the plant tissues. The effect of temperature and of inhibitors on peroxidase activity were measured and analysed. Financial support for this work was obtained by ‘Seconda Università di Napoli’, ‘Ministero dell’Università’ and ‘Ricerca Scientifica e tecnologica’ of Italy (Progetto PRIN 2006077008).

P11-062 Transcriptome analysis of different cell types from glandular trichomes of plants; a model study with Artemisia annua
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Multicellular glandular secretory trichomes (GSTs) are the green factories of many plants. In order to investigate the function of different cells of multicellular GSTs, a method based on the laser microdissection capture pressure catapulting technique has been developed. Using a modified tissue preparation method, one outer pair of apical cells and two pairs of sub-apical, chloroplast containing cells, were isolated from GSTs of Artemisia annua; the source of the widely used antimalarial drug artemisinin. The biosynthesis of artemisinin has been proposed to be located to the GSTs. The first committed steps in the conversion of farnesyl dipiphosphate to artemisinin is catalyzed by amorpha-4,11-diene synthase and a cytochrome P450 monooxygenase (CYP71AV1). First strand cDNA synthesized from RNA extracted from the different cell types was used as template in the PCR amplifications of these two transcripts. Our experiments showed expression of the two genes in the apical cells with no detectable amplification in the sub-apical cells. Elongation factor 1α was used as control and it was shown to be expressed in both cell types. We conclude that the two outer apical cells are the site for the initial steps of artemisinin biosynthesis while the two pairs of chloroplast-containing cells have other functions in the overall metabolism of glandular trichomes. Further studies on terpene metabolism in trichomes are in progress. The results of these extended studies will be discussed.
157 proteins were modified by treated plants, especially at membrane level (cell membrane identified by MS/MS analysis. Cell damages were detected in As (TEM). For proteomic analysis, leaf proteins were extracted by the interest was focused on cytological and proteomic changes in the Populus deltoides. The genetic variation of leaf proteome was studied from two genotypes. Two experiments were carried out; the first one was conducted in glasshouse using 4 months each experiment. Leaf proteome analysis was performed using two-dimensional gel electrophoresis. Whatever the experiment, the difference of predawn leaf water potential between well-watered and drought-stressed plants was of about −0.4 MPa. Productivity never varied between genotypes whereas most of the leaf traits differentiated significantly the two genotypes. Whether the experiment was conducted in glasshouse or in nursery, under optimal irrigation or drought stress condition and whatever the sampling date, 36 proteins allowed to differentiate the two genotypes. Among these proteins 15 were currently identified and 10 concern the carbohydrate metabolism. In response to water deficit, 41 proteins differentiated the two genotypes; 22 were currently identified and concern the secondary metabolism, the mechanisms of plant defence against constraints or the carbohydrate metabolism.

The fern Pteris vittata can tolerate soil As concentration up to 1500 ppm and rapidly accumulates the metalloid in its fronds. However, its tolerance to As has hitherto not been completely explored yet. Arbuscular mycorrhizae (AM) are known to influence plant performance in many ways, including enhancement of heavy metal/metalloid stress tolerance. The aim of the present work was to study the effects of the AM fungi Glomus mosseae and Gigaspora margarita on P. vittata plants treated with As. The interest was focused on cytological and proteomic changes in the fronds. Cytology was investigated by ultrastructural techniques (TEM). For proteomic analysis, leaf proteins were extracted by the TCA-acetone method and separated by 2DE, spots of interest were identified by MS/MS analysis. Cell damages were detected in As treated plants, especially at membrane level (cell membrane proliferation, tylosis disorganization), partly and differently restored by AM symbiosis. At molecular level, the expression of 165 and 157 proteins were modified by G. mosseae and G. margarita, respectively: in particular, AM fungi influenced glycolysis, photosynthesis, tricarboxylic acid cycle, malate metabolic process and electron transport. In non colonized plants, As induced the change of 179 proteins, some of which involved in the above mentioned biological processes. The two AM fungi differently modulated the As-induced modifications, suggesting improved tolerance, especially by G. mosseae.

The genetic variation of leaf proteome was studied from two Populus deltoides x P. nigra genotypes. Two experiments were carried out; the first one was conducted in glasshouse using 4 months old rooted-cuttings and the second one, in open field using 4 years old trees coppiced each year. In both experiments, a set of plants was well-watered whereas a second one was drought-stressed. Drought intensity was estimated from leaf predawn water potential. Productivity, structural and functional leaf traits were measured in each experiment. Leaf proteome analysis was performed using two-dimensional gel electrophoresis. Whatever the experiment, the difference of predawn leaf water potential between well-watered and drought-stressed plants was of about −0.4 MPa. Productivity never varied between genotypes whereas most of the leaf traits differentiated significantly the two genotypes. Whether the experiment was conducted in glasshouse or in nursery, under optimal irrigation or drought stress condition and whatever the sampling date, 36 proteins allowed to differentiate the two genotypes. Among these proteins 15 were currently identified and 10 concern the carbohydrate metabolism. In response to water deficit, 41 proteins differentiated the two genotypes; 22 were currently identified and concern the secondary metabolism, the mechanisms of plant defence against constraints or the carbohydrate metabolism.

We (K.U.Leuven, Belgium) host the global in vitro collection of banana varieties. The aim of this international gene bank is to conserve all banana genetic resources safely and to supply the germplasm to any bona fide users. We were one of the pioneers to explore the possibilities of storing germplasm in liquid nitrogen. For a successful storage at −196°C, the meristematic cells need to survive a severe dehydration process prior to freezing. Dehydration tolerance is achieved by an osmotic stress acclimation. However, more than half of the collection consists of varieties that show a low survival rate. Hence, there is a need to unravel the mechanisms behind acclimation and to get insight into the genotype specific diversity. Protein separation via two-dimensional gel electrophoresis (2DE) and protein identification via tandem mass spectrometry (MS/MS) is the most informative approach for a poorly characterized organism like banana (Carpentier et al. 2005, Carpentier et al. 2007a, b, Carpentier et al. 2008a, b, Samyn et al. 2007). Using the DIGE approach we consider different time points during acclimation and show that 4 days of acclimation is significantly correlated to the highest post-thaw survival. Insight into the complex data confirms that the proteome at 4 days is clearly different from the other sample points.

NACs are plant-specific transcription factors (TFs) that are among the most numerous in the green lineage. Functional analyses indicate that NAC TFs play an important role in tissue formation, but also in abiotic and biotic stress responses. Their absence from algae and their role in cell differentiation led to the proposal that NACs are important for plant multicellularity. Uncovering the ancestral relationships of these genes will assist in rationalizing functional
studies and shedding light on the acquisition of a new TF family during plant evolution. We have identified a putative non-redundant set of 101 NAC genes in Arabidopsis, 120 in Oryza sativa, 161 in Populus trichocarpa, 38 in Selaginella moellendorfii and 32 in Physcomitrella patens. Phylogenetic analyses allowed identifying 22 groups of homologous genes in angiosperms, from which nine can be traced back to the most recent common ancestor with bryophytes and lycophytes. Additionally, there are 27 lineage-specific groups, which reflect the recruitment of NACs for lineage- or species-specific processes. NAC-regulated senescence also appears to be a conserved process in all embryophytes. Based on our results we propose an updated NAC classification and a model of their evolution.

P13-015 Comparative quantitative proteomics — The elucidation of adaptation mechanisms in Chernobyl grown plants
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Twenty two years passed since the worst environmental disaster in human history — the explosion of one of the four reactors of the Chernobyl Nuclear Power Plant (CNPP). As consequence, huge amount of radioactive material have been released to the atmosphere. Presently, nearby lands remains significantly polluted with long living isotopes such as $^{137}$Cs and $^{134}$Sr. Interestingly, plants in Chernobyl area were able to adapt to such environment. However, decades of phenotypic and physiological research did not bring clear answers on mechanisms of plant ability to survive and successfully reproduce in such harmful conditions. To create system-wide overview on biochemical pathways potentially connected with the adaptivity of plants, we started comparative proteomic investigation. During 2007, soybean (Glycine max var. ‘Soyachna’) and flax (Linum usitatissimum var. ’Kievskiy 2000’) plants were grown in ‘polluted’ ($\approx 5$ km from CNPP) and ‘clear’ ($\approx 100$ km from CNPP) experimental plots. Protein extracts from mature seeds were subjected to proteomics approach based on two-dimensional protein electrophoresis connected with tandem mass spectrometry. After Coomassie Coomassie Blue staining of the gels and identification of proteins by MALDI-TOF MS, we identified a total of 566 spots. The quality and quantity of protein spots were increased; the background was reduced especially at the acid end of the gels. A total of 566 spots were detected in 2-DE gels with SyproRuby-staining over a pH range from 4 to 7. Proteins were identified with MALDI-TOF mass spectrometry and three proteins with matched database are pyranose-2-oxidase, malate dehydrogenase and ACL196Wp. This study provided an effective 2-DE proteomic method for the identification of novel proteins from fungi with medical potentials in future studies.

P13-016 Proteome analysis of the medicinal mushroom: Coriolus versicolor
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Thousands of mushrooms exist in the world, but only hundreds of them are edible, and less possess medicinal properties. The current issue is the lack of sensitive and fast methods in discovering and identifying the microbial species with anticancer potentials. Proteomic analysis has been increasingly used in search of novel proteins in plants during current years but very few data on mushrooms are available. This study developed a two-dimensional electrophoresis (2-DE) proteomic methodology and successfully analyzed the proteomes of the Coriolus versicolor (CV), or best known as Yun Zhi, a Chinese mushroom with anticancer properties. Our data showed that the acidic condition was the best choice for the extraction and separation of proteins from the cultivated mycelium extract of CV. The quality and quantity of protein spots were increased; the horizontal streaking and spot smearing, usually associated with 2-DE protein separation of plants because of the large cell walls, were dramatically improved and the dark background was reduced especially at the acid end of the gels. A total of 566 spots were detected in 2-DE gels with SyproRuby-staining over a pH range from 4 to 7. Proteins were identified with MALDI-TOF mass spectrometry and three proteins with matched database are pyranose-2-oxidase, malate dehydrogenase and ACL196Wp. This study provided an effective 2-DE proteomic method for the identification of novel proteins from fungi with medical potentials in future studies.

P13-017 NAC (NAM, ATAF and CUC) transcription factors in rice (Oryza sativa L.): gene organization, phylogenetic relation and gene expression
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With the availability of rice genomic sequence, full length cDNA sequences (35 187 clones) and expression profiling using 22K oligoarray system, we focused our holistic approach to study the plant specific transcription factors- the NAC (NAM, ATAF and CUC). In rice genome, 124 NAC loci have been found by the gene prediction and full-length cDNA mapping. Eighty three predicted transcripts are supported by 74 full-length cDNA clones. There are nine regions in rice genome where NAC genes are located close to each other (within 25–100 kb) and these might have arisen by tandem duplication. 16 pairs of NAC loci are assigned to segmentally duplicated block presented by TIGR. Alternatively spliced variants (more than 15 loci) showed both redundancy of genes and diversity of transcripts. Eight of alternatively spliced loci were confirmed by RT-PCR experiments. Five of the NAC members have strong transmembrane helices. Phylogeny of NAC genes revealed two major groups I and II. Group I consists of the NAC genes with high homology with many NAC genes of other plants, many expressed genes and located on the segmentally duplicated blocks, on the other hand Group II consists of the NAC genes rice specific and relatively no cDNA evidence and many of them are located close to each other. 22K oligoarray covers the transcripts from 63 loci. Microarray analysis revealed the differentiation of the gene expression on the segmentally duplicated gene pairs under several stress condition.

P13-018 Temporal progress of Arabidopsis thaliana defence responses during the early phase of infestation with aphids
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With the availability of rice genomic sequence, full length cDNA sequences (35 187 clones) and expression profiling using 22K oligoarray system, we focused our holistic approach to study the plant specific transcription factors- the NAC (NAM, ATAF and CUC). In rice genome, 124 NAC loci have been found by the gene prediction and full-length cDNA mapping. Eighty three predicted transcripts are supported by 74 full-length cDNA clones. There are nine regions in rice genome where NAC genes are located close to each other (within 25–100 kb) and these might have arisen by tandem duplication. 16 pairs of NAC loci are assigned to segmentally duplicated block presented by TIGR. Alternatively spliced variants (more than 15 loci) showed both redundancy of genes and diversity of transcripts. Eight of alternatively spliced loci were confirmed by RT-PCR experiments. Five of the NAC members have strong transmembrane helices. Phylogeny of NAC genes revealed two major groups I and II. Group I consists of the NAC genes with high homology with many NAC genes of other plants, many expressed genes and located on the segmentally duplicated blocks, on the other hand Group II consists of the NAC genes rice specific and relatively no cDNA evidence and many of them are located close to each other. 22K oligoarray covers the transcripts from 63 loci. Microarray analysis revealed the differentiation of the gene expression on the segmentally duplicated gene pairs under several stress condition.

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Despite their sophisticated feeding strategy, phloem sucking insects are strong stimulants of plant defence system. We present an investigation of Arabidopsis Ler responses to infestation with a Brassicaceae specialised aphid, Brevicoryne brassicae. Intensities of the aphid-induced changes were assessed at four time points after infestation: 6, 12, 24 and 48 h. Gene expression profiling with the use of full genome microarrays revealed a large scale transcriptional reprogramming, progressing with the time of aphid attack. Induction of genes whose products are involved in the generation and detoxification of reactive oxygen species (ROS) and transcripts coding for calcium binding proteins indicated an important role of ROS and calcium signalling in regulation of plant defences. Furthermore, transcriptional changes indicated mobilization of jasmonic acid and salicylic acid signalling pathways and enhanced biosynthesis of defensive compounds such as anti-insect proteins, indolyl glucosinolates and camalexin. Secondary metabolite profiling revealed accumulation of 4-methoxy-3-3-imethyl glucosinolate and camalexin 48 h after infestation. The role of these compounds in defence against aphids was verified in fitness experiments where elevated levels of camalexin negatively influenced aphids' fecundity. Our integrated approach combining transcriptional and metabolomic data allows for a comprehensive characterization of early responses to aphid infestation.

P13-019 The study of plastid genome of non-photosynthetic orchid Neottia nidus-avis

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The information on chloroplast genome sequences is essential in different fields of plant biology: molecular phylogenetics and evolution, plant physiology and transgenic research. The sequence and order of plastid genes are highly conserved across species. Non-photosynthetic plants are the exception from this rule – they have highly reduced and sometimes rearranged plastome. We have studied plastid genome of Neottia nidus-avis, a mycorrhizal monocot species from Orchidaceae. The availability of the plastome genome sequence can be used for the study of plastome evolution, plant physiology and transgenic research. The plastome genome sequence of Neottia nidus-avis has elucidated some details of its structure. It is in many respects similar to those of Phalaenopsis. The most conserved part of these two genomes is the inverted repeat region. However Neottia plastome has specific features due to its non-photosynthetic nature. It lacks most of photosynthetic genes; they are absent of represented by pseudogenes. This is also concerns RNA polymerase genes and chlororespiratory genes. The latter is characteristic also for Phalaenopsis and for the gymnosperm Pinus thunbergii, indicating that the loss of these genes is not necessarily correlated with the heterotrophy. In contrast, a large number of genes for translational apparatus are found to be intact. This provides strong evidence for the retention of translational activity in Neottia plastids.

P13-020 Characterization of barrel medic mutants affected in flavonoid synthesis

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Anthocyanins are secondary metabolite products of plants with important antioxidant activity. In this work six mutants of barrel medic (Medicago truncatula) affected in anthocyanins biosynthesis have been characterized. Mutants were obtained after chemical (tiling) or physical (fast-neutron radiation) mutagenesis and they showed either an altered pattern or an absence of pigmentation in leaves and flowers compared to wild-type plants. A strong reduction of the total amount of anthocyanins present into mutant leaves was also found. We analysed the expression of structural genes and selected transcriptional factors (Myb, Myc and MADS-box genes, WD40 protein) involved in flavonoids biosynthesis by RT-PCR and qPCR and we measured altered expression profiles in the mutant compared to wild-type leaves. For instance, when the amount of anthocyanins was very low, the GST expression was strongly reduced; one mutant showed a complete suppression of the 5GT expression (the last enzyme of anthocyanin biosynthesis). The expression of transcriptional factors was also very different between the mutants: a correlation was observed between the amount of anthocyanins and the expression of PAP1, a specific myb gene related to anthocyanin synthesis. At metabolite level, the amount of flavones measured with LC-MS was also affected by the mutations. The tricin-3GluAc was the most accumulated product in mutant leaves compared to apigenin-3GluAc in wild-type.

P13-021 Architecture of microcenosis of a root zone as a structured element of a plant-microb community system

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Every alive thing exists in contact with microflora. But plant-microb interactions are the most multifaceted and abundant. Real microb cenosis remains inaccessible for study due to methodological difficulties. Thus, rhizosphere microb cenosis is studied via both classic microbiological and modern genetic methods. But 99–99% of microorganisms cannot be cultivated using microbiological mediums. And genetic methods for cenosis studying can show only the whole pool. But the main problem of those methods is both their failure to study microb cenosis in its connection with the plant and disability to show the spatial cenosis organization. We have elaborated a new approach which lets microb cenosis studying on the level of the plant-microb interactions. The method generally can be described as plant cultivation on a special carrier (using either the carrier only or the carrier filled with natural substratum); the carrier can be named as ‘quasi-substratum’ which is made with polymeric material. Applying the approach it is possible to obtain real microb cenosis images which are placed on polymeric components of the quasi-substratum which can be studied via broad range of different.
methods: from light and electron microscopy to molecular methods. Arabidopsis rhizosphere microcnosis was studied via this new technique. The spatial microorganisms arrangement according to the root system was shown. The rhizosphere censosis structure was analysed; its zoning was classified.

P13-022 Comparative analysis of transcription factor genes in organisms

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We have analyzed known transcription factor genes in plants (Arabidopsis, rice), animal (human, nematode, fly), yeast, fungi and Escherichia coli to detect the specificities of transcription regulation in each organism. For better understanding, we have classified them based on the features of their DNA-binding structures: basic domain, helix-turn-helix domain, zinc-coordinating factor domain, beta-scaffold factor domain, and others. There is a difference between the two kingdoms in animals preferentially adapted and customized their zinc-coordinating systems while plants have evolved diversity across several transcription factor families. Transcription factors in rice can be divided into two types by the way of diversification of protein structure. One is by the alternative splicing and the other is by the gene duplication. The AP2/EREBP, NAC, Ringfinger indicates high ratio at the alternative splicing and the bZIP, bHLH and Myb genes are highly segmental duplicated in rice. Parallelism of DNA binding domain structure and protein function is observed in some family of TF proteins such as AP2/EREBP to the hormonal response and MADS to the flowering meristem development.

P13-023 Morphologic and palynologic observation of Oxytona section belongs to Papaver kind which grows up in natural flora of Turkey

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Turkey is considered to be rich with regard to Papaver species. The three species in Oxytona section of Papaver genus are commonly found in natural flora of our country. In this study, belong to 3 species 46 characters as morphologic and 9 characters as palynologic were observed. They were identified that the plant is woody; the plant is dark brown, the shape of pollen is triporate, rarely tricolpate, the kind of pollen is oblong, the surface of bud is reticulate; the filaments are linear, the edge of leaf is serrate, the bud is ovoid, oblong and erect; the petals are dark red and tile red and spotless or found at the top of the base; stigma is smooth and the middle is a bit sharp, the edges are smooth, convex; the bud is brown, the shape of bud is oblong, the surface of bud is reticulate; the filaments are linear, thethers are linear or oblong, dark purple; stomas are amphistomatic and thick in the bottom epiderma; the pollens are dark lilac, the kind of pollen is triporate, rarely tricolpate, the shape of pollen is generally spheroid.

P13-024 Differential protein expression of Conference pear slices submitted to gas stresses

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The pear variety ‘Conference’ is the most important cultivar in Europe. A two dimensional differential in gel electrophoresis (2DE-DIGE) approach was undertaken to study the metabolic responses of Conference pears slices submitted to high oxygen, high carbon dioxide or anoxia compared to control or optimal storage conditions. Selection of proteins for LC-ESI-MS/MS identification for a biological interpretation was based on two independent statistical approaches. The univariate statistical included one-way ANOVA together with the false discovery rate methodology (q value) to account for false positives. The multivariate approach included partial least squares discriminant analysis and the variable importance in projection methodology for the selection of the most relevant proteins. Both approaches taken independently revealed 50 confirmed proteins involved in responses to specific gas stresses. By combining both statistical approaches not only differences in terms of absolute expression were revealed but also in terms of small changes and correlations. Anoxia led to an up-regulation of enzymes such: alcohol dehydrogenase and transketolase. Allergenic proteins, molecular chaperones and proteins involved in trafficking such as vacuolar ATP synthases were down regulated under anoxia. In conclusion, short term exposure of pear slices to gas stresses showed to be relevant in several metabolic processes which in turn might end up in the appearance of physiological processes.

P13-025 Inference of transcription factor target genes with condition-dependent Bayesian networks

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One of the key interests in biological research is the inference of a gene regulatory network, especially the local structure with respect to a certain gene under inspection. Here we model the local regulatory network of the gene RCD1 in A. thaliana using condition-dependent Bayesian networks. RCD1 is a protein of unknown function which has been found to interact with several transcription factors in our yeast 2-hybrid experiments. We perform a meta-analysis that combines publicly available microarray data from several experimental conditions to predict sets of genes that are co-regulated by RCD1 and the interacting transcription factors.

P13-026 Finding PIN1 interacting proteins using the Y2H membrane split ubiquitin system

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PIN proteins play an important role in auxin transport. Polar localization of PIN proteins is relatively well understood. In contrast, the underlying protein-protein interactions remain uncharacterized. Clues to the function of a newly discovered gene product can be obtained by investigating its interaction with other proteins. To screen for proteins which interact with members of the PIN protein family, the split-ubiquitin membrane Y2H system was established and optimized. The system is based on the ability of the N- and C-terminal halves of ubiquitin to reassemble into a functional moiety when brought into close proximity, leading to the release of a reporter enzyme. AtPIN1 was fused to the C-terminal half of ubiquitin and used as a bait to screen a normalized Arabidopsis cDNA library fused to the N-terminal half. A range of interactors, currently being validated by BiFC and co-IP were found, illustrating the pathway of PINs to and from the plasma membrane.

P13-027 Cloning and characterization of miRNAs and endogenous siRNAs from grape
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The molecular determinants of grape berry development are poorly known, but its strictly regulated metabolism point to a possible role of miRNAs in grape berry ripening. We have constructed a small RNA library from low MW-RNA extracted from mixed-stage grape berries. About 150 plasmids from this library were sequenced, yielding about 900 small RNA clones of average length 22 nt. Thirteen miRNAs belonging to 15 conserved different families were isolated. The expression of vv-miR160, vV-miR164 and miR167, which target genes involved in the auxin signal, was reduced in the berries comparing to leaf and root. Seven grape-specific miRNAs were isolated and their expression was profiling in root, leaves and berries at three stages of development. Target genes were predicted and validated for six of these miRNAs, and included different NBS-LRR R-like proteins, a heavy metal ion transport/detoxification protein and an AMP-dependent ligase protein. An endogenous siRNA matching a cyokinin synthase gene transcript in antisense orientation was isolated and shown to be specifically expressed in mature berries. Degradation fragments from the cyokin synthase transcript were mapped by 5' RACE and found to be arranged in a 21-nucleotide phase register starting from the predicted siRNA-guided cleavage site. Endogenous siRNAs that matched in sense orientation to a functional moiety when brought into close proximity, leading to the release of a reporter enzyme. AtPIN1 was fused to the C-terminal half of ubiquitin and used as a bait to screen a normalized Arabidopsis cDNA library fused to the N-terminal half. A range of interactors, currently being validated by BiFC and co-IP were found, illustrating the pathway of PINs to and from the plasma membrane.

P13-028 Endogenous peptides from the Physcomitrella patens moss
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Peptides are major signaling molecules in animals and yeasts. In plants, only a few peptides are known that control physiology, growth and development. Full sequencing of genome of the moss Physcomitrella patens (Hedw.) B.S.G. together with recent advances in peptide mass-spectrometry and bioinformatics opened new possibilities for conducting genome-wide search of peptides that perform signaling role in plants. In attempt to clarify the role of peptides in moss physiology we started our studies on isolation, identification and further elucidation of physico-chemical and biological properties of peptides from protoplasts, protonemata and gametophores of Physcomitrella patens. We have elaborated the strategy of extraction and fractionation of peptide-containing material from moss protoplasts and tissues. Total mass-spectral analysis by MALDI-TOF-TOF of HPLC fractions revealed over 400 individual components with molecular masses below 10 kD. Individual fractions were analyzed by LC-MS-FTI CR mass-spectrometry. A number of moss endogenous peptides has been sequenced mainly derived from chloroplast proteins.

P13-029 Genomic resources at national center for biotechnology information
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The National Center for Biotechnology Information (NCBI) provides integrated systems for storage, analysis and retrieval of data pertaining to genomes, genes, and proteins. The most widely used interface for the retrieval of information is the Entrez system that enables text searches across various databases at NCBI. Map Viewer is the genome browser that allows for the aligned display of different maps of a genome, with many different objects like Genes, STs, markers, and probes. There are currently 41 higher plants, two algae and the nucleomorph genomes of Guillardia theta and Hemiselmis anderseni displayed in the Map Viewer. A specialized plant query in Map Viewer allows for all the plants to be queried and the output displayed as aligned maps of different plants. Genome Project database is a collection of all large scale sequencing and mapping projects that allows for the display of project specific data and provides for the status of the various sequencing projects. In addition to organism specific BLAST for plants that have their genomes sequenced, there is PlantBLAST for BLAST against accessions associated with mapped loci and plant EST BLAST that BLASTs against ESTs from those plants with more than 50 000 ESTs. Plant Research in the context of these resources will be discussed.

P14-011 Plant and mammalian calreticulin exhibit similar functions
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The chaperone calreticulin (CRT) plays important roles in a variety of processes in the ER of animal cells, such as Ca\(^{2+}\) signaling and protein folding. Although the functions of CRT are characterized in animals only indirect evidences are available for plants. To increase our understanding of plant CRT’s we introduced one of the Arabidopsis isofoms, AtCRT1a, into CRT deficient (crt\(^{-/-}\)) mouse embryonic fibroblasts. As a result of CRT deficiency the mouse crt\(^{-/-}\) cells have decreased levels of Ca\(^{2+}\) in the ER and impaired protein folding abilities. Expression of the AtCRT1a in mouse crt\(^{-/-}\) cells rescued these phenotypes, that is AtCRT1a restored the Ca\(^{2+}\)-holding capacity and chaperone functions in the ER of the mouse crt\(^{-/-}\) cells, demonstrating that the animal sorting machinery also was functional for a plant protein, and that basic CRT functions are conserved across the Kingdoms. Expression analyses using a CLUS-AtCRT1a promoter construct revealed high expression of CRT1a in root tips, floral tissues and in association with vascular bundles. To assess the impact of AtCRT1a, we generated Atcrt1a mutant plants. The Atcrt1a mutants exhibited increased sensitivity to the drug tunicamycin, an inducer of unfolded protein response. We suggested that AtCRT1a is an alleviator of the tunicamycin-induced unfolded protein response, and propose that the use of the mouse crt\(^{-/-}\) fibroblasts as a CRT expression system may prove useful to assess functional differences of CRT’s from different species.

### Abstracts

**P14-012** Confocal microscope as a tool for the study of chloroplast avoidance movements in Arabidopsis  
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Upon strong illumination, chloroplasts tend to move away from light-exposed areas of the cell to avoid photodamage. This phenomenon, induced by blue light via the phototropin signalling pathway and known as the chloroplast avoidance movement, has been studied in a number of models ranging from algae to Arabidopsis. Although chloroplast avoidance movements can be observed even macroscopically in plants exposed to specific light regimes (Oikawa et al. 2003, Plant Cell 15: 2805), dedicated custom-built equipment is nowadays usually used in studies of this reaction, such as modified spectrometers (Trojan and Gabrys, 1996, Plant Physiol 111: 419) or microbeam illuminators (Kagawa and Wada 2000, Plant Cell Physiol 41: 84). We have successfully used a modified FRAP protocol on a standard Leica TCS SP2 confocal microscope to induce and document the chloroplast avoidance reaction in Arabidopsis leaves after localized illumination with blue laser light in the range between 405 and 488 nm. These observations open a possibility to use the sophisticated but relatively widely available confocal microscopy technique to examine chloroplast avoidance on single cell (and single chloroplast) level, including pharmacological studies and characterization of mutants. This work was supported by the MSM 0021620858 and MSM LC06004 projects.

**P14-013** In vivo visualization of secGFP-CesA6 sorting in tobacco protoplasts  
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The aim of this work was to visualize the in vivo sorting of an Arabidopsis cellulose synthase catalytic subunit (CesA6) fusing to the Green Fluorescent Protein (GFP). The fluorescent pattern of the chimera secgfp-CesA6 was followed in transiently transformed tobacco protoplasts. 18 hours after transient expression, confocal observations showed that secgfp-CesA6 labelled ER, highly motile dictiosomes and the plasma membrane. Test of plasmolysis and co-localization with a plasma membrane specific dye FM4-64 confirmed the localization of secgfp-CesA6 in plasma membrane. Experiments with cycloheximide showed that the final destination of secgfp-CesA6 was plasma membrane and dictosomes. To validate the chimera functionality, wt and secgfp-CesA6 transformed protoplasts were incubated in the presence of D-[U-14C]glucose. The remarkable differences between the amount of newly synthesised cellulose in wt and transformed protoplasts showed that secgfp-CesA6 was a functional fusion protein. Analyses of RNA expression levels showed that secgfp-CesA6 overexpression determined an increase of CesA1 RNA expression level. CesA1, CesA3 and CesA6 are the cellulose synthase subunits that interact for a stable complex formation and are involved in the cellulose synthesis of the primary cell wall. The results showed that secgfp-CesA6 was correctly inserted into the plasma membrane interacting with other subunits to form a functional cellulose synthase complex.

**P14-014** Determination of genetic diversity in populations of Iranian Aegilops tauschii using karyotypic studying  
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_Aegilops tauschii_ is an herbage, diploid and autogame plant that is origin of D genome in _Triticum aestivum_. The studied Iranian populations were seven and three populations related to _Aegilops tauschii_ and _Aegilops strangulata_, respectively. In order to cultivation of seeds, we used filter paper on plate to produce root tips. Selected roots with 1.5–2.5 cm length were used for slide providing from root tips. Some chromosomal traits were calculated such as: length of short arm and long arm, total length of chromosome, relative length of chromosome and arm ratio. In this research, _A. tauschii_ and _A. strangulata_ were diploid and their base chromosome numbers were seven. Karyotypic formula in these populations was metacentric and submetacentric. According to A1 and TF%, population 840 related to _A. strangulata_ had more asymmetric chromosomes. Also population 641 related to _A. tauschii_ had the symmetric chromosomes and the other populations had an average level in respect of symmetry. Cluster analysis based on morphological data and comparison of theses clusters with sources of populations, indicated that no relationship was observed between clustering and geographical regions of populations. _A. tauschii_ and _A. strangulata_ didn’t differ from chromosomal number and indices, because they didn’t locate in separately groups after cluster analysis.
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P14-015 Role of ABI3 interacting protein 2 (AIP2) in root development in Arabidopsis
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AIP2 is an E3 ubiquitin ligase from Arabidopsis, which regulates protein stability of the transcription factor ABI3 during germination. Added evidence showing that AIP2 is expressed in other tissues, suggest that AIP2 also affects other biological processes. In this work we studied the potential role of AIP2 in root development. We analyzed transgenic plants expressing AIP2 promoter::GUS fusion, to characterize the exact timing and localization of AIP2 expression in roots. Our results demonstrate that after elongation begins, AIP2 is expressed in the epidermis of the main root. Immediately after germination, AIP2 expression is localized in root hairs and the root-stem junction, and later changes to epidermal cells localized along the main root, always excluding the root tip. Due to the relevance of auxin to root development, we also present the effect of NPA (auxin transporter inhibitor) and auxin treatments in the expression of AIP2. To explore the biological relevance of this expression pattern, we analyzed and quantified the root phenotype of 35S:AIP2 and aip2 mutant plants and confirmed AIP2’s role in root development. Finally to gain insight into the molecular action mechanism of AIP2, we performed a two hybrid and a TAP screening from which we isolated several AIP2 interacting proteins that are currently under analysis.

P14-016 Plant subcellular dynamics prefigure arbuscular mycorrhizal colonization of the root cortex
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Arbuscular mycorrhizas (AM) are widespread, ancient endosymbiotic associations that contribute significantly to soil nutrient uptake in plants. Initial fungal penetration of the host root is mediated via a specialized cytoplasmic assembly called the pre-penetration apparatus (PPA), which directs AM hyphae through the epidermis. In vivo confocal microscopy studies performed on Medicago truncatula and Daucus carota, host plants with different patterns of AM colonization, now reveal that subsequent intrarootal growth across the root outer cortex is also PPA-dependent, while inner root cortical colonization and arbuscule development involve more complex PPA-related mechanisms. In particular, a striking alignment of polarized PPAs can be observed in adjacent inner cortical cells of carrot, correlating with the intrarootal root colonization strategy of this plant. Ultrastructural analysis of these PPA-containing cells reveals intense membrane trafficking and nuclear enlargement and remodeling, typical features of arbusculated cells. Taken together, these findings imply that pre-penetration responses are both conserved and modulated throughout the AM symbiosis as a function of the different stages of fungal accommodation and the host-specific pattern of root colonization. We propose a model for intracellular AM fungal accommodation integrating peri-arbuscular interface formation and the control of functional arbuscule development.

P14-017 Protein-protein interaction in membranes stacking phenomena in thylakoids and artificial systems
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The stacking phenomena of thylakoid membranes isolated from two plant species with different susceptibility to chilling – bean (Phaseolus vulgaris) – CS (chilling sensitive) plant and pea (Pisum sativum) – CT (chilling tolerant) plant were investigated in vitro as a function of Mg2+ concentration. An attempt was made to characterize protein-protein interaction in stacked and non-stacked thylakoid membranes isolated from bean and pea and in stacked and non-stacked artificial systems of liposome. Liposome containing chloroplast membranes’ lipids, such as MGDG, DGDG, PG, and incorporated LHCCI were use as a semi-lamellar system to study by FTIR method. FTIR spectra of biological and artificial membranes were analyzed in the Amid I region providing data on membrane proteins’ secondary structure. Especially the level of β-sheet compound of Amid I spectra indicates a degree of membrane proteins aggregation. Experiments revealed that a reorganization of the biological membrane structure caused by changes in Mg2+ concentration is different in pea and bean. Comparison of the type of protein-protein interaction applied for artificial, less complicated systems and native, biological thylakoid membranes gave a possibility of determination specific thylakoids organization in vivo.

Acknowledgements – This work was supported by University of Warsaw intramural grant BW #1755-18 (KG).

P14-018 Impact of glutathione reduction on cellular redox homeostasis in Arabidopsis
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The redox state of glutathione and its exchange between compartments need to be known to understand redox homeostasis in relation to reactive oxygen species and signal transduction. To this end Arabidopsis T-DNA mutants were used in combination with a redox-sensitive GFP (roGFP) as a probe to analyse glutathione and redox homeostasis. To investigate the role of GRs in the three compartments, T-DNA insertion lines of the two GR genes of Arabidopsis were characterized. GR1 encodes plastid and mitochondrial GR by way of a dual target sequence. A null allele of gr1 was embryo lethal, while inactivation of gr2 encoding cytosolic GR2 caused no visible phenotype. The cytosol of gr2 plants had significantly increased contents of oxidized glutathione and consequently a lowered glutathione redox potential in the cytosol as shown by ratiometric fluorescence measurement of roGFP targeted to the cytosol of gr2 plants. The role of GR1 in organelle redox homeostasis was investigated by supplementation of the gr1 mutant with plastid- or mitochondria-specific constructs. Expression of GR1 only in plastids was sufficient for survival while exclusive targeting to mitochondria was not. Thus,
P14-019 Flavonoids redirect PIN-mediated polar auxin fluxes during root gravitropic responses
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The rate, polarity and symmetry of the flow of the plant hormone auxin are determined by the polar cellular localization of PIN-FORMED (PIN) auxin efflux carriers. In roots, gravitropism is a result of the asymmetric auxin distribution to the lower side of epidermal cells. However, the regulation of auxin transport during root gravitropic responses is still unclear. Flavonoids, a class of secondary plant metabolites, have been suspected to modulate auxin transport and tropic responses. Nevertheless the identity of specific flavonoid compounds involved and their molecular function and targets in vivo are essentially unknown. Here we show that gravitropic pin2/eir1/wav6/agr1 roots have altered patterns of flavonol glycosides. Application of non-inhibitory concentrations of flavonols to pin2 roots is sufficient to restore root gravitropism. By employing a quantitative cell-biological approach, we demonstrate that flavonoids restore the formation of lateral auxin gradients in the absence of PIN2. Chemical complementation by flavonoids strictly correlates with an asymmetric re-distribution of the PIN1 protein. Pin2 complementation does not result from inhibition of auxin efflux, as supply of the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) failed per se to restore pin2 gravitropism. We propose that flavonoids promote asymmetric PIN shifts upon gravity stimulation, thus redirecting basipetal auxin streams necessary for root bending.

P14-020 Changes in the functioning of the WMC continuum in suspension-cultured tobacco by-2 cells adapted to hyperosmotic conditions
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A special case of the cell’s response to stress is the adaptation to conditions that are lethal to non-adapted cells. We have applied a stepwise approach that allowed prolonged adaptation of suspension-cultured tobacco BY-2 cells to altered osmotic conditions, evoked by ionic (NaCl, KCl) or nonionic agents (mannitol, sorbitol, polyethylene glycol) at high concentrations. Nonionic and ionic osmotaic act in different manner inducing specific responses of adapted cells, that differ from responses of cells transiently exposed to the osmotic stress. Ionic agents increase adhesive properties of the cells, and formation of cell aggregates, whereas nonionic agents stimulate ordered cell divisions and thus induce formation of cell files. Composition of proteins and polysaccharides in walls of adapted cells is specifically modified, whereas organization of cytoskeleton seems to be unaffected. Several cell wall modifying enzymes were overexpressed exclusively in adapted cells. It seems that upon prolonged exposure to osmotic stress conditions adaptive alterations in cell wall composition are occurring. This might change anchoring of the cytoskeleton in the walls and modify functioning of the whole cell wall-plasma membrane-cytoskeleton continuum. In that way, cell’s mechanical balance restoration will be ensured and cell will be able to resist osmotic pressure. This research was funded by the Polish Ministry of Science and Higher Education grant PBZ-KBN-11/10/P04/2004.

P14-021 Effects of global inhibition of DNA methylation/histone deacetylation on the gene expression during transdifferentiation into tracheary elements
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To understand the relationship between the dynamics of chromatin and the profile of gene expression during differentiation of plant cells, we have used a Zinnia elegans cell culture system, in which isolated mesophyll cells transdifferentiate into tracheary elements. Treatment of Trichostatin A (TSA) or 5-azacytidine (AzaC) as inhibitors of histone deacetylase and DNA methyltransferase, respectively, suppressed the transdifferentiation into tracheary elements depending on the concentration of inhibitors. Then we performed GeneChip analyses using newly developed Zinnia GeneChip to reveal changes in the global gene expression patterns in the inhibitor-treated cells and found sixty-three transcription factor genes were up-regulated over two-fold after 6 h of TSA treatment. Based on these results we discuss the gene regulation in relation to histone modification during tracheary element transdifferentiation.

P14-022 Nitric oxide (NO) donor mediates Arabidopsis thaliana root development via cortical microtubules reorganisation
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NO in plants mediates the processes ranging from growth and development to biotic and abiotic stress responses. Cytoskeleton components, particularly microtubules, are involved in the majority of those processes. It was shown later that α-tubulin can be identified as the major target of C-nitration of tyrosine residues in animal cells. Functional role of plant tubulin nitrotyrosination remains unknown. Our experiments were aimed to in vivo study of the effects of SNP, NO-donor sodium nitroprusside (10, 100, 250 and 500 μM) on cortical microtubules (CMTs) organization in root cells of Arabidopsis thaliana line expressing (GFP-MAP4) in time range of 3, 6, 12, 24, 48 and 72 h. The application of all tested SNP concentrations during 24 h caused the enhancement of primary root growth, while prolonged treatment (48–72 h) resulted in its significant inhibition. Treatment with 250 and 500 μM SNP during 24 h stimulated the root hairs initiation in trichoblasts. SNP treatment with the same concentrations (48–72 h) initiated root hair number increase, and also lateral and adventitious roots formation. Alterations of native CMTs orientation in both epidermal and cortical cells of different root zones to randomized, oblique or longitudinal in time- and space.
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dose-dependent manner were observed. We suppose that NO is one of intracellular triggers of cell differentiation mediated with participation of CMTs reorganization.

P14-024 Role of arabidopsis β-N-acetylhexosaminidases in the formation of paucimannosidic N-glycans

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Plant glycoproteins contain substantial amounts of paucimannosidic N-glycans lacking terminal GlcNAc residues at their non-reducing ends. It has been proposed that this is due to the action of β-hexosaminidases during late stages of N-glycan processing or in the course of N-glycan turnover. We have now cloned the three putative β-hexosaminidase sequences present in the Arabidopsis thaliana genome. When heterologously expressed as soluble forms in Spodoptera frugiperda cells, the enzymes (termed HEXO1-3) could all hydrolyze the synthetic substrates pNP-GlcNAc, pNP-GalNAc, MU-GlcNAc and MU-GlcNAc-6SO4, albeit to a varying extent. HEXO1-3 were further able to degrade chitotriose-PA, whereas chitobiose-PA was only cleaved by HEXO1 and HEXO3. With N-glycan substrates, HEXO1 and HEXO3 displayed a much higher specific activity than HEXO2. To get an idea of the in vivo function of the different HEXO enzymes β-hexosaminidase activity was analyzed in different hexo knockout plants. In planta, HEXO1 and HEXO3 could hydrolyze pNP-GlcNAc as well as N-glycan substrates, whereas HEXO2 did not display any specific activity. Subcellular localization studies with HEXO-fluorescent protein fusions showed that HEXO1 is a vacuolar protein. In contrast, HEXO2 and HEXO3 are located at the plasma membrane. These results indicate that HEXO1 participates in N-glycan trimming in the vacuole, whereas HEXO2 and/or HEXO3 could be responsible for the processing of N-glycans present on secretory glycoproteins.

P14-025 Anatomical studies on the genus Limonium (Plumbaginaceae) in Turkey

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The genus Limonium Mill. (the sea lavenders), the largest genus of the family, has a worldwide distribution with the largest number of species found particularly in the Mediterranean as well as in the saline habitats in the Irano-Turanian Phytogeographic region where they have an important role in the coastal ecosystem. In Turkey, as a result of recent revisional study carried out recently Limonium covers 23 species, 13 of which are endemic to Turkey. These species were classified under five sections, namely sect. Pteroclados (including L. sinuatum), sect. Limonium (incl. L. vanense, L. caspium, L. smithii, L. davisii, L. didimense, L. guenerii, L. gmelinii, L. angustifolium, L. meyeri, L. eufusum, L. virgatum, L. graecum, L. sieberi, L. bellidifolium, L. iconicum and L. tamaricoides), sect. Sphaerostachys (incl. L. lilacinum, L. pycnanthum ve L. globuliferum), sect. Serophyllum (incl. L. anatomicum and L. oxyphilum) and sect. Schizyphymenum (incl. L. echioides). The main aim of the work is to illustrate the anatomical properties of the three endemic species in relation with their habitat preferences. So far, L. eufusum, L. iconicum and L. smithii have been chosen for achieving the objectives of the study. Therefore, an attempt is made to illustrate the main anatomical properties revealed by means of using paraffin method. The results of this study show that these three species are quite distinct from each other on the bases of their leaf and stem anatomies.

P14-026 Cell-wall redox activities along growing shoots of maize and arabidopsis

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Apoplastic [H+] content and enzymatic activities catalyzing its production and utilization were estimated in the course of baspetal decrease of cell elongation rate in maize mesocotyls and arabidopsis internodes. [H+] was measured using 1) enzymatic method (HRP-catalyzed oxidation of DMAB and MBTH), 2) FOX method with xylene orange reagent. Peroxidase activity was examined with guaiacol. NADH, oxalate, polyamine oxidase activities were estimated by the rate of [H+] production. CW plastic extensibility was...
estimated by the rate of CW creep in vitro; elastic extensibility – by the extent of frozen-thawed tissue contraction. $f_{\text{h}}$ had ambivalent action on CW extensibility. At concentration 0.1–10 mM, it stimulated peroxidase-mediated formation of oxidative cross-links between polymers that led to CW rigidification. At higher concentrations, $f_{\text{h}}$ increased CW extensibility, causing production of hydroxyl radical, which disrupted CW polymers. Basipetal decrease of cell elongation rate was accompanied with CW rigidification, increase of CW peroxidase activity and decrease of apoplastic $\text{H}_2\text{O}_2$. Activity of enzymes catalyzing $f_{\text{h}}$ production did not substantially change along growth zones. It could be supposed that basipetal decrease of cell elongation rate was connected with gradual acceleration of peroxidase-catalyzed oxidative reactions leading to CW rigidification. Work was supported by RFBR Grant 08-04-00566-a.

P14-027 The role of ADP-ribosylation factor 1 in redistribution of membrane proteins between the Golgi and ER in tobacco leaf epidermal and BY2 cells

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ADP-ribosylation factor 1 (ARF1), a small GTP-binding protein, has been implicated in the formation of COP-coated vesicles and ARF-GEFs which carry a catalytic sec7 domain, are a target of BFA. We have investigated the interaction between ARF and BFA and the effect of a dominant inhibitory ARF [TN] mutant on Golgi-ER transport in tobacco epidermal and BY2 cells. Our data show that N-ST-GFP and GmMan1-RFP markers were transported to Golgi in tobacco leaves and BY2 cells when expressed with wild type ARF1 or ARF1 [QL]. Most of the N-ST-GFP and GmMan1-RFP markers were redistributed in ER when leaves or BY2 cells were infiltrated with BFA or ARF-TN mutant DNA constructs. The BFA effect was suppressed when the tobacco leaves expressed with either ARF1 wild type or ARF1 [QL]. ARF1 wild type and ARF1 [QL] also rescued the ARF1 [TN] phenotype in both tobacco leaves and BY2 cells. Co-expression of either N-HDEL-GFP or N-Sec-YFP with ARF [TN] mutant in tobacco leaves resulted the appearance of an Endo H resistant population of GFP and YFP. This effect was overcome when leaves were co-expressed with ARF wild type or ARF [QL] mutant and was enhanced by low concentrations of BFA. Additionally, the expression of ARF1 both at mRNA and protein level in the roots, nodules and shoots of Medicago truncatula, a model leguminous plant, has been studied. The role of ARF1 in retrograde traffic will be discussed in the light of recent models put forwarded for vesicular trafficking in plant cells.

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P14-029 Cell biological approaches to understand stomatal receptor function
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In addition to leucine-rich-repeat (LRR) receptor-like kinases, plants also possess a large number of proteins lacking the kinase domain. These LRR-receptor like proteins (RLPs) are involved in regulation of many aspects of plant development including response to pathogens (CT-9), meristem development (CLV2) and stomatal patterning (TOO MANY MOUTHS (TMM)). The TMM eLRR-RLP is plasma membrane protein localized in specific stomatal lineage cells. TMM plays central role in the decision of which cells enter the stomatal pathway and also regulates stomata patterning. Mutation in this gene results in developmental aberrations within the leaf epidermis including excessive stomata. In stems, however, tmm-1 mutants have no stomata. The mechanisms controlling TMM functions in different organs are unknown. To fill this gap we performed genetic screens and identified suppressors of tmm-1. Preliminary analysis suggests that one of these suppressors is a regulator of vesicular trafficking. This points toward novel mode of regulation of the TMM receptor at the cellular level. As we continue our analysis of this suppressor, we have also taken a complementary approach to better characterize cellular behavior at many stages during stomatal development. We used stomatal lineage specific promoters to visualize different endomembrane compartments within the stomatal lineage. This will allow us to investigate relationships between cell division and cell fate acquisition during stomata development.

P14-030 Analysis of Arabidopsis thaliana exocyst complex using blue native PAGE method
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A blue native polyacrylamide gel electrophoresis (BN-PAGE) is based on the mild solubilization in detergent and treatment with the dye...
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Coomassie Blue G250, which adds negative charge to proteins promoting their unidirectional mobility in the electric field. BN-PAGE has been the method of choice for study of high molecular weight protein complexes in different organisms, including plants. We wanted to test if this method could be used to unravel the structure of A. thaliana exocyst, from other organisms known to be an octameric protein complex involved in targeting and tethering of secretory vesicles to the plasma membrane. Using a dodecylmaltoside (DDM) as a detergent for solubilization of cell suspension proteins, we were able to visualize co-migration of the SEC3, SEC6, SEC8 and EXO70A1 subunits. After the solubilization with mild detergent concentrations (total protein (TP):DDM ratio of 1.33) and low NaCl concentrations (10 mM and 30 mM), the range of high molecular mass complexes of approximately 300–700 kDa was detected for tested exocyst components. Under the conditions of high detergent concentrations (TP:DDM ratio of 0.67) and no salt added, SEC6 and EXO70A1 seemed to be present in small complexes, while the SEC3 seemed to stay in high molecular weights complexes. The fact that a range of high molecular weight complexes, not a discrete one, was detected by BN is according to our opinion the consequence of the high dynamicity of exocyst structure in plant cells.

P14-031 Probing endocytosis with FM-dyes in plants: tracking or dragging?
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Amphiphilic styryl dyes of FM family (FM1-43, FM4-64 and FM5-95) are very often used in tracking endocytosis in plants. Their application to plant tissue or cell cultures results in their insertion into plasma membrane (PM), where they start to be fluorescent after incorporation into lipid bilayer. Depending on cell type, they are quickly internalized into cells by active processes of endocytosis followed by their incorporation into endomembrane system including tonoplast and the whole PM recycling machinery. Here we show that besides tracking endocytosis in plant cells FM-dyes FM4-64 and FM5-95 but not FM1-43 stimulate transient invagination of plasma membrane vesicles containing PM-integral proteins (PIP2-GFP, PIN1-GFP and others). Treatment with specific inhibitors of clathrin-mediated endocytosis (dynasore and tyrphostin A23) suggested the involvement of this type of endocytosis in the process(es) triggered by FM dyes. This work was supported by the Grant of the Ministry of Education, Youth and Sports of the Czech Republic, project no. LC06034, by the Grant Agency of the Academy of Sciences of the Czech Republic project no. KJB601080604 and Grant Agency of Charles University project no. 43-25245S.

P14-032 UDP-glucose dehydrogenase: an important enzyme for plant cell wall biosynthesis
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Plant primary cell walls contain large amounts of hemicellulose and pectins. 50% of them are synthesised via the conversion of a common precursor UDP-glucuronic acid into sugar nucleotides such as UDP-galacturonic acid or UDP-xylene. The purpose of my study is to better understand the enzyme UDP-glucose dehydrogenase which catalyses the oxidation of UDP-glucose into UDP-glucuronic acid. This enzyme has a crucial role in the formation of the cell wall. One tool for the comprehension of UGD was the establishment of single and double T-DNA Arabidopsis knockout mutants for the four existing isoforms. Single mutants show little visual phenotypic differences compared to wildtype plants. Therefore we focus on double knockouts: ugd1ugd4 is bigger than the wildtype and shows thinner, stretched cell walls, whereas ugd2ugd3 has a dwarf plant phenotype, dark green leaves, reduced root-lengths, longer life cycles and low reproduction rates. Its cell wall composition displays a significant reduction of galacturonic acid, xylose and arabinose. In contrast, ugd1ugd4 shows minor changes compared to wildtype. This suggests a distinct role of each UGD-isoform for normal plant development. Immuno-fluorescence using monoclonal antibodies against cell wall epitopes and protein activity measurement are being done to get a better idea of the characteristics of these mutants.

P14-033 PIPK family in the moss Physcomitrella patens. PpPIPK1 is required for normal cell growth and differentiation
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Phosphoinositides (Pis) are minor lipids in eukaryotic cells but play a major role in many cellular processes. Phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P2] plays a key role in PI metabolism not only because is the precursor of inositol-1,4,5-trisphosphate, dia-cylylglycerol and PtdIns(3,4,5)P3, but also due to its involvement in several cellular processes such as exocytosis, cytoskeletal regulation and intracellular vesicular trafficking. We are focusing on phosphatidylinositol bisphosphate kinase (PIPK), which catalyzes the production of PtdIns(4,5)P2. P. patens has two PIPK genes, PpPIPK1 and PpPIPK2, with differently regulated expression and protein sequences displaying a conserved PIPK catalytic domain and eight MORN (Membrane Occupation Recognition Nexus) domains in accordance with the description of PIPKs class II/B in higher plants. In vitro biochemical characterization showed that the two enzymes exhibited different substrate specificities. Interestingly, PpPIPK1 can synthesize PtdIns(3,4,5)P3, a PI which has not yet been detected in plant cells. In order to study the physiological role of these proteins, we have disrupted PpPIPK1 and PpPIPK2 by gene targeting and our preliminary results show a strong phenotype for pippk1 but not for pippk2. Pippk1 lines are delayed in growth, protonemal filaments show irregular branching patterns, and gametophores are impaired in rhizoid development. Our data support an essential role for PpPIPK1 in cell growth and differentiation.

P14-034 Use of protein-based nanosensors for monitoring metabolite fluctuations in vivo
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Self-reporting cells will be an important tool to monitor metabolite fluxes in a plant non-invasively, since they will uncover how plant cells are adjusting their cytosolic metabolite concentrations to a set value. Metabolite homeostasis is maintained by transport processes at plasma membrane and tonoplast. Uptake and release of metabolites are not only controlled by metabolite-specific transporters, but also by the activity of the proton pump energizing them. This control has a high significance for root hairs, endodermis and xylem parenchyma which are key interfaces for uptake, long distance transport and partitioning of nutrients. In sequence they coordinate the flux of e.g. inorganic phosphate (Pi) from root to leaf cells. The role of plasmodesmata in regulating symplasmic transport between key interfaces is basically unknown. In order to develop self-reporting cells for Pi, we have designed low and high affinity nanosensors, consisting of two GFP variants coupled to a bacterial binding protein. Changes in the efficiency of fluorescence resonance energy transfer allowed us recording changes in Pi in vitro and in vivo. For monitoring apoplastic and intracellular pH values, we have coupled a pH-sensitive GFP variant to an insensitive one. Ratiometric fluorescence changes indicated transient pH modulations. The nanosensors were incorporated into cells at key interfaces using protein transduction domains as well as different transient transformation methods.

P14-035 Closed unequal division of nuclei, precedes incomplete cytokinesis, cell cooperative totipotency and PCD in palisade parenchyma
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The plant parenchyma cell is particular because of its totipotency, i.e. the ability to unlimited change of the developmental program – both to develop an entire plant and to carry out several ways of cell death. Young, expanded and yellow leaves of tobacco (Nicotiana tabacum L.) plants, grown under controlled conditions were analysed by light, confocal laser scanning and transmission electron microscopy. The nuclear division in parenchyma cells is typical with compulsory presence of intact nuclear envelope – like to ancient closed mitosis. An unequal division of nuclei into two or more different parts involving dictyosomes in leaf parenchyma resembles both amitosis and neosis typical for the stem cells, tumorgenesis and PCD. Cytokinesis in parenchyma is particular with difference and subordination of daughter cells due to asymmetric outset of cell plate during anticline division and formation cell kins under direct subjection. The development of cell plates and cell walls is variable – centripetal, centrifugal, complete or partial. Lasting intra- and intercellular communication of daughter nuclei by stretched constriction and via vesicular pathway and plasmodesmata is typical after division. Frequently joined twin cells are typical with asynchrony in cell death realised with unequal decrease of nuclei, volume of ER around the nucleus, size, number of chloroplasts and an active vesicular transport among cells.

P14-036 Chloroplast transglutaminases in leaf senescence delay by kinetin
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There is literature indicating the involvement of polyamines (PAs) and transglutaminases (TGases), enzymes catalysing linking of polyamines to proteins, in cascade of reaction leading to apoptotic cell death. Structural modifications of proteins by TGases are a possible reaction in cell death programs. TGases may alter protein function by “cationisation” and crosslinking formation with an obvious structural consequence. These studies have been well-conducted with animal system. In contrast to the considerable number of reports concerning TGase in dying animal cells, almost no information is available regarding these enzymes during programmed cell death (PCD) in plants. We analysed the level of PAs bound to thylakoids and the level and activity of TGases throughout the barley leaf senescence process, which is considered as PCD, retard by kinetin. Increase in the level of PAs and in the level and activity of TGases throughout the senescence was observed. The data also demonstrated the kinetin down-regulation of TGases protein expression. It appears that chloroplast TGases are involved in PCD, similarly to the TGases studied in animal apoptosis. Furthermore, preservation of the low TGases activity by kinetin may represent an important component of the mechanism of kinetin action in the retardation of leaf senescence.

P14-037 Characterization of the CRM1/Xpo1 nuclear export pathway in plants by identifying different classes of interacting proteins in Arabidopsis thaliana
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Fast and efficient transport of macromolecules and complexes across the nuclear envelope is required to exchange materials and information between the nucleus and the cytoplasm. We have previously characterized the ortholog of the vertebrate nuclear export receptor CRM1 in Arabidopsis, Exportin 1 (Xpo1). Analyses of T-DNA insertion lines of the two Xpo1 genes in Arabidopsis provide evidence for overlapping but distinct functions. Vertebrate CRM1 shows a broad cargo spectrum and plays a key role in nucleo-cytoplasmic partitioning of proteins, a fast and versatile regulatory mechanism to control gene expression and signaling. To assess the regulatory potential of nuclear export in plants, we identified Xpo1-interacting proteins, including different transcription factors that contain a nuclear export signal (NES) and shuttle between the nucleus and the cytoplasm. We have previously characterized novel proteins of the plant nuclear transport machinery, and provide evidence for the involvement of Xpo1 in the nuclear export of ribosomal subunits and mRNA in plants. In sum, we present different classes of proteins that interact with Arabidopsis Xpo1 and thereby characterize the Xpo1-dependent nuclear export pathway that is essential in plants. The identification of NESs in different transcription factors and other regulatory proteins clearly reflects a significant contribution of nucleo-cytoplasmic partitioning in different cellular processes and signal transduction pathways in plants.

P14-038 A role of Rab escort protein in Arabidopsis
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Post-translational geranylgeranylation of Rab proteins is necessary for their membrane association and function as regulators of intracellular vesicle trafficking. This modification is catalyzed by Rab geranylgeranyltransferase (RGGT) and Rab escort protein (REP) complex. Only one REP gene exists in Arabidopsis genome. Here, we report studies on the AIREP insertion mutant of A. thaliana. Activity of RGGT was examined in vitro by incorporation of [3H]GGPP to recombinant Rab3a in the presence of 5100 fractions (source of RGGT). The level of radioactivity in mutant was very low (background level) in comparison with wild type plants. This suggests that RGGT was not active when AIREP gene expression was abolished. After addition of recombinant AIREP enzyme activity was partially restored. Ultrastructural studies of mutant organs showed significantly increased number of lipid bodies in the cytoplasm and plastoglobules in the plastids of roots and much higher number of starch granules in the chloroplasts of stems. Comparison of endocytosis of FM 1-43 dye in mutant and wt cotyledons revealed decrease of fluorescence in mutant. This suggests reduced efficiency of vesicular transport when AIREP function is disordered. Phenotype comparison of wt and mutant plants grown on MS medium containing NaCl, mannitol or sorbitol revealed lower tolerance of mutant under the salt and osmotic stress conditions. This work was supported by Ministry of Science and Higher Education grant PBZ/MEiN/01/2006/45.

P14-039 Identification of chaperone networks in the endoplasmic reticulum of Arabidopsis
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The protein disulfide isomerases (PDIs) stabilize intermediate protein conformations through formations and alterations of disulfide bridges, and play an active part in the endoplasmic reticulum (ER) chaperone network. To identify the complete set of PDIs in higher plants, a search for novel family members at the National Center for Biotechnology Information was undertaken. Amino acid sequences were aligned and were used to construct a phylogenetic tree. Expression patterns in different tissue types of Arabidopsis suggest that the PDI family members are ubiquitously expressed throughout the plant. Since the chaperones are part of a large ER folding machinery it appears likely that the proteins do not work as separate entities, but rather as components of complex chaperone networks. Co-expression studies based on labeled microarray data mining was therefore undertaken. Several typical chaperones, such as calreticulin, calnexin and ERp57, appear to form a larger co-expressed entity. Mutants generated of components in this cluster exhibit endoplasmatic stress, retarded seedling growth and constitutive tunicamycin stress, with increased severity correlated to number of silenced genes. Smaller co-regulated networks including less well-characterized chaperones was also identified. These data suggest an interesting formation of specific chaperone matrices in higher plants and may reveal intricate connections to other cellular components.

P14-040 Exudates released by Desmodesmus subspicatus to medium affect cell cycle of the producer
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Conditioned media (CM), containing exudates from cells of D. subspicatus grown one week in a batch culture, revealed autoinduction activity. CM diluted with fresh BBM medium enhanced proliferation of the producer in a dilution-dependent manner and the most effective appeared two-fold diluted CM (CM2). Synchronized cultures of D. subspicatus were used to explain the effects of CM2 on cell cycle of the producer. Growth was monitored by changes of cell volume and dry matter production. The photosynthetic activity was characterized by the chlorophyll fluorescence (OJIP). The timing of the commitment points triggering the reproductive processes and their termination were characterized by the commitment curves and autospores release curves. At the light period, all control cells attained 3 commitment points and 20% of them additionally the forth one. This resulted in a formation of mainly 8 and partly 16 autospores released from the parent cell during the dark period of cell cycle. CM2 markedly increased the number of cells that attained the forth commitment point. Growth rate as well as quantum efficiency of PS II was markedly stimulated by CM2 at the beginning of D. subspicatus cell cycle. Cells quickly produced and released to the culture medium CM factor (CMF) and its maximal activity was observed in the middle light phase of cell cycle. Isolation and preliminary characterization using HPLC-MS has revealed that CMF is a small peptide with molecular weight about 500Da.

P14-041 Imaging of ABC transporter and regulatory immunophilin interaction by bioluminescence resonance energy transfer (BRET)
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Protein-protein interactions are crucial for many biological processes of living cells. The interaction between Arabidopsis ABC transporter ABCB1/PGP1 and its regulatory immunophilin, FKBP42/TWISTED DWARF1 (TWD1), was previously verified in vitro and in planta. One useful technique for studying protein-protein interactions is bioluminescence resonance energy transfer (BRET), a naturally occurring biophysical phenomenon, which can be used for real-time monitoring of the interactions in living cells. Here we report the usage of BRET to monitor relevant functional interaction between ABCB1 and TWD1 in yeast cells. Yellow fluorescent protein (YFP) tagged ABCB1 and Renilla luciferase (rLuc) tagged TWD1 were expressed in yeast cells. ABCB1-YFP and TWD1-rLuc fusion proteins co-localized and were functional as shown by analysis of IAA export. Control experiments using a set of mutant versions of both proteins ensured that BRET signals of ABCB1-YFP/TWD1-rLuc interaction were specific, stable and linear over the time. Interestingly, this interaction quantified by BRET was disrupted by auxin transport inhibitors, like N-1-naphtylphthalamic acid (NPA) and flavonoids. Currently, we are imaging ABCB1-TWD1 interaction by BRET using Arabidopsis and tobacco Bright Yellow-2 cells.

P14-042 Regulation of auxin transport
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Active transport of the essential signaling molecule auxin is essential for plant physiology and development. Many aspects of these are controlled by cell-to-cell or polar auxin transport, which is primarily determined by auxin efflux complexes, characterized by PIN and ABCB (PGP/MDR) auxin exporters. Here, we will summarize recent biochemical and genetic studies indicating that both types of proteins appear to act independently but—at least in certain cell files—perform specific interactions that determine the specificity and direction of auxin efflux. Moreover, we summarize recent progress of ABCB interaction with immunophilin-like FKBP42, TWISTED DWARF1, which functions as a sensor in ABCB-mediated auxin transport. Our data suggest that a combined action of several components forming an auxin efflux complex is needed for the establishment and control of asymmetric auxin fluxes.

Poster Presentations, Topic 15: Water, Minerals and Transport

P15-011 Uptake of metallic nutrients by the traps of carnivorous pitcher plants
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Carnivorous plants use traps to absorb nutrients from prey animals to supplement their mineral nutrition. So far, research has focused on N and P. This study deals with the uptake of three essential metallic elements, i.e., K, Fe and Mn. Traps of four species of pitcher plants (Cephalotus follicularis, Cephalotaceae; Darlingtonia californica, Sarraceniaceae; Sarracenia purpurea and Heliamphora nutans, Sarraceniaceae) were fed with radioactive isotope of these elements. The limit of detection was lowered by the application of highly active, short-lived isotopes (42K and 59Fe) prepared by neutron activation. In case of the trace element Mn, sensitivity was further increased by converting 54Fe into carrier free 54Mn. Uptake was detected and quantified by γ-spectroscopy. K was readily absorbed by all traps of all species. In Cephalotus, Darlingtonia and Heliamphora, more than 80% maximum were taken up within 72 h. Fe and Mn were poorly absorbed by all three Sarraceniaceae. Cephalotus incorporated more than 70% of Fe and up to 100% of Mn. Even traces down to 10–11 g were completely absorbed. Physiological investigations indicate that nutrients are actively absorbed via ion pumps in Cephalotus localised in glands at the bottom of the pitcher. Taking into account typical trapping rates, the K, Fe and Mn content of the prey cannot cover the plants’ need. However, under the highly oligotrophic conditions of the habitat, even small amounts of prey-derived nutrients may result in increased fitness.

P15-012 The effect of foliar application of urea on the composition of amino acids of the must of two grapevine varieties during veraison
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The gradual increase in temperature due to the climatic change is a factor that threatens the quality of the wines. This temperature rise causes changes in the production cycle of the grapevine. The influence of the amino acids on the aromas and final quality of the wines is known, but normally no specific nitrogen fertilization is done and also its repercussions are not well known. The aim of this study is to evaluate the influence of nitrogen fertilization on the amino acid content and other parameters of the must of two grapevine varieties by means of the foliar application of urea during veraison. The research was done on two varieties of grapevine (V. vinifera L.), one red (Merlot) and one white (Sauvignon blanc) during the summer of 2007 at a commercial vineyard. A nitrogen fertilizer treatment was carried out during veraison by means of the foliar application of urea at a rate of 10FU/ha (split up into two applications). The applied urea was isotopically labelled with 15N at a concentration of 1 and 2% respectively. The amino acid content was determined by capillary electrophoresis and δ15N by mass spectrometry. The foliar application of urea produces significant changes in the proline content of both varieties, as well as an increase in total acidity and degree Baumé in the Merlot variety. The increase of δ15N in the plants treated with labelled urea demonstrates the existence of a significant translocation of urea nitrogen towards the berries in both varieties.

P15-013 Positive effects of a plant growth promoting rhizobacteria on root hydraulic properties under salt stress by regulation of aquaporins
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It is largely known that plant growth promoting rhizobacteria (PGPR) enhance plant salt tolerance. However, how PGPR modify root hydraulic properties under control or salt stressed conditions have not been addressed yet. Here, maize plants were inoculated with two different strains of Bacillus megaterium with different origins (Bm1 and Bm2). Under control conditions, inoculation with Bm1 strain enhanced root hydraulic conductance (L), but this did not happen with Bm2. Under salt conditions (60mMNaCl), Bm1 plants showed again higher L than the other two groups of plants. At the same time, Bm1 inoculated plants showed better leaf water status after salt stress than the others treatments. Since L is in part regulated by aquaporins, we analyzed at mRNA and protein levels their regulation. Under control conditions, the expression of ZmPIP2;1 and ZmPIP2;6 was higher in Bm1 roots than in the others. However, under salt stress almost all aquaporin PIP genes analyzed increased their expression in Bm2 roots. This enhancement was confirmed in
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part at protein level. On the contrary, the expression of ZmPIP2;1 decreased dramatically in Bm1 roots. In conclusion, the two different strains had a contrary effect on L under salt stress, as well as on PIP gene expression and abundance. The expression analysis may indicate the pivotal role of ZmPIP2;1 on salt tolerance. At the same time, here it is reported for the first time a positive effect of inoculation with a PGPR on L under salt stress.

P15-014 Regulation of iron acquisition in roots by basic helix-loop-helix transcription factors

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Iron (Fe) is an essential micronutrient. However, despite of being present in high quantities in soils, Fe has a very limited bioavailability. Therefore, to sustain general iron supply of living organisms efficient mechanisms are needed for extracting Fe from the soil. Dicots and nongranimaceous monocots employ a Fe-acquisition mechanism termed strategy I. Strategy I plants display proton extrusion in the rhizosphere, Fe•+ reduction capacity at the root surface due to the activity of a Fe-regulated ferric-chelate reductase such as FRO2 (Robinson et al., Nature 1999), followed by uptake of Fe•+ via the ferrous iron transporter in the root plasma membrane IRT1 (Eide et al., PNAS 1996; Vert et al., Plant Cell 2002). Granimaceous plants on the other hand acquire iron by chelation to phytosiderophores termed strategy II. Previous work in our group has identified the tomato FER and Arabidopsis FIT genes encoding bHLH transcription factor proteins as major regulators controlling Fe uptake in the roots under Fe-deficiency conditions (Jakoby et al., FEBS Lett 2004; Ling et al., Proc Natl Acad Sci USA 2002). FER/FIT activity is induced upon iron deficiency and it is regulated at transcriptional and post-transcriptional level by iron supply (Brumbarova et al., Plant Physiol 2005; Jakoby et al., FEBS Lett 2004). Here, we present novel results regarding the interactions and regulation of these transcription factors in the molecular network that controls iron acquisition in the root.

P15-015 Capacity of some mushrooms species as biosystems for accumulate heavy and rare metals, in order to be used in environmental biotechnologies

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It is necessary today to encrase the efforts of researches in direction to identify those biosystems which are hyperaccumulators for heavy and rare metals in order to be used as instruments in environmental clean biotechnologies. That is the reason of our determinations of chemical content in heavy and rare metals of some mushrooms species autochtonous in forestry ecosystems of Dambovita county: Armillariella tabescens, A. mellea, Fistulina hepatica, Lactarius volenus, Amanita rubescens, A. phalloides, Russula virescens, Macrolepiota procera, Agaricus canepstrius, Pleurotus ostreatus.

The determinations were made by spectrometry advanced method in our own laboratories. It were identified heavy metals as Mn, Fe, Zn, Cu and others, in different concentration from a species to other. It was determined very rare metals as Europium, 11,50% in Pleurotus ostreatus, this fact encouraged us to continue the researches. It is well known that Eu is a metal rarely present in wild organisms, but used today in lasser technology necessary to establish genetical disease as Down syndrom. Finally, by modern statistical methods it will be established the species which are hyperaccumulator for heavy metals in the view to be considered bioindicators and why not one instrument in bioremediation technology, and the species which are hyperaccumulator for rare metals in the aim to be used as natural resources for exploiting them.

P15-016 Influence of foliar urea on antioxidant response and fruit quality of sweet pepper under limited N supply

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The method of N application greatly influences plant quality, through effects on growth and storage of N. Thus, reducing the rate of soil N application combined with foliar N may reduce the amount of N lost to the environment and increase yield by applying N at a critical time. N deficiency invokes oxidative stress in plants as one of the early rapid responses including lipid peroxidation and consequently membrane injury. One aspect, still little studied, is the regulation of the activity of the antioxidant enzymes, by the supply of foliar nitrogen to the plant when low N is applied to the roots. Thus the studied treatments consisted of two different nutrient solutions (standard = 12.5 mM NO3 and deficient = 3.5 mM NO3) to those plants irrigated with the N-deficient solution, 13 g l-1 foliar urea was applied at different frequency: every fortnight, every week, once a week until 150 DAT (days after transplanting) and twice a week after this date (split frequency), and twice a week. Results showed a significant colorimetric fruit response to foliar urea (CIELAB L*a*b*-color space) compared with N-deficient and non-sprayed fruits. Additionally in these fruits, lipid peroxidation and catalase and ascorbate peroxidase activity were significantly higher whilst no difference in lipid peroxidation was found between control and foliarly applied urea at any frequency. Anthocyanins and flavonoids were not significantly affected when urea was applied compared with control fruits.

P15-017 Stimulative effect of HgCl2 on root pressure of Zea mays L.

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We have examined root exudation on excised roots 5–7-day-old etiolated corn sprouts (Zea mays L.) under effect of 10 nM–10 mM HgCl2. No significant changes in the exudation rate were found when the roots were treated with HgCl2 concentrations up to 1 μM. 1–20 μM HgCl2 decreased exudation rate for all time of observation (22 h). The hydraulic conductivity was decreased to 40% for all time. It was suggested that HgCl2 affected aquaporins specifically. 40 μM–1 mM HgCl2 for the first 30 min inhibited exudation rate but later markedly stimulated it in 20 times as much. Root pressure was increased in 10 times. The osmotic pressure of exudate was increased no more than in 1,5 times. The hydraulic conductivity was decreased to 70% for the first 30 min and increased in five times in the moment of exudation rate stimulation. The temperature coefficient of exudation rate (Q10) reached up to 14 in separated experiments to 20 as much. That gives evidence of channel desensitization or any disruptions, e.g. protein denaturation in the moment of stimulation. (>1 mM HgCl2 had immediate stimulatory effect and next exudation drop. These data prove that mercuric-sensitive processes are connected with driving forces of root pressure. The rise in exudation rate was not correlated with that in hydraulic conductivity and osmotic pressure. That could be explained by local changes of osmotic pressure gradient.

P15-018 Identification of novel plant secondary metabolite transporters

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Plants produce a vast number of natural products collectively called secondary metabolites. These may be involved in any of a number of diverse activities, such as mediating interactions with the environment or symbionts, stress responses, protection against pathogens, or internal signalling. Often these compounds have spatially distinct sites of biosynthesis and storage or usage. However, very little is currently known about the specific transporters involved in the translocation of secondary metabolites and their biosynthetic intermediates between these sites. Identification of such transporters has important implications for metabolic engineering of valuable natural products in plants. We have developed a novel functional genomics approach to identify such transporters. A full-length Arabidopsis cDNA library presently comprising c. 250 putative or confirmed transporter genes has been constructed for expression in Xenopus oocytes. This library can then be screened for transport activity in the presence of a substrate. The approach has been validated and used to successfully identify a number of transporters (Nour-Eldin et al. 2006). We have selected a number of secondary metabolites that lack corresponding transporter genes with which to screen our library. These are mainly high-value compounds with relevance to metabolic engineering. Genes identified in the screens as encoding transporters for these metabolites will be presented.

Nour-Eldin et al. (2006) Plant Methods, 2 (17)

P15-019 Variation in roots and rhizosphere: potential for improving resource capture in crop plants

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P15-020 Study of surface waters quality by AAS and TDS measurements

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Water quality is affected by the many substances water contacts during its movement through the hydrologic cycle. Water dissolves a wide variety of minerals, nutrients, and other substances from soils, rocks, and the atmosphere, and carries them in solution. Surface water is an important component of fresh water systems and surface water monitoring is essential to attain a comprehensive understanding of the physical, chemical and biological characteristic of aquatic systems. The impurification of water surface with heavy metals has disastrous effects on environment. In this paper are presented the results of total dissolved solids (TDS) and atomic absorption spectrometry (AAS) measurements of surface water samples from two affluent of Arges River in Dambovita County, Romania. The AAS measurements were performed using an Atomic Absorption Spectrometer with flame AVANTA GBC at Valahia University of Targoviste. The TDS measurements were performed at the sampling sites by means of HACH CO150 conductivity, three months running.

P15-021 Water dynamics in dormant buds of Norway spruce (Picea abies L.) – study by magnetic resonance imaging

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Barley and potatoes are two of the major crops produced in Scotland, but the effects of global environmental change on the sustainability of their production is unknown. The predicted warmer wetter winters and hotter drier summers in northern Europe mean that crop cultivars will need to become more efficient at capturing resources (water and nutrients) in order to maintain current yields or take advantage of the improved conditions for plant growth. Plants which have large root-soil interfaces (e.g. longer thinner roots, more root hairs or symbiotic relationships with mycorrhizae) or improved rhizosphere biochemistry are likely to be more efficient in capturing resources. Currently, little is known about the genetics of root and rhizosphere properties of potatoes or barley. Our research has focused on using genetically well defined populations of both crop species to elucidate genotypic variation in these traits in plants grown in the field, under glass and in vitro. Our results demonstrate meaningful genotypic variation in a range of traits important for improved resource capture including root length, root proliferation in patches of resource, mycorrhizal symbiosis and rhizosphere biochemistry. Ultimately, this research will potentially identify the genetic control of traits involved in resource capture and inform breeding programmes or biotechnological approaches which will produce more resource efficient cultivars of common crops.'
P15-022 Plasma membrane aquaporins play a central role in root cell membrane water permeability control as revealed in WT and transgenic maize lines
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Transmembrane water movement is facilitated by water channels called aquaporins. Using immunodetection approaches, we have characterized the expression of plasma membrane aquaporins (PIPs) in Zea mays primary roots grown either aeroponically or hydroponically. Such growth conditions influence differently the development of apoplastic barriers. Whereas aeroponically grown roots develop a suberized exodermis, hydroponically grown ones fail to do so. Immunocytochemical localization of four ZmPIP isoforms in the vicinity of suberized cell layers in the root cortex indicated that they are involved in radial water movement. The diurnal expression of ZmPIP proteins was also investigated in hydroponically grown roots and their expression was found to be higher during the day than at night. Interestingly, this variation of expression could be correlated to changes in the cell membrane water permeability as determined with a cell pressure probe. Finally, the root cell water permeability of transgenic plants partially downregulated in ZmPIP expression through RNA silencing decreased significantly highlighting the key role of ZmPIP aquaporins in the control of cell water permeability.

P15-023 Multiscale modelling of gas exchange in relation to storage condition and physiological disorders of pear
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A multiscale gas exchange model was developed to perform in silico experiments to evaluate the effect of external storage conditions, fruit size and maturity to the intra-cellular respiration and risks of occurrence of physiological disorders. Pear fruit was chosen as a model system. The approach consists of interconnected models that describe the transport phenomena at the macro and the microscale. First, macromodel predictions of the most sensitive fruit regions (regions of the lowest and highest O2 and CO2 concentration of intact fruits) were performed. Next, the microscale model was applied to quantify intra-cellular metabolic gas concentration of the sensitive regions of the tissue. The in silico study revealed that O2 concentration of optimal picked pear stored at typical controlled atmosphere condition (2.5 kPa O2, 0.7 kPa CO2 at –1°C) were higher than the Michaelis-Menten constant for cytochrome c oxidase Km,c, the rate limiting enzyme of the respiration pathway. In contrast to small pears, large pears and extreme low O2 storage conditions lead to O2 concentrations well below the Km,c. This most probably leads to fermentation and physiological disorders which have been observed under such conditions. Ripening of the fruit increased the risk of physiological disorders since increased respiration resulted in anoxia in the fruit center even at the typical storage conditions.

P15-024 Increased substrate salinity impact on nutrient balance in coastal species Aster tripolium and Hydrocotyle vulgaris
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The aim of the present experiments was to study the effect of increased substrate salinity on nutritional status of two endangered species from coastal marsh – Aster tripolium and Hydrocotyle vulgaris. In A. tripolium root growth was stimulated by 25–200 mM NaCl while leaf growth was significantly reduced by 200–400 mM NaCl. Concentrations of several elements (K, Ca, Fe, Zn) increased in leaves of A. tripolium due to decrease in the leaf mass with no stimulation of the uptake. On contrary, Mn uptake as well as leaf and root concentrations were stimulated by NaCl. Concentrations of N in leaf tissue was increased by 100 and 200 mM NaCl salinity. In H. vulgaris, decrease in K, Ca and Mg concentration by NaCl was state in leaves, petioles and stolones. Similar to A. tripolium, accumulation of Mn was stimulated by NaCl treatment in all the analyzed tissues of H. vulgaris. The data show different adaptation mechanisms to increased substrate salinity in the two species possibly related to various developmental strategies.
P15-025 Abrogated guard cell anion channel functioning of slac1 influences also stomatal opening in response to low CO2 and light

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Stomatal pore, surrounded by a pair of guard cells, regulates plant gas-exchange. Size of a pore is regulated by changing the amount of osmotically active ions in guard cells. We have recently identified a guard cell specific plasma membrane protein SLAC1 required for 3-type anion channel functioning and showed that functional SLAC1 is essential for stomatal closure in response to CO2, ABA, ozone, light/dark transitions, humidity change and Ca2+ (Vahisalu et al. 2008, Nature 452: 487–491). However, stomatal closure is a process different from stomatal opening and it is not yet known how SLAC1 affects the opening of stomata. We studied the stomatal opening of slac1 mutants in response to reduction of air CO2, and light/dark transitions. We found that stomatal opening induced by reduction of CO2 is dependent on air humidity in slac1 mutants. Reduction of CO2 from 420 to 110 ppm at 70–80% air relative humidity caused distinct stomatal opening in wild type plants while slac1 mutants displayed only weak response. However, when same experiment was carried out at 30–40% air humidity, distinct stomatal opening response to CO2 was observed in slac1 as well. Stomatal opening caused by onset of light was also slower in slac1. Interestingly, guard cells extracted from the slac1 mutants had significantly reduced K+in currents compared to WT. Our results suggest that SLAC1 is also involved in the regulation of stomatal opening.

P15-027 Characterisation of TcHMA4, a Cd/Zn ATPase purified from roots of the hyperaccumulator Thlaspi caerulescens

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TcHMA4 belongs to the family of P1-type ATPases, which are known from genomics to occur in all organisms from bacteria to plants and animals, but their protein biochemistry is still largely unknown. TcHMA4 transports zinc as well as cadmium over the cytoplasmic membrane. We isolated it from natural overexpression in roots of the Cd/Zn hyperaccumulator Thlaspi caerulescens grown on 100 μM Zn2+. The natural C-terminal His tag of TcHMA4 consisting of nine histidines was used for purification via metal affinity chromatography and it was found that the natural form of this transporter is modified by post-translational processing as SDS gels and western blots did not show the full size protein that was predicted from the gene sequence. After reconstitution in artificial lipid vesicles, ATPase activity tests of the purified protein showed that it is in its active state and its activity can be strongly increased by the addition of metal (cadmium, zinc and even copper) during the activity test. Further, EXAFS measurements revealed that the ligands of TcHMA4 involved in the binding of cadmium are mainly cysteins (according to the sequence, 58 Cys residues are present in TcHMA4), histidines are only little involved in cadmium binding. Currently we are further characterising the binding and transport of different heavy metals by purified TcHMA4 in order to elucidate the metal specificity, the number and affinity of different binding sites, and kinetic constants of the metal transport.

P15-028 Regulation of root nitrate uptake at the NRT2.1 protein level in Arabidopsis thaliana

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In Arabidopsis the NRT2.1 gene encodes a main component of the root high-affinity nitrate uptake system (HATS). Its regulation has been thoroughly studied at the mRNA level, showing a strong correlation between NRT2.1 expression and HATS activity. Despite its central role in plant nutrition, little is known concerning localization and regulation of NRT2.1 at the protein level. By combining a GFP fusion strategy and an immunological approach, we showed that NRT2.1 is mainly localized in the plasma membrane of root cortical cells, and unravelled an unexpected structural complexity of this protein, with at least three different forms in cell membranes (the monomer and two higher molecular weight complexes). The...
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monomer is the most abundant form of NRT2.1, and seems to be the one involved in NO₃⁻ transport. It strictly requires the NAR2.1 protein to be expressed and addressed at the plasma membrane. No rapid changes in NRT2.1 abundance were observed in response to light, sucrose or nitrogen treatments that strongly affect both NRT2.1 mRNA level and HATS activity. This suggests the occurrence of posttranslational regulatory mechanisms. One such mechanism could correspond to the cleavage of NRT2.1 C-terminus, which results in the presence of both intact and truncated proteins in the plasma membrane.

P15-029 Evidence for the interaction of plasma membrane-bound redox proteins with high molecular mass protein complexes
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Plant plasma membranes contain a constitutive transmembrane electron transport system, the so-called standard-system (Lüthje 2008). This redox system was suggested to have important functions in redox regulation and cell detoxification. Experiments with NAD(P)H-loaded and sealed right-side-out plasma membrane vesicles of maize (Zea mays L.) roots suggest a major function of NAD(P)H for the standard-system (Menckhoff and Lüthje 2004). Meanwhile several constitutive and inducible redox components (i.e. NAD(P)H oxidoreductases and b-type cytochromes) have been purified from plasma membranes and characterized in more detail (refs in Luthje (2008)). In the present work we demonstrate the occurrence of high molecular mass protein complexes in the plasma membrane of dicotyledoneous and monocotyledoneous plants by native polyacrylamide gel electrophoresis. Protein profiles depend on the state of development, plant material and species investigated. Proteins of Arabidopsis thaliana have been identified by peptide mass analysis (ESI-Q-ToF-MS/MS). In addition interaction of protein complexes with redox proteins has been demonstrated by specific staining procedures and for all materials investigated.


P15-031 Purification, identification and biochemical characterisation of iron reductase (FRO 1) from plasma membranes of pea (Pisum sativum L.) roots
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Iron uptake strategy I plants like pea reduce iron by a transmembrane Fe³⁺-chelate reductase (FRO) before uptake. Initial attempts at the purification of FRO (81 kDa) have been published for tomato (Holden et al. 1991, 1995, Bagnaresi et al. 1997). Meanwhile the tomato gene coding for LeFRO1 (81 kDa) was isolated and characterized (Li et al 2004). PsFRO1 (flavocytochrome b family) was identified as a pea Fe⁺⁺-chelate reductase involved in iron deficiency (Waters et al. 2002). The predicted protein has a molecular mass of 81 kDa, 10 transmembrane helices, heme, FAD and NADPH binding-sites. In the present study a significant transmembrane Fe³⁺-chelate reductase activity was demonstrated in sealed and NAD(P)H-loaded apoplastic-side-out plasma membrane (PM) vesicles. The protein corresponding to this activity was partially purified from PM of iron deficient pea roots. Fe⁺⁺-chelate reductase activity of the purified protein was compared to that of PM isolated from roots of both iron-sufficient and iron-deficient pea. Enzyme kinetics (Km, pH-optima, and inhibitors) of Fe⁺⁺-chelate reductase activity will be shown. Properties like MW, pl, co-factors, etc. were investigated by in-gel staining procedures and absorbance spectra. Possible protein-protein interaction was analysed by native PAGE and Western-blot analysis.


P15-032 Uptake and partitioning of 15N protein and not-protein amino acids in barley as affected by grain protein synthesis
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The Na⁺ concentration in common reed roots is similar to that in rice roots, while common reed shoots have lower Na⁺ concentration than rice shoots. It has been suggested that in common reed, Na⁺ translocation to the shoot is inhibited at the shoot base (SB). To evaluate the SB contribution to the inhibition of Na⁺ translocation to the shoots and determine the Na⁺ translocation pathway in the SB, we analyzed the kinetics of ²²Na⁺ in common reed and in rice under the 50 mM NaCl stress condition by performing a traditional tracer experiment and a non-invasive tracer experiment with a positron-emitting tracer imaging system (PETIS). The traditional tracer experiment revealed that the ²²Na⁺ translocation rate via the xylem at the SB in common reed was slower than that via the xylem at the SB in rice, while the ²²Na⁺ translocation rate via the xylem in the roots of common reed was more rapid than that via the xylem in the roots of rice. The PETIS experiments showed that ²²Na⁺ accumulation is slower in the SB and the shoots of common reed than in those of rice. The ²²Na⁺ accumulated in the shoots of both species was not actively excluded under the continuous mild saline condition. Modeling of the Na⁺ fluxes within the SB revealed that common reed strongly retrieved Na⁺ from the xylem at the SB.


P15-030 Common reed has efficient Na⁺ retrieving mechanisms at the shoot base to maintain low Na⁺ concentration in the shoot
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The Na⁺ concentration in common reed roots is similar to that in rice roots, while common reed shoots have lower Na⁺ concentration than rice shoots. It has been suggested that in common reed,
Barley plants were grown in a growth chamber. 15 days after anthesis the second leaf below the ear was supplied with either 2% 15N-DL-alpha-alanine (15N-ala), a protein amino acid (AA) or 2% 15N-alpha-amino isobutyric acid (15N-aib), a non-protein AA (95.6% 15Nexc). After an 8 day feeding period the uptake and distribution of 15N were investigated. 15N-ala has been more exported from feed leaf and transported into ear thia 15N-aib. Subsequently, a significant higher amount of 15N-aib remained in the feed leaves compared to 15N-ala. This indicated that the ear acted as the major sink organ for both AA, but they showed allocation differences linked to substrate specificity. 15N amount in the ear protein was significantly higher by feeding 15N-ala than 15N-aib. 15N incorporation into ear protein seems not to be influenced by cycloheximide (CH = Protein synthesis inhibitor of 8 = S ribosomes) spraying onto ear, but CH reduced the transport of 15N into ears. The current results show that both fed AA were transported into ears but the rate of this targeted transport is related to their proteinogenic nature. This indicated that the transport of N compounds into ears do not seems to be intrinsically controlled by the grain protein synthesis but partly regulated by it. The incorporation of the not-protein aib was not awaited. Further studies are necessary by using analogous protein AA which amino groups are completely protected for preventing amide linkages between adjacent amino acids.

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P15-034 Guard cell plasma-membrane protein, SLAC1, and its family members are essential for malate/anion transport
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Stomatal pores in the epidermis enable gas exchange between plants and the atmosphere, a process vital to plant life. Stomatal movements (opening and closing) are induced by environmental factors, including light, CO2, and humidity. Closure of the stomatal pores is driven by the efflux of osmoregulatory anions (Cl- and malate2-) from guard cells. However, central question regarding the molecular basis of the process remains unanswered. Through a leaf thermal imaging screen, we isolated the Arabidopsis mutant slac1 (slow anion channel associated 1) that is impaired in CO2-induced stomatal closure. The SLAC1 protein is a distant homologue of the C4-dicarboxylate transporter, and is localized specifically to the plasma-membrane of guard cells. It belongs to a protein family which in Arabidopsis comprises of four structurally related members that are common in their subcellular localization, but show distinct tissue-specific expression patterns. The loss-of-function in the slac1 mutant was accompanied by an overaccumulation of the osmoregulatory anions in guard cell protoplasts. Guard cell-specific expression of SLAC1 or its family members resulted in restoration of the wild-type stomatal responses, and also in the dissipation of the overaccumulated anions. These results suggest that SLAC1 family proteins have an evolutionarily conserved function that is required for the maintenance of ion homeostasis across the plant cell plasma-membrane.

P15-035 The effect of short period of carrot psyllid (Trioza apicalis) feeding and weed competition on carrot yield and nutrient uptake
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Carrot psyllid (Trioza apicalis) is a severe carrot pest in northern Europe causing significant yield loss after a few days feeding period at early growth stages. The mechanism of the damage is unknown. However, damage symptoms including fibrous root proliferation and leaf discoloration could refer to nutrient deficiency in the plant. Therefore, we studied nutrient uptake of carrot roots exposed to carrot psyllid feeding and/or weed competition. One carrot seedling was grown together with no, two or four seedlings of fat-hen (Chenopodium album) for 4 weeks from sowing in 3-l pots in greenhouse. At 1-leaf stage, half of the carrots were exposed to a 6-day feeding period of one carrot psyllid. Carrot psyllid feeding reduced the yield significantly whereas weed competition did not. In relation to dry weight, root concentrations of several nutrients increased.

Aquaporin is responsible for water transport across cell membrane and play a crucial role in the regulation of water status in plants. Water transport activity of plasma membrane intrinsic protein 2 (PIP2) is considered to be regulated by protein phosphorylation and some serine residues, such as Ser115 and Ser280, are suggested to be phosphorylation sites. We made mutant cDNAs of pear PIP2 (PcPIP2;2) to substitute the Ser115 and Ser280 to Ala or Asp. Substitution to Ala mimics non-phosphorylational state and those to Asp mimics phosphorylational state. cRNAs from the cDNAs were injected into Xenopus oocytes and water transport activity was measured. Ala115/Ala280 was significantly lower water transport activity compare with wild-type PcPIP2;2, although Ala115/Asp280, Asp115/Ala280 and Asp115/Asp280 showed almost the same water transport activity as wild-type. We tried to prepare antibodies, which recognize phosphorylated Ser115 and Ser280 specifically, and only antibody for phosphorylated Ser280 was obtained. With this antibody, phosphorylational state of Ser280 of PIP2 in per fruit development and in flower opening of Japanese morning glory was determined. In pear fruit, phosphorylational state of Ser280 changed diurnally depending on water status of the fruit. In Japanese morning glory flower, phosphorylational state of Ser280 increased during flower opening. Our results show the involvement of phosphorylational regulation of PIP2 and its physiological importance in plants.
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were significantly increased by carrot psyllid feeding but unaffected by weed competition. However, carrot psyllid feeding significantly reduced dry weight of the roots. In relation to fresh weight, root concentrations of K and Mg were significantly reduced by carrot psyllid feeding. The shoot:root ratio was significantly increased by carrot psyllid feeding but unaffected by weed competition. Increased shoot:root ratio could be due to K or Mg deficiency in carrot since they are known to affect photosynthetic export and therefore their role in damage formation should be more thoroughly studied.

P15-036 Non-invasive, quantitative and repetitive imaging of photoassimilate flow after source and sink manipulation

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Higher plants regulate photoassimilate flow from source to sink organ in order to respond to environmental or developmental changes. An understanding of the source-sink interrelationship requires an experimental system which can measure the change of photoassimilate flow corresponding to various conditions in source and sink organ. In this study, we adapted 11C-tracer and the positron emitting tracer imaging system (PETIS), which can obtain carbon dynamics in intact plants non-invasively and quantitatively. To manipulate the conditions in source and sink organ, we treated leaves or roots of plants (rice and soybean) with p-chlorobenzenesulfonic acid (PCMBS), an inhibitor of sucrose transporters. 11CO2 was supplied to leaves after PCMBS treatments and the translocations of 11C-photoassimilate were monitored using PETIS repetitively with the same plants. We have developed an analytical method to estimate the velocity of 11C-photoassimilate flow from PETIS data. As a result, a gradual change in the velocity after PCMBS treatments was successfully detected. The effect of source/sink strength on the velocity of photoassimilate flow will be discussed.

P15-037 The effect of salinity on mineral nutrition is modified by elevated CO2

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Under future environmental conditions, the increase of the concentration of ions such as Na+ and Cl− in the soil is predicted. Together with this rise in the saline surface it is expected that for the end of the century the atmospheric concentration of CO2 will double. In saline soils, the Na+ and Cl− ions provoke changes in the uptake of nutrients observed differences between cultivars. Could high level of CO2 mitigate the negative effects provoked by salinity on mineral nutrition in Alpha and Iranis barley cultivars? In our cultivars we observe that NaCl reduced the K+ and Ca2+ levels and increased the Na+ concentration in the leaf at 350 ppm of CO2. However, variations between cultivars were observed being the levels of Na+ and Ca2+ higher in Iranis than in Alpha. Under 700 ppm CO2 conditions and salinity, the uptake and translocation rates of Na+ and K+ and the translocation rate of Ca2+ were higher than at ambient CO2. Nevertheless, due to a dilution effect the levels of Na+ and K+ in the leaf were similar to the ones observed at 350 ppm CO2, whereas the concentration of Ca2+ was higher. In conclusion, higher uptake and/or translocation rates were observed under salinity and elevated CO2 conditions, probably as a consequence of a greater proportion of biomass allocated to fine roots and/or higher availability of ATP, both needed to increase the nutrient uptake. This work has been supported in part by grants of UPV 118.310-G07/2001, MEC BFU2007-60523/BFI and UNESCO 07/02.

P15-038 Multiple phosphorylation sites regulate the plasma membrane H+-ATPase

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The plasma membrane H+-ATPase couples ATP hydrolysis to the transport of protons out of the cell, thus generating an electrochemical gradient used by secondary transporters to move ions and metabolites through the membrane. In addition, H+-ATPase is involved in several physiological roles as stomata opening and cell elongation. How does this enzyme coordinate and regulate all these roles? We already know that it is activated by phosphorylation of its penultimate residue (a threonine) and the subsequent binding of 14-3-3 proteins to the enzyme C-terminal auto-inhibitory domain. However, mass spectroscopy analysis of the purified PMA4 and PMA2, the major Nicotiana plumbaginifolia H+-ATPases, allowed us to identify additional phosphorylated residues. Three of them have been studied by their mutation into aspartate or alanine and the expression of the mutant H+-ATPases in S. cerevisiae as well as in N. tabacum suspension cells. Three conclusions can be inferred from these data. (1) Phosphorylation of Ser448, located in the PMA4 nucleotide binding domain, interferes in the coupling between ATP hydrolysis and proton transport; (2) Phosphorylation of PMA2 Thr938, located in the auto-inhibitory domain, inactivates the enzyme by reducing 14-3-3 binding; (3) Phosphorylation of PMA2 Thr889, also located in the inhibitory domain, activates the enzyme in spite of reducing 14-3-3 binding. These results indicate that the H+-ATPase regulation is complex and involves several phosphorylation events.

P15-039 Metallothioneins – what are their physiological roles in barley?

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Metallothioneins (MTs) are a family of low molecular mass metalloproteins (Mr < 10 kDa) that are present in all living organisms except Eubacteria. Due to their high cysteine content, MTs have a strong capacity for binding of transition metals. In vivo MTs have

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been found to coordinate Zn, Cu and Cd. Metallothioneins in mammals have been associated with heavy metal detoxification, scavenging of reactive oxygen species, and transfer of essential metals to metalloenzymes and transcription factors. Although transcripts of plant MTs are induced by a wide variety of stressors such as metal ions, hormones, salt and oxidative stress, there is still limited knowledge regarding their physiological functions. Unique structural features of plant MT proteins are a long cysteine-free spacer region with a few aromatic amino acids. Possibly, this region has resulted in difficulties in the purification of native MTs from plants.

In order to investigate the physiological functions of barley MTs we have identified and cloned the entire family of MTs from the cultivar Golden Promise. The family consists of several genes and we are currently profiling their expression in different plant organs in response to metal (Zn, Cu, Cd) treatment. Promoter analyses are carried out to reveal regulatory elements controlling MT expression in barley. In parallel, barley MTs are expressed in E. coli and purified for further metal binding studies and competition assays in vitro.

**P15-040 Zinc and iron distribution and speciation in the cereal grain**


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Phytic acid and proteins are believed to be important for the distri-
bution and bio-availability of minerals in the cereal grain but little quantitative information is available on the relative importance of different organic ligands. We used a micro-scaled digestion procedure and ICP-MS for analysing the elemental profile of the four major tissue types in the barley grain. The embryo and the aleurone layer were clearly the most mineral dense tissues, exceeding endosperm concentrations with a factor 5 to 10. The chemical speciation of water-soluble Zn, Fe, P and S was investigated using a novel extraction technique (accelerated solvent extraction), which improved both extraction efficiency and reproducibility. Metal complexes in the extracts were identified by SEC-ICP-MS followed by LC-ESI-MS. Phytic acid and its derivatives were the major Fe-binding ligands, eluting as clusters of 15.7 and 5 kDa, respectively. In contrast, Zn co-eluted with S in a single 7 kDa peak, clearly showing preferential protein speciation of this element. The results contradict the current dogma on Zn mainly being bound to phytate in cereal grain and could therefore have a major impact on future research regarding bio-availability of Zn. Spectro-microscopic in situ techniques (PIXE, S-XRF and XAFS) with a lateral spatial resolution of a few micrometers are currently used to further elucidate the distribution and solid phase speciation of Zn and Fe in barley and rice grains.

**P15-042 Understanding the role of two ZIP transporters in Arabidopsis metal homeostasis**

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Members of the ZIP family of metal transporters are involved in the transport of cations across biological membranes in most kingdoms of life. There are 15 members of the ZIP family in Arabidopsis thaliana. Previous work has shown expression of ZIP1 and ZIP4 in yeast can rescue zinc- and copper-deficient mutants respectively. T-DNA mutants zip1-1 and zip4-1 were obtained. Homozygous single mutants had no observable phenotype either on plates or in soil. The homozygous zip1/zip4 double mutant has impaired fertility. Zn levels in zip1/zip4 shoots were decreased to 75% of wild-type levels. No difference was observed in roots or for other metals. Current work involves replicating this result with additional alleles, creating promoter-GUS constructs to examine expression in planta, as well as 35S-cDNA constructs to try to perturb zinc homeostasis further. Multiple mutant combinations of other ZIP genes are also being generated.

**P15-043 Determination of elemental content of vegetables by EDXRF spectrometry**

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In this work the non-destructive methodology based on energy dispersive X-ray fluorescence (EDXRF) has been applied for the

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determination of some major and minor elements (K, Ca, Fe, Mn, Cu, Zn, Sr, Pb) in different vegetation species: Atriplex hortensis, Rumex patientia, Lactuca sativa, Urtica dioica, Spinacea oleracea and Allium ursinum. From data obtained about the mineral content of analyzed samples it could be concluded that all of these plants can be use in supplementary mode as a very high nutritional foodstuffs. The EDXRF measurements were made using the Elvax spectrometer having an X-ray tube with Rh anode and 140 μm Be window and a solid state Si-pin-diode X-ray detector with 200 eV at 5.9 keV (Fe Kα line) energy resolution.

P15-045 Anion channels in vacuoles of the liverwort Conocephalum conicum
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Vacuoles isolated from the liverwort Conocephalum conicum were examined using the patch-clamp technique. Two class of ion channels were identified: slow vacuolar channel (SV) and anion-selective channel (Trebacz et al. 2007. Plant Cell Physiol. 48: 1747–1757). SV channels were activated at high cytoplasmic Ca2+ activity. They exhibit typical slow kinetics and outward rectification allowing cation transport from the cytoplasm to the vacuole at positive tonoplast potentials. Anion channels were activated at high cytosolic Mg2+, Sr2+ or Ba2+. Al3+ activated the channels at 0.5 mM concentration. The other necessary condition of activation was reduction of Ca2+ concentration to the submicromolar level. This indicates the possibility of replacing calcium by Mg2+, Sr2+ or Ba2+ in a putative regulation place of the channel. Currents flowing through anion channels show strong inward rectification at negative voltages which means that they conduct anions from the cytoplasm to vacuole lumen. Single channel recordings revealed a 32 pS channel, whose kinetics corresponds to whole-vacuole currents. The anion channel is almost equally permeable to Cl−, NO3−, SO42− and much less to malate2−. Anion currents are strongly reduced by anion channel inhibitors: A9C (2 mM), DIDS (1 mM) and ethanetric acid (0.5 mM). Once activated, they were weakly calcium dependent and remained active at physiological Ca2+ concentration. ATP, cAMP and protein kinase A had no significant effect on the channels.

P15-044 UV induced PDR-type ABC transporter of grape
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Resveratrol, is one of the functional compounds for human health and is a phytoalexin against plant pathogens such as Botrytis cinerea. Resveratrol is a member of polyphenols, synthesized by stilbene synthase (STS) and accumulated in grape berry skin. On the other hand, PDR-type ABC transporters have been reported as transporters for plant secondary metabolites. NpABC1, which is a PDR-type ABC transporter in Nicotiana plumbaginifolia, is suggested to relate to resistance against B. cinerea. B. cinerea strain lacking PDR-type ABC transporter (BcatB) is sensitive to resveratrol. Therefore we predicted the existence of PDR-type ABC transporter for resveratrol secretion in grape berry skin. We found an orthologue (VvPDR1) of NpABC1 in grape genome (Genoscope, http://www.genoscope.cns.fr/spip/) and its full length cDNA was cloned with total RNA from grape skin by RT-PCR. VvPDR1 is categorized the same clade of NpABC1 and AtPDR12 induced by pathogen in Arabidopsis. Gene expression of VvPDR1 and stilbene synthase (VvSTS) was determined by Real-time PCR. VvPDR1 was expressed in various organs except for berry flesh and the expression pattern of VvPDR1 was similar to that of VvSTS. In grape berry skin, resveratrol accumulation is increased by ultraviolet (UV) irradiation. UV irradiation induced both VvPDR1 and VvSTS expressions in grape berry skin. These results suggest the involvement of VvPDR1 in resveratrol accumulation in grape skin.

P15-046 A novel function of a nitrate transporter with Glutathione transport activity in Arabidopsis
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Members of the PTR family (Peptide Transporter or NRT1 for Nitrate transporter 1) transport diverse substrates, including nitrate, histidine, various dipeptides and phaseolotoxin secreted by plant pathogen. In Arabidopsis, the PTR homolog At5g14940, when overexpressed in roots (At5g14940OE), displayed a cadmium (Cd)-sensitive phenotype with root growth suppressed by 20 μM of CdCl2 treatment. To understand the in vivo functions, the transport properties for dipeptides (such as Leu-Leu, Gly-Gly, and L-EC) and glutathione (GSH), and HNO3 in Xenopus oocytes using two-electrode voltage clamping were tested. Transporter At5g14940 is capable to transport GSH, L-EC as well as NO3− with little or none dipeptide transport activity. Measurements of the Cd content in at5g14940OE revealed that both roots and shoots contain higher Cd compared to that in wild-type. High levels of GSH and GSH-derived peptides phytochelatins were identified in root parts of at5g14940OE using combined mass spectrometry and fluorescence HPLC analyses. Phylogenetic analysis of 53 homologous genes reveals that At3g01350 and At5g14940 belong to the same subgroup of the PTR family. Intriguingly, At3g01350 and At5g14940 are redundant genes due to the chromosome duplication event. A double knock-out mutant was then created and showed Cd-resistant phenotypes with longer primary roots and higher shoot weights to 72 hr of Cd exposure.

P15-047 Functional analyses of two NRT1 family genes, At1g18880 and At5g62680 in nitrate transporter in Arabidopsis thaliana
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Nitrates is a vital nitrogen source for plant growth. CHL1, the first member identified in NRT1 family, is a dual-affinity nitrate transporter. The predominant root-expressed and nitrate-inducible expression pattern of CHL1 correlates its function in plant-uptake nitrate from soil. Based on protein homology, there are 53 homologs in NRT1 family. NRT1:5, another member in NRT1 family, is also expressed more in roots, especially in the pericycle cells close to xylem. In nrt1:5 mutant, less nitrate was transported from root to shoot, suggesting that NRT1:5 is involved in nitrate transport from root to shoot. After analyzing RNA expression by RT-PCR, we found that several genes in NRT1 family have predominant root-expressed pattern. The RNA expression of At1g18880 and At5g62680 is just below that of CHL1, and is not induced by nitrate. The protein products of these two genes have nitrate uptake ability in Xenopus oocytes, and localized to the plasma membrane of Arabidopsis mesophyll protoplasts. According to the cell type-specific microarray data from Benfey and his colleagues, At1g18880 and At5g62680 seem expressed more in phloem tissues, suggesting the functions of the two genes may be involved in nitrate transport between root and shoot. T-DNA insertion mutant lines were obtained and analyzed for the nitrate uptake, transport, and growth phenotypes.

Manganese (Mn) deficiency is a widespread plant nutritional problem resulting in substantial yield and quality reductions. Barley genotypes differ considerably in their tolerance to grow in soils with low Mn2+ availability. The physiological basis for this tolerance to Mn deficiency, termed Mn efficiency, is not yet fully understood. However, we have demonstrated that two barley genotypes, the Mn efficient Vanessa and the inefficient Antonia, differed in their high affinity Mn2+ uptake kinetics when exposed to physiologically relevant Mn2+ concentrations. Mn2+ is often present in the soil solution in exceedingly low concentrations, implying that plants must use high affinity transport systems to accumulate Mn2+, but the molecular basis for this Mn2+ transport is still poorly understood. Here we report the identification and characterization of the first barley gene encoding a plasma membrane localized metal ion transport protein with specificity for Mn2+, named HvIrt1. RT-PCR analysis showed that Mn and Fe deficiency induced an up-regulation of HvIrt1 in the two barley genotypes. The expression level was under these conditions significantly higher in Vanessa compared to Antonia. The higher expression of HvIrt1 correlated with an increased Mn2+ uptake rate. It is therefore suggested that HvIrt1 is an important factor controlling Mn2+ uptake in barley roots. Furthermore, HvIrt1 may play a role in controlling differential Mn efficiency among barley genotypes.
M1-3 The polyphenol oxidase multigene family of Physcomitrella
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Polyphenol oxidases (PPOs) are copper-binding enzymes of the secondary plant metabolism that oxidise polyphenols to quinones. Characterisation of PPO proteins and transcript analyses from many secondary plant metabolism that oxidise polyphenols to quinones. Phylogenetic analyses of the gene family revealed PPOs to cluster in five groups with 2-3 PPOs each and that the PPO gene duplications in Physcomitrella occurred after separation from the seed plant lineage. Expression of PPO genes was determined by RT-PCR: nine of the twelve analysed PPO genes were found to be expressed in protonema tissue. Irradiation with strong light intensities and subsequent semi-quantitative RT-PCR revealed PPO4 and -12 to be up-regulated, while expression of PPO1 decreased. PPO1-knockout plants showed 30% reduction of extracellular PPO activity compared to wild type. Phenotypic changes observed in PPO1-knockout plants will be discussed with respect to a possible causal connection to PPO activity.

M1-4 Rescue and characterization of shuttle plasmids from Physcomitrella
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A highly useful tool in yeast research is the availability of stably replicating shuttle vectors that can be rescued back into E. coli. This has made it possible to clone genes by complementation and to screen for dosage suppressors, i.e. genes that can suppress the effect of a mutation when overexpressed. Previous work has shown that foreign DNA can replicate stably in Physcomitrella, but it can still be lost in the absence of selection, consistent with an episomal mode of replication (Ashton et al. 2000, Schaefer 1994). We have now studied this phenomenon in more detail, using different plasmids. We find that plasmids transformed into moss can be rescued back into E. coli without restriction digestion of the moss DNA, similar to plasmid rescue in yeast. Significantly, we recovered the original plasmids without rearrangements or insertions of moss DNA from moss transformants generated after transformation with circular (uncut) plasmids. This suggests that shuttle plasmid strategies similar to those used in yeast should be feasible in Physcomitrella. If the plasmids were linearized prior to transformation into moss, we found that they were recircularized either by cohesive end ligation or by deletions involving short direct repeats on the plasmid. The latter process appears to be highly efficient, consistent with the high frequency of homologous recombination in Physcomitrella.

MOSS 2008: SESSION 2 ON SATURDAY, 16 AUGUST 2008

M2-1 From genes to proteins, the Arp2/3 and WAVE/SCAR complexes in P. patens
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Control of actin filament nucleation, polymerization and organization plays an important role in cell development of eukaryotes affecting different mechanisms such as growth, morphology, as well as the establishment and integration of external cues. The Arp2/3 complex, composed of seven subunits (Arp2, Arp3 and ARPC1-5), initiates upon activation actin nucleation and allows branching of such new filament on existing filaments. The only known Arp2/3 activator in plant is the WAVE/SCAR complex, composed of five proteins, PIR121, NAP125, ABI SCAR and BRICK1. In plants, neither complex has been isolated and characterized biochemically. But, different genetic and in vitro biochemical studies suggest a common function in actin regulation.

We are trying here to answer the lingering question of the actual presence of these two complexes in plants. We took advantage of two existing P. patens strains, yfp-arpc4 and brkt1-yfp. Both strains exhibit no morphological abnormalities but express a single copy of the native gene tagged with YFP that localizes at the tip of growing caulonemal cells. Using an antibody raised against YFP, we performed immunoprecipitation (IP) assay on protein extracts from each strain. LC/MS-MS analysis of IP products identified unambiguously the presence, not only of the two tagged proteins but components of each complex, providing for the first time direct evidence of the presence of the Arp2/3 and WAVE/SCAR complexes in plants.

M2-2 PIPK family in the moss Physcomitrella patens. PpPIPK1 is required for normal cell growth and differentiation
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Phosphoinositides (PIs) are minor lipids in eukaryotic cells but play a major role in many cellular processes. Phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P2] plays a key role in PI metabolism not...
only because is the precursor of inositol-1,4,5-trisphosphate, diacylglycerol and PtdIns(3,4,5)P3, but also due to its involvement in several cellular processes such as exocytosis, cytoskeletal regulation and intracellular vesicular trafficking. We are focusing on phosphatidylinositol phosphate kinase (PIPK), which catalyzes the production of PtdIns(4,5)P2. P. patens has two PIPK genes, PpPIPK1 and PpPIPK2, with differently regulated expression and protein sequences displaying a conserved PIPK catalytic domain and eight MORN (Membrane Occupation Recognition Nexus) domains in accordance with the description of PIPK class I/IB in higher plants. In vitro biochemical characterization showed that the two enzymes exhibited different substrate specificities. Interestingly, PpPIPK1 can synthesize PtdIns(3,4,5)P3, a PI which has not yet been detected in plant cells. In order to study the physiological role of these proteins, we have disrupted PpPIPK1 and PpPIPK2 by gene targeting and our preliminary results show a strong phenotype for pipk1 but not for pipk2. Pipk1 lines are delayed in growth, protoneural filaments show irregular branching patterns, and gametophores are impaired in rhizoid development. Our data support an essential role for PpPIPK1 in cell growth and differentiation.

**M2-3  Specificity of DICER-LIKE genes in Physcomitrella patens**

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Dicer proteins are essential enzymes in RNA interference pathways. From the Physcomitrella genome four DICER-LIKE genes (PpDCL1a, PpDCL1b, PpDCL3 and PpDCL4) have been identified and confirmed through cDNA sequencing and protein domain prediction. On the basis of sequence homology PpDCL1a and PpDCL1b are homologs of AtDCL1. The other two genes PpDCL3 and PpDCL4 are homologs of AtDCL3 and AtDCL4, respectively. Functional characterization of Dicer genes in Physcomitrella patens is successfully achieved by targeted knockout generation. Complete loss of PpDCL1a transcript results in retarded growth, abnormal cell shape and size and developmental arrest at the protonema stage. Molecular analysis revealed that PpDCL1a is the functional homolog of AtDCL1 and is required for the biogenesis of microRNAs (miRNAs) and trans-acting siRNAs (ta-siRNA). The expression analyses of miRNA and ta-siRNA target genes demonstrated elevated transcript levels in ΔPpDCL1a mutants. Currently ΔPpDCL3 and ΔPpDCL4 mutants are being analyzed. Phenotypic analysis is carried out by protoplast regeneration studies. Furthermore, we are analyzing the role of PpDCL3 and PpDCL4 in the biogenesis of different classes of small RNAs in P. patens. The phenotypic and molecular analysis of ΔPpDCL3 and ΔPpDCL4 mutant lines will be presented.

**M2-4  SHI-genes and regulation of local auxin biosynthesis in the moss Physcomitrella patens**

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In Arabidopsis, the formation of local peaks of the plant hormone auxin is crucial for development, differentiation and growth. The establishment of these peaks is known to depend on polar auxin transport but recent reports have revealed local expression of the YUCCA gene family, encoding rate limiting enzymes for auxin production, suggesting that local auxin biosynthesis might be equally important. YUCCA4 was recently identified as a direct target of the SHI/STY-related transcriptional activators suggesting that this family might be crucial for the establishment of auxin peaks by controlling local auxin biosynthesis. In an effort to learn more about the importance of local auxin biosynthesis in moss, we have initiated a characterization of two SHI/STY-homologues in Physcomitrella, PpSHI1 and PpSHI2. Analysis of the expression patterns revealed maxima in caulonemata, rhizoids and axillary hairs which are all suggested sites of auxin activity. Single knockout lines exhibit short gametophore stems and an apparent stimulation of rhizoid growth. In PpSHI1 overexpressing lines, transcript levels correlate with elevated levels of auxin, misense expression of a GH3-GUS reporter and the severity of a detrimental phenotype affecting aged chloronemata and basal gametophore leaves. In summary, our data indicates that PpSHI1 and PpSHI2 serve a function analogous to the Arabidopsis SHI/STY gene family in controlling auxin biosynthesis.

**M3-1  Adaptation of bryophyte reproductive structures to environmental stress**

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Successful sexual reproduction in bryophytes requires adequate humidity for motile sperms to travel along a water film to the egg. However, many bryophytes inhabit environments with high risk of desiccation, and sexual reproduction is therefore a hazardous event. Consequently, many bryophyte species reproduce sexually very seldom if at all. In some species reproductive organs are protected by gametophytic structures decreasing the risk of desiccation, often followed by decreased ability of sperms to reach the egg. Such structures cause among-species variation in reproductive success, and consequent species and/or population level variation in evolutionary potential. In our study, the structural differentiation among species in liverwort genera Jungermannia and Lophozia is compared with data on reproductive success in order to find potential evolutionary processes leading to loss of sexual reproduction in harsh environments.

**M3-2  The plant heat-shock response is controlled by specific and sensitive calcium channels in the plasma membrane**

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Plants cannot escape environmental stresses and must detect mild temperature increments and efficiently induce defence mechanisms,
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like heat shock proteins (HSPs), before forthcoming heat-damages. To address how plants perceive and react to mild physiological temperature variations, we designed several Physcomitrella patens reporter lines to monitor, in vivo, the cytosolic calcium concentrations, the stability of a heat-labile reporter luciferase and the activation of a heat-shock promoter. Mild temperature upshifts and treatments with membrane interfering compounds resulted in significant heat shock response (HSR) that depended on transient entry of extracellular calcium into the cytoplasm. EGTA pre-treatment drastically reduced heat and chemical-induced calcium influxes and correspondingly blocked the HSR. The release of calcium ions from intracellular stores, using ionomycin, did not trigger the activation of heat shock promoters and neither heat denatured luciferase nor treatment with Hsp90 inhibitors were sufficient to restore the HSR. In contrast, the anti-inflammatory drug, celestrol, activated a significant HSR in a calcium-independent manner indicating that it is likely acting downstream the plasma membrane. Thus, under mild heat-shock conditions, heat-induced changes in the plasma membrane can be perceived by specific calcium channels allowing calcium influx and activating chaperone-independent signalling cascade, leading to the activation of heat shock promoters.

MOSS 2008: SESSION 4 ON SATURDAY, 16 AUGUST 2008

M4-1 Signalling and adaptation: essential genes underlying abiotic stress tolerance in Physcomitrella patens
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Two stress-induced genes encoding a Ca2+-ATPase (PpPCA1) and a homolog of the tryptophan rich sensory protein (PpTSPO1), respectively, were identified by differential display RT-PCR. ATP-driven Ca2+ pumps are discussed to restore stimuli-induced elevated cytosolic Ca2+ ([Ca2+]cyt) levels. PpPCA1 encodes a Ca2+-ATPase with an N-terminal autoinhibitory domain and is localised to membranes of small vacuoles. PpPCA1 null mutants exhibit an enhanced susceptibility to salt stress. ΔPpPCA1 lines show sustained elevated ([Ca2+]cyt) in response to salt-treatment in contrast to wild type indicating a direct role for PpPCA1 in the restoration of pre-stimulus ([Ca2+]cyt). The disturbed Ca2+ response of the ΔPpPCA1 mutant lines correlates with altered expression levels of stress-induced genes providing evidence for a disrupted stress-associated signalling pathway. TSPO proteins are highly conserved from bacteria to eukaryotes and act in the transport of tetrapyrroles. Like its mammalian homolog PpTSPO1 is localised to mitochondria. Analysis of PpTSPO1 null mutants revealed an essential role of PpTSPO1 in salt stress adaptation. Under stress conditions, the ΔPpTSPO1 mutants show elevated H2O2 levels, enhanced lipid peroxidation and cell death, pointing at a role of PpTSPO1 in redox homeostasis. We hypothesize that PpTSPO1 acts to direct porphyrin precursors to the mitochondria for heme formation, and is involved in the removal of photoreactive tetrapyrrole intermediates.

M4-2 The capacity of the moss Physcomitrella patens to repair DNA damage
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In comparison to other plants the moss Physcomitrella patens is often mentioned for its efficient homologous recombination (HR) that might take part in removal of various types of DNA damage. We were particularly interested to compare Physcomitrella and Arabidopsis repair of double strand breaks (DSB) and DNA-DNA cross-links (CL). For this purpose we used single cell gel electrophoresis (Comets) assay that enables detection of global damage in genomic DNA to study removal of UV pyrimidine dimmers (Py•Py), Mitomycin C induced CL and Bleomycin induced DSB at the stage of protonema. We found out that repair of Py•Py and CL has similar kinetics in Physcomitrella as in Arabidopsis. Nevertheless in comparison to Arabidopsis repair of DSB is surprisingly much slower (see Fig right). Also DSB repair is slower in protonema 2 weeks after plating in comparison to 1 week old tissue. This raises an important question whether Physcomitrella repairs DSB exclusively by ‘slow’ HR pathway and alternative pathways e.g. NHEJ or direct ligation are attenuated or missing, at least in haploid protonema? Research in Physcomitrella is in our Lab supported by Czech Science Foundation (project # 521/04/0971) and Ministry of Education, Youth and Sports of the Czech Republic (projects # 1M0505 and LC06004).

M4-3 Physcomitrella patens responds to chitosan with release of a specific peroxidase
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Class III peroxidases have versatile functions in plants. While required for normal physiological processes, peroxidases also participate in various biotic and abiotic stress reactions. Generation of reactive oxygen species (ROS), which involves peroxidases, is one of the first events occurring in defence of seed plants. On the other hand, peroxidases are capable of detoxifying the harmful ROS. We studied Physcomitrella patens to find out whether similar defence mechanisms are present in bryophytes. Chitosan was used as an elicitor to induce possible defence responses. Chitosan application to the liquid culture of moss caused a rapid increase in peroxidase activity in the medium. Browning and cell death were observed later, mainly in the protonema and rhizoids and at the base of the gametophores. Peroxidase activity was due to a single secreted peroxidase. The protein was isolated, sequenced and the gene identified based on the genome sequence of P. patens. The gene has two copies in the moss genome. Induction of the peroxidase gene by chitosan treatment, as measured by quantitative PCR, occurred later than the observed increase in peroxidase activity in the culture medium. Both copies of the gene were removed by targeted gene replacement. The liquid cultures of the knock-out lines did not have peroxidase activity but appeared more necrotic following chitosan
knockout of the corresponding genes is feasible. The first steps towards the ‘humanisation’ of moss-produced biopharmaceuticals production. The availability of the
P. patens genome sequence enables the identification of genomic loci for all the enzymes responsible of these plant-specific modifications. Due to the high degree of homologous recombination in moss, targeted enzymes responsible of these plant-specific modifications. Due to the small differences in the product compared to the original protein can hamper the biotechnological use of plants for biopharmaceutical production. The availability of the P. patens genome sequence enables the identification of genomic loci for all the enzymes responsible of these plant-specific modifications. Due to the high degree of homologous recombination in moss, targeted knockout of the corresponding genes is feasible. The first steps towards the ‘humanisation’ of moss-produced biopharmaceuticals were already achieved by creating P. patens knockout strains which lack the activities of the α1,3-xylosyltransferase and the α1,3-fucosyltransferase, enzymes which are responsible for the introduction of allergenic sugar residues in glycoproteins. Here we will present the targeting of additional plant-specific post-translational modifications on a recombinant pharmaceutical protein in moss.


MOSS 2008: SESSION 5 ON SUNDAY, 17 AUGUST 2008

M5-1 The moss bioreactor: improved conditions for biopharmaceuticals production
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The potential of Physcomitrella patens (moss) to produce pharmaceutically valuable proteins has already been demonstrated (reviewed by Decker and Reski 2007). As opposed to microorganisms, proteins produced in plants are modified post-translationally in a similar pattern as their human counterparts. Nevertheless, the small differences in the product compared to the original protein can hamper the biotechnological use of plants for biopharmaceutical production. The availability of the P. patens genome sequence enables the identification of genomic loci for all the enzymes responsible of these plant-specific modifications. Due to the high degree of homologous recombination in moss, targeted knockout of the corresponding genes is feasible. The first steps towards the ‘humanisation’ of moss-produced biopharmaceuticals were already achieved by creating P. patens knockout strains which lack the activities of the α1,2-xylosyltransferase and the α1,3-fucosyltransferase, enzymes which are responsible for the introduction of allergenic sugar residues in glycoproteins. Here we will present the targeting of additional plant-specific post-translational modifications on a recombinant pharmaceutical protein in moss.


MOSS 2008: SESSION 6 ON SUNDAY, 17 AUGUST 2008

M5-2 The Washington university in St. Louis T-DNA mutagenesis program, – update
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At Moss 2007, we outlined our plans and initial progress for a program of forward gene identification via T-DNA tagging. From January 2008, we have been able to allocate two people to this program and so are now in a position to assess more clearly its feasibility as a high throughput procedure. The reproducibility of Agrobacterium-mediated T-DNA transformation has been low with recovery of stable transgenics varying between experiments from 0 to 750 transgenics per 10^6 regenerating protoplasts. We are investigating the reasons for this variability and will report new findings. We are transforming both P. patens and C. purpureus. P. patens generates a higher frequency of stable transgenics per regenerating protoplast, but a lower absolute number of transgenics, since a higher proportion on C. purpureus protoplasts survive co-cultivation with Agrobacterium. Our screening procedure detects various phenotypic changes, and we have so far identified gravitropically-abnormal mutants of P. patens and a C. purpureus mutant that does not grow in darkness. Progress towards identifying the genes responsible will be reported.

M6-1 Lineage analyses in the moss, Physcomitrella patens
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Flowering plants grow from shoot meristems that are multicellular and have layers and zones of specialised function. A central stem cell zone supplies cells to a peripheral zone from which pools of cells from several layers are recruited to form leaves. In contrast to flowering plants, representatives of the earliest vascular plant lineages such as Selaginella have a more simple meristem structure. In Selaginella just two epidermal cells give rise to the leaf, and two clearly identifiable stem cells generate the shoot. Leafy shoot growth in basal non-vascular plants is in the haploid rather than the diploid phase of the life cycle. We have developed clonal analysis and live imaging techniques to determine how leaves and shoots develop in Physcomitrella. These suggest that leaves each initiate from a single cell that retains stem-cell like properties as the leaf grows, and illustrate subsequent cell division patterns in the leaf.

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Our results provide a robust descriptive basis on which to found future functional studies in Physcomitrella.

M6-2 Live cell analysis of transdifferentiation from a leaf cell to an apical stem cell in Physcomitrella patens
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Transdifferentiation of a differentiated non-stem cell to a stem cell have been observed in both animals and plants, although the molecular mechanisms are not well known. One of the technical problems to pursue the mechanisms is a difficulty to trace the transdifferentiation process in cellular and subcellular levels. A leaf of the moss Physcomitrella patens, that is composed of a single layer of cells, is a suitable material for cellular and subcellular observation. Excision of a leaf makes cells facing to the excised plane transdifferentiate to a protonemal apical cell, a stem cell with apical growth, within 48 h. We established a multiple-position fluorecent timelapse imaging system, which enabled us to sequentially observe the transdifferentiation. Prior to starting an apical growth and a cell division into an apical cell and non-stem subapical cell, nuclear together with nucleolus swelled approximately two times in area within 24 h after excision under light condition. We found that nuclear swelling was mainly due to the enlargement of a nucleolus rather than the increase in nuclear DNA contents with fluorescent image analysis. We also showed that the process of transdifferentiation was regulated by light and the responsible photoreceptors were cryptochromes and phytochromes.

M6-3 Molecular mechanisms of transdifferentiation from a differentiated leaf cell to a stem cell
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Differentiated cells can be transdifferentiated to be pluripotent stem cells that are undifferentiated type of cells with abilities for both self-renewing and giving rise to most cell types in the organism. An induction of the transdifferentiation is more easily manipulated in plants than in animals, although genetic and molecular bases of the difference are mostly unknown. The moss Physcomitrella patens has a high ability of reprogramming: i.e. cells in a dissected leaf segment can be reprogrammed in water without any exogenous chemicals within 24 h. Here we show that light and auxin are indispensable for the transdifferentiation. As a down-stream of these signaling pathways, special and temporal patterns of cell cycle-related genes are dramatically changed and cell cycle re-enter into G1 and S phases. To connect the former signaling pathways and the cell-cycle machinery, expression patterns of transcription factors and epigenetic regulators were analyzed using a digital expression profiling with SOLiD. Candidate genes with conspicuous change of their expression between the signalings and cell cycle re-entry have been characterized in detail using inductive overexpression and disruption systems.

MOSS 2008: SESSION 7 ON MONDAY, 18 AUGUST 2008

M7-1 KNOX functions in Physcomitrella and the evolution of terrestrial plants
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Embryophytes but not charophyan algae, their closest relatives, possess a multicellular, diploid sporophytic generation. The adaptive advantage of this feature was the ability to generate greater numbers of genetically diverse spores, thereby promoting terrestrial colonisation through enhanced dispersal and increased genetic variability of progeny. The most widely accepted theory concerning the mechanism of evolution of a multicellular sporophyte in the first embryophytes implicates a delay in meiosis resulting in proliferative mitotic growth between fertilisation and sporogenesis. While specific vascular plant class 1 KNOX gene functions were almost certainly not implemented in the earliest land plants, the common theme in class 1 KNOX gene functions is the prevention of premature differentiation. Thus, a plausible ancestral and fundamental role for class 1 KNOX genes is to postpone spore differentiation, allowing the formation of a multicellular diploid generation. This view is supported by the discovery that mature spores were often present in apparently immature sporophytes of Physcomitrella lines mutated in MKN2 (one of four class 1 Moss KNOX genes). Correlated with this was a reduction in size of the spore mass and number of spores in mature mutant sporophytes. Physcomitrella class 2 KNOX functions will also be discussed within the context of the evolutionary conquest of land by plants.

M7-2 Functional analysis of class 2 KNOX gene in Physcomitrella patens
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KNOX homeobox genes of land plants can be divided into two subfamilies, class 1 and 2. Based on loss- gain-of-function mutant phenotypes, class 1 genes have an important role in initiation and maintenance of shoot apical meristems in flowering plants and are involved in sporophyte development in Physcomitrella. In contrast, the functions of class 2 genes are unknown, as loss-of-function mutant phenotypes have not been reported and gain-of-function alleles do not differ significantly from the wild type. Physcomitrella patens Bruch & Schimp subsp. patens is a suitable model plant to analyze genes with unknown function, since gene targeting techniques have been established. Two class 2 KNOX genes, MKN1 (Champagne and Ashton 2001) and 6, are found in Physcomitrella genome. Based on RT-PCR analysis, mRNA for both genes is expressed in embryonic sporophytes, but not in protonemata, nor...
in gametophores. The expression pattern of MKN1 as revealed by a transgene, in which uidA (GUS gene) is inserted at the end of coding sequence, is limited to sporogenous tissues within the developing sporophyte. The analyses of MKN6 expression and loss-of-function phenotypes are ongoing and will be presented.

M7-3 Evolution of the target of rapamycin (TOR) signaling pathway in land plants
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As multicellular organisms with a sessile life style and an indeterminate growth, land plants constantly adapt their development to environmental stresses. However, the mechanisms that sense this environment changes are not well characterized in plants. The TOR (Target Of Rapamycin) kinase is a conserved central regulator of eukaryotic growth in response to environmental stresses that has integrated new functions through evolution of different organisms. It is thus an entry point towards understanding the evolution of land plant adaptation to environmental changes. We are studying the TOR pathway in the moss Physcomitrella patens which belongs to a very basal group of land plants. We are taking advantage of new genetic tools developed in P. patens to create plants in which P. patens TOR (PpTOR) activity can be rapidly activated or inhibited. Such plants will be used to determine the function of PpTOR during the developmental response of the moss to environmental changes and to dissect the PpTOR signalling pathway. The results will be compared with data obtained in parallel in the recently diverged angiosperm Arabidopsis thaliana and in other eukaryotes in order to obtain a global picture of the evolution of the mechanisms that integrate environmental signals into growth and development in land plants.

M8-1 Are the leaves of Physcomitrella homologues or analogues of vascular plant leaves?
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Leaves are the predominant feature of some gametophyte-dominant plants including Physcomitrella and sporophyte-dominant plants such as Arabidopsis. Whether leaves of gametophyte-dominant plants and sporophyte-dominant plants are homologous or analogues is a perplexing question. Investigation of leaf development in Physcomitrella can provide clues to this unsolved mystery. Heteroblasty, lanceolate leaf shape and spiral phyllotaxy are common to Physcomitrella and many vascular plants. Unlike the leaves of higher plants, however, the leaves of Physcomitrella are composed of a simple midrib and a unistratose lamina. To further explain similarities and differences in leaf morphology in Physcomitrella and in higher plants, the developmental patterns that result in the different leaf forms of Physcomitrella will be described. The contributions of cell divisions and cell expansion to leaf size and morphology will be quantified. As in higher plants, mutagenesis and perturbation of development through, as an example, alteration of hormone levels in Physcomitrella frequently results in the production of leaves of abnormal size and form. Therefore, leaf morphogenesis in mutagenised strains of Physcomitrella and plants exposed to exogenous auxins, cytokinins or their inhibitors will be described. The results of an in silico search of the Physcomitrella genome for genes that are homologous to higher plant genes known to be involved in leaf development will be presented.

M8-2 Evolution of plastid peptidoglycans in plants
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The consensus is that a cyanobacterium with cell wall phagocytosed by a host cell evolved into plastids. We reported that the moss Physcomitrella patens has all Mur gene homologs related to peptidoglycan (PG) biosynthesis. Gene knockout experiments for the Mur genes in P. patens showed appearance of macrochloroplasts in protonema and leaf cells, suggesting relationship between PG synthesis and plastid division in moss. Plastids of glaucophytes, red algae, and green plants have evolved as siblings from a primary endosymbiont. It is known that glauco-phytes have a PG-armed plastid. The inhibition of PG synthesis by ampicillin caused the inhibition of septum formation of plastids in the glaucophyte Cyanophora paradoxa. On the other hand, the red alga Cyanidioschyzon merolae had no Mur genes based on its entire genome sequence, suggesting loss of PG in red algae. Bryophytes represent the first divergence in land plants. Lycophytes including Selaginella are the earliest branch of vascular plants. While ampicillin showed no effects on plastids of three species that are not classified in lycophytes, ampicillin-treated cells of S. nipponica had a smaller number of macroplastids than the untreated cells. Moreover, S. moellendorffii draft genome sequence suggests existence of all Mur genes. With the data for no effects of antibiotics on plastids of seed plants, these results strongly suggest that several independent losses of PG occurred in the evolution of plants.

M8-3 Racomitrium canescens as a material for greening and physiological study
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Racomitrium canescens (Hedw.) (Grimmiaceae) is studied as greening material for roof and wall surfaces of buildings and for covering many other artificial surfaces and slopes to prevent so-called ‘heat island phenomenon’. This moss is tolerant against...
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Enzymes of the chalcone synthase (CHS) superfamily catalyse production of a variety of secondary metabolites in bacteria, fungi and plants. It is generally accepted that some of these secondary metabolites have played an important role during the early evolution of land plants by providing protection from various environmental assaults including UV irradiation. The Physcomitrella genome incorporates a chs multigene family comprising 18 putative chs genes of which at least 10 are expressed. Our bioinformatic and biochemical analyses indicate that these genes probably encode enzymes belonging to the CHS superfamily. Chs8, chs9 and chs11 are especially intriguing since they appear to encode non-CHS enzymes that may be ancestral to plant CHS enzymes. Additionally, our study of Physcomitrella chs genes is providing insights into their evolutionary expansion, functional diversification and regulation. From phylogenetic reconstructions coupled with linkage data, we have mapped the most probable path of chs gene duplications. Comparative analysis of sequence data from Physcomitrella and other plants has enabled us to infer plausible gene models for the moss chs genes and to annotate them. Within the moss chs genes, we have identified cis-acting elements (G-box, W-box, etc) known to be involved in regulating higher plants chs genes. We are using these discoveries as a platform for the further investigation of chs gene control in Physcomitrella.

P04-012 Cell wall architecture of Physcomitrella revealed by atomic force microscopy

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Physcomitrella serves as a nonvascular plant model system suitable for studying many developmental phenomena. The tip-growing filamentous protonemal stage of its life cycle exhibits polarised growth and various tropic responses. Conventional staining and light microscopy were used to provide the first direct evidence that Physcomitrella protonemal cells lack a cuticle. Atomic force microscopy images revealed detailed surface structures identified by scanning electron microscopy. The cell wall ultrastructure is characterised by rounded protrusions that are uniformly distributed along each caulonemal filament, and longer fibrillar structures, which are disorganised at the apex, but become oriented in longitudinal arrays parallel to the growth axis in more proximal regions of caulonemal apical cells. The subapical cells are characterised by a polylamellated texture. There was no difference in gross surface ultrastructure between light-grown and dark-grown filaments, but the dimensions of the rounded protrusions at the apices of caulonemata cultured in the light and in darkness were significantly different. The convex and concave cell wall surfaces of a curved, gravitropically responding dark-grown caulonema appear structurally different. The data further elaborate a simple model of cell wall development in caulonemata of Physcomitrella that was proposed for other tip-growing filamentous plants.

P04-013 Characterization of the trehalose biosynthetic genes from Physcomitrella patens and their response to drought and ABA

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Trehalose is a non-reducing disaccharide consisting of two glucose molecules linked by a 1α–1α bond. It is present in very different organisms such as bacteria, fungi, nematodes, insects and plants. Several functions have been described for trehalose in nature, being a compatible solute, storage compound and structural part of cell walls. So far, five trehalose biosynthesis pathways have been described. Of these, the most widely distributed and best-characterized pathway consists of two steps mediated by the trehalose 6-phosphate synthase (TPS) and trehalose 6-phosphate phosphatase (TPP) enzymes, where the first is catalyzing the synthesis of trehalose 6-phosphate (T6P) while TPP dephosphorylates T6P to form trehalose and Pi. The plant TPS and TPP genes form multigene families where members are divided in classes I, II and III. Recent findings show the importance of trehalose and its intermediate T6P in plant sugar metabolism, gene regulation and development, suggesting T6P as a new plant hormone. In the present work, we characterized the whole trehalose biosynthetic gene family from Physcomitrella.
patens, and show that members of the three gene classes are present in Physcomitrella and are being differentially regulated by drought and ABA. We will use these simple plants as a model to investigate the role in sugar signalling, development and complex gene regulation of the different genes of the large gene family (12 members in P. patens).

**P04-014 Identification of adenosine nucleosidase in Physcomitrella**

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Adenosine nucleosidase (ADN) is an enzyme of purine metabolism catalyzing the hydrolysis of purine nucleosides to the corresponding bases. Although the activity of this enzyme has been described in several plant species, no ADN encoding genes were identified to date. Here we report on ADN genes from Physcomitrella. Using amino acid sequences of related enzymes from various sources, three genes were identified from the Physcomitrella genome, designated as PpADN1, -2, -3. PpADN1 was selected for functional analysis and expressed in E. coli. Enzyme assays using recombinant PpADN1 demonstrated the conversion of radioactive adenosine to adenylic acid. Metabolisation of cytokinin riboside (iPR) suggests an involvement of PpADN1 in regulation of cytokinin activity. Experiments to determine substrate specificity revealed PpADN1 as a purine nucleoside-prefering enzyme. Based on the sequence of PpADN1 we identified two putative ADN genes from Arabidopsis (AtADN1, -2) sharing up to 58% identity with the Physcomitrella genes. Transient heterologous expression of AtADN:GFP fusion proteins in tobacco leaf cells exhibited cytoplasmatic localisation of both ADNs. T-DNA insertion mutants for each gene were crossed to generate a loss-of-function line for this enzyme activity. Further characterisation of ADNs will provide novel insights into the physiological role of these enzymes including their possible function in adenosine recycling and cytokinin homeostasis.

**P04-015 SHI-genes and regulation of local auxin biosynthesis in the moss Physcomitrella patens**

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In Arabidopsis, the formation of local peaks of the plant hormone auxin is crucial for development, differentiation and growth. The establishment of these peaks is known to depend on polar auxin transport but recent reports have revealed local expression of the YUCCA gene family, encoding rate limiting enzymes for auxin production, suggesting that local auxin biosynthesis might be equally important. YUCCA4 was recently identified as a direct target of the SHI/STY-related transcriptional activators suggesting that this family might be crucial for the establishment of auxin peaks by controlling local auxin biosynthesis. In an effort to learn more about the importance of local auxin biosynthesis in moss, we have initiated a characterisation of two SHI/STY-homologues in Physcomitrella, PpSHI1 and PpSHI2. Analysis of the expression patterns revealed maxima in caulonemata, rhizoids and axillary hairs which are all suggested sites of auxin activity. Single knockout lines exhibit short gametophore stems and an apparent stimulation of rhizoid growth. In PpSHI1 overexpressing lines, transcript levels correlate with elevated levels of auxin, missense expression of a GH3-GUS reporter and the severity of a detrimental phenotype affecting aged chloronemata and basal gametophore leaves. In summary, our data indicates that PpSHI1 and PpSHI2 serve a function analogous to the Arabidopsis SHI/STY gene family in controlling auxin biosynthesis.

**P04-016 Spatio-temporal accumulation of proteins during asymmetric stem cell division in Physcomitrella patens**

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Asymmetric cell division in a stem cell generates two different daughter cells; one is a self-renewed stem cell daughter and the other is a differentiated, non-stem cell daughter. Although unequal distribution of mRNA or proteins plays a pivotal role to specialize each daughter cell, such molecules in plant stem cells remain largely unknown. The moss, Physcomitrella patens assures a good system to study molecular mechanisms for asymmetric cell division of a stem cell. A single cell isolated from a protonema as a protoplast shows polar outgrowth and divides asymmetrically to generate an apical stem cell with pluripotency and a differentiated, non-stem cell. The apical stem cell, then, continues to divide asymmetrically to regenerate a row of differentiated, non-stem cells. We previously reported 59 genes as candidates that affected the asymmetric cell division of the stem cell. For those, we made citrine, which is a modified YFP, knock-in transgenic plants by using gene targeting technique to express the fusion proteins under a control of native promoters. We found nine of the citrine-tagged proteins accumulated preferentially in the apical stem cells but less in differentiated, non-stem cells. We will present time-lapse imaging of some of these proteins during the asymmetric cell division.

**P04-017 Vitrification of Splachnum ampullaceum: the effect of plant material source in the success of cryopreservation**

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Mosses show a high desiccation tolerance, in their biological cycle many of them develop asexual propagules, which present this characteristic even more accentuated. Cryopreservation of mosses can be enhanced selecting properly the kind of material that is going to be use as germplasm source. 

Splachnum ampullaceum is an endangered species in the Iberian Peninsula. The high plasticity of this moss has been revealed in vitro with the formation of brood cells from their protonemal cells when the nutritive resources and water availability scarce in the culture medium. Samples of gametophore, protonemata and brood cells obtained from in vitro cultures were vitrified after the exposure to PVS2 during two different times (5 and 10 min) at 0°C. The half of the samples were pre-treated with a loading solution consisted of 2 M glycerol and 0.4 M sucrose before their exposure to PVS2. After their introduction in cryovials, samples were plunge into liquid nitrogen and kept stored for a week. After this period, S. ampullaceum germplasm was recovered on the same growing medium. Loading solution exposure was shown as indespensible for a high recovery of all samples. After a 4-week-period, 92.29% of brood cells, 60% of gametophores and 46% of protonemata were recovered from loaded samples. The high levels in endogenous ABA of brood cells can be presumed the cause of their resistance to cryopreservation without desiccation pre-treatments.

**P04-018 Acclimation to UV, irradiation and climate – mosses as model plants**

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Polytrichastrum alpinum (Hedw.) G. L. Smith, the mountain hair moss, is a polar alpine species found over large areas and a key species of special interest in view of enhanced temperatures and moss, is a polar alpine species found over large areas and a key species of special interest in view of enhanced temperatures and water availability scarce in the culture medium. Samples of gametophore, protonemata and brood cells obtained from in vitro cultures were vitrified after the exposure to PVS2 during two different times (5 and 10 min) at 0°C. The half of the samples were pre-treated with a loading solution consisted of 2 M glycerol and 0.4 M sucrose before their exposure to PVS2. After their introduction in cryovials, samples were plunge into liquid nitrogen and kept stored for a week. After this period, S. ampullaceum germplasm was recovered on the same growing medium. Loading solution exposure was shown as indespensible for a high recovery of all samples. After a 4-week-period, 92.29% of brood cells, 60% of gametophores and 46% of protonemata were recovered from loaded samples. The high levels in endogenous ABA of brood cells can be presumed the cause of their resistance to cryopreservation without desiccation pre-treatments.

**P04-019 UV-B-triggered changes in Sphagnum sp. chemistry and cell wall ultrastructure: a research plan**

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Sphagnum peatland is a subtly balanced sensitive environment, favourable to few plant species. Specific structure makes Sphagnum mosses susceptible to environmental stress. Changes in UV radiation environment may be detrimental for the plant and the ecosystem where it dominates. Little is known about the impact of UV-B radiation on Sphagnum mosses. It is discovered that the plant's response to UV radiation is species-specific. Since Sphagnum role in peatlands is fundamental, precise knowledge about how UV-B radiation affects the plant is vital. Responses of Sphagnum species to different UV-B levels might be used to predict changes in fragile subarctic bog ecosystems. By using a UV-B exclusion experiment in Finnish Lapland we will aim at studying the impact of UV radiation on: (1) the concentration of various UV-B absorbing compounds in Sphagnum species; (2) the ultrastructure of Sphagnum's cell wall; and (3) the chemical and structural responses of different Sphagnum species. UV-B absorbing compounds in bryophytes can be synthesised in less than a day, whereas their half-life is much longer, 3–15 days. Thus frequent monitoring is needed to follow short term changes in Sphagnum phenolics. The total content of UV-B absorbing compounds and the content of a number of separate methanol soluble and cell wall bound phenolics will be defined using high performance liquid chromatography. The ultrastructure of Sphagnum cell walls will be studied with electron microscopy.

**P04-020 Studies on cytoplasmic phytochrome signalling in Physcomitrella patens**

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Physcomitrella are red light photoreceptors mediating numerous developmental and physiological responses. Upon red light absorption the P; ground state converts into the photosynthetically-active P; state, which converts back to P; upon absorption of far-red light. In higher plants P; translocates into the nucleus whereupon it regulates transcription of light-responsive genes. However, in higher plants some red/far-red reversible responses occur far too quickly to derive effects from transcription. In lower plants, moreover, red/far-red reversible directional responses to vential characteristics of light are well established. These cannot be based on a transcriptional mechanism, but rather imply a yet unknown cytoplasmic function. Four Physcomitrella phytochrome genes have been identified and disrupted by gene targeting. In particular phy4; showed defects in phototropism, polarotropism and chloroplast photorelocation in red light. Thus it seems that PHY4 mediates directional light sensing and is probably connected to a cytoplasmic signalling pathway. Physcomitrella thus represents an excellent object for characterising this likely cytoplasmic phytochrome function. In order to elucidate the molecular mechanisms involved in early signalling events, we are focussing both on the identification of phytochrome interacting partners using yeast two-hybrid methods as well as on the intracellular localisation of phytochrome under different light conditions via fluorescence tagging.

**P04-021 Analysis of the evolution of MYB-like regulatory gene function; comparison of genes in Physcomitrella and Arabidopsis**

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Phytochromes are red light photoreceptors mediating numerous developmental and physiological responses. Upon red light absorption the P; ground state converts into the photosynthetically-active P; state, which converts back to P; upon absorption of far-red light. In higher plants P; translocates into the nucleus whereupon it regulates transcription of light-responsive genes. However, in higher plants some red/far-red reversible responses occur far too quickly to derive effects from transcription. In lower plants, moreover, red/far-red reversible directional responses to vential characteristics of light are well established. These cannot be based on a transcriptional mechanism, but rather imply a yet unknown cytoplasmic function. Four Physcomitrella phytochrome genes have been identified and disrupted by gene targeting. In particular phy4; showed defects in phototropism, polarotropism and chloroplast photorelocation in red light. Thus it seems that PHY4 mediates directional light sensing and is probably connected to a cytoplasmic signalling pathway. Physcomitrella thus represents an excellent object for characterising this likely cytoplasmic phytochrome function. In order to elucidate the molecular mechanisms involved in early signalling events, we are focussing both on the identification of phytochrome interacting partners using yeast two-hybrid methods as well as on the intracellular localisation of phytochrome under different light conditions via fluorescence tagging.
MYB transcription factors have a conserved N-terminal MYB DNA-binding domain and comprise a large gene family in plants implicated in a number of diverse processes. We aim to elucidate how responses to abiotic stress have evolved in plants through the comparative analysis of selected MYB transcription factors. Identification of the molecular adaptations and the patterns of molecular evolution associated with the adaptations of plants to life on land should help us understand some of the diversity in the plant kingdom and how plants have adapted to life in very different environments. Two MYB-related genes encoding structurally similar MYB transcription factors in Arabidopsis thaliana, AtMYB6 and AtMYB8 (HOS10), show strong similarity to proteins encoded by two putative MYB genes found in Physcomitrella patens, PpMYB4 and PpMYB5. AtMYB8 mutants are claimed to have an altered response under some abiotic stress conditions. AtMYB6 mutants do not respond to abiotic stress in the same way as hom10. This suggests that AtMYB6 could have evolved other functions during its evolution. Microarray analysis will be undertaken to identify possible genes under AtMYB6 regulation. Targeted knockout of PpMYB4 and PpMYB5 will shed light on the roles of these transcription factors in Physcomitrella and elucidate whether their functions are similar to those of AtMYB6 and AtMYB8 (HOS10) and have been conserved during the evolution of plants or whether they diverged to regulate other pathways.

P04-022 An overexpression screen for early developmental phenotypes in moss
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We are interested in how plants have evolved and how morphogenesis and pattern formation can be achieved with a minimal set of building blocks. The moss is build up of filamentous protonemata with its two distinct cell types, caulonema and chloronema, and the upright gametophore. Presumably the protonemal phase is more ancient in its origin with green alga and the switch between caulonema and chloronema represents a truly ancient example of cell type differentiation. We will screen a moss cDNA expression library in regenerating moss protoplasts to identify genes that control the switch between the two cell types. The cDNA library, which represents genes expressed under many different conditions, was normalized in order to reduce abundant transcripts. This normalized cDNA library contains 11.9 million primary clones where the average insert size is 1.1 kb and the size range is from 0.7 to 2.6 kb. It was made in a Gateway vector, so that it easily can be moved to other vectors. For the screen, we will use an overexpression vector which targets the inserts to a neutral locus in Physcomitrella with the aim to obtain stable integrants that overexpress each cDNA. Transformants will be screened for early onset of caulonema formation, and for other developmentally interesting phenotypes. Plasmids from such colonies will be rescued into E. coli after restriction and recircularization of moss DNA, sequenced to identify the cDNAs involved, and retested by re-transformation into moss.

P04-023 Meiotic recombination genes involved in gene targeting in Physcomitrella patens
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The moss Physcomitrella patens exhibits high frequencies of gene targeting. The mechanism by which targeted integration occurs is believed to be through the homology-dependent (HR) DNA double-strand break repair pathway. In flowering plants, the activity of this pathway is subordinate to the non-homologous end joining (NHEJ) pathway, except during meiosis, when the HR pathway is required for meiotic recombination. We have examined the roles of conserved DNA repair genes by targeted knockout of their Physcomitrella orthologs. Detection and stabilisation of double-strand breaks is mediated by the heterotrimeric Mre11-Rad50-Nbs1 (MRN) complex. Disruptants of both PpMRE11 and PpRad50 are viable, but display severe growth defects, and are hypersensitive to the DNA damage. By contrast, disruption of the PpNBS1 gene has no effect. Meiosis-specific recombination genes also act in the somatic DNA repair and recombination pathway in Physcomitrella. In yeast and mammals, the genes MND1 and HOP2 are expressed exclusively during meiosis, where their products interact with each other, with the meiosis-specific Rad51 paralog Dmc1, and with single-stranded DNA to promote strand invasion in meiotic synapsis. Targeted gene knockout of PpMND1 and PpHOP2 produces meiotically incompetent mutants, as expected. They are also compromised in somatic DNA repair, as evidenced by hypersensitivity to genotoxic treatment, and exhibit a significantly reduced gene targeting activity.

P04-024 The ectohydric moss Pleurozium schreberi (Britt.) Mitt. after 5 years of enhanced UV-B radiation in situ
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Northern ecosystems are adapted to low levels of UV radiation, and therefore considered especially vulnerable to increasing UV-B radiation. The majority of bryophytes are believed to be particularly susceptible to UV-B radiation due to their simple structure. The enhanced UV experiment was begun in spring 2002, where ozone depletion of approximately 20% was simulated. Samples were collected on October 1, 2006. The UV-absorbing compounds were extracted from the youngest top and the following older green part of each moss shoot. A significant increase in the UV-B-absorbing compounds under enhanced UV-B was detected in the new segments. No treatment effect was found in the UV-A-absorbing compounds, or in the UV-B-absorbing compounds of the old segments. No additional accumulation of compounds in the old green segments was found. Earlier findings showed that UV-B enhancement treatment affected the UV-B-absorbing compounds only transiently. However, the concentrations of these compounds correlated with the amount of radiation received (Lappalainen et al. 2007). Our results show that even though P. schreberi seemed to adapt to the enhanced UV-B radiation after the first year, the effects on the UV-B-absorbing compounds can still be found after 5 years.
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P04-025 MIKCw MADS proteins with a putative involvement in regeneration and rhizoid development in Physcomitrella patens

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In previously generated Physcomitrella knock-out mutants for several MIKCw MADS box genes expressing reporter proteins under the control of the respective promoter no major phenotypic aberration compared to wild type could be observed. This indicated that a putative effect on development was either not apparent under normal conditions or very subtle hypothetically due to redundancy in the MIKCw family as suggested by sequence similarity and overlapping expression as well as protein interaction patterns of at least some of the members. However analysis of the reporter gene activity in those lines for the genes PPM3, PPM4, and PPM9 revealed an expression pattern associated with the site of rhizoid attachment and emergence respectively, giving a possible hint at the function of these genes. Together with certain abnormalities in regeneration observed in some of the mutant lines this led us to a closer analysis of a putative involvement of the proteins PPM3, PPM4, and PPM9 in rhizoid development and regeneration processes. The findings are discussed.

P04-026 The evolution of ROS scavenging in plants

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Physcomitrella patens is highly tolerant to extreme environments like drought, osmotic and salt stress and identifying the underlying mechanisms giving this tolerance will supply valuable information on stress tolerance in higher plants. Doing comparative genomics across this ancient non-vascular plant and angiosperms will also allow us to determine which genes have been retained during the evolution of plants and which protective mechanism vascular plants have developed to deal with their specific environmental challenges. A common denominator under abiotic stress is the generation of reactive oxygen species (ROS) and efficient removal of these damaging radicals is essential for plant survival. The ascorbate-glutathione pathway is one of the main scavenging pathways and is found in the cytosol, chloroplast, mitochondria and peroxisomes in angiosperms. Initial studies have shown that a key enzyme in that pathway, monodehydroascorbate reductase (MDHAR), appears to be absent in the chloroplast, mitochondria and peroxisomes in Physcomitrella, hence, ROS scavenging is likely to rely on different enzymes in these organelles in moss. The main target of this project is to determine (1) if the functional role of cytosolic MDHAR has been conserved throughout plant evolution; (2) why vascular plants require extra ROS scavenging in the chloroplast, mitochondria and peroxisomes; and (3) if Physcomitrella has maintained or developed a unique ROS scavenging system not present in higher plants?

P04-027 Identification of genes contributing to gene targeting efficiency in Physcomitrella patens

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The high gene targeting (GT) efficiencies in P. patens are unique among plants and comparable to those found in yeast and mammals. GT is based on homologous recombination (HR) but the mechanisms underlying this process are mostly unknown. By studying the HR apparatus in moss we are aiming to gain insight into the biological basis of GT. After generating a complete dataset of all recombination related genes in P. patens based on the complete genome sequence we will select genes of interest for gene knockout and perform functional analysis of the respective proteins. As differential transcriptional regulation may be crucial for high GT efficiencies, we aim to analyze the transcriptional patterns of recombination genes using a whole genome microarray. Expression profiling and SuperSAGE analysis of diploid and haploid lines will be performed as well. The project is part of the GABI-PRECISE (precision engineering of genes in barley) initiative which aims at introducing GT technology to barley. At present, crop plant transformation lacks precision and thus hampers the application of modern plant biotechnology. The stimulation of GT in barley via knowledge transfer from models like P. patens will as a long term goal allow directed modification of crop plants for the production of high-value plant products and ensure sustainable agriculture. We will present recent progress in the GABI-PRECISE project using the P. patens system. Financial support by the BMBF is gratefully acknowledged.

P04-028 Retrospective bioindication of total ozone and UV radiation using hydroxycinnamic acid derivatives of herbarium samples of an aquatic liverwort

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Due to the anthropogenic ozone depletion, the amount of solar ultraviolet (UV) radiation in the biosphere has increased. In this context, we have analyzed both the bulk UV absorbance of methanolic extracts and the levels of five UV-absorbing compounds in 135 herbarium samples of the aquatic liverwort Jungermannia exsertifolia subsp. cordifolia from northern Europe. Our objective was to reconstruct past levels of ozone and UV radiation using, for the first time to our knowledge, individual UV-absorbing compounds. The samples were collected in the period 1850–2006, and the compounds analyzed were five hydroxycinnamic acid derivatives. Both the bulk UV absorbance and the concentrations of all the individual compounds increased in recent decades. A multiple regression model was used to predict the level of p-coumaroylmalic acid as a function of the collection year, the collection month, latitude and altitude.
P04-029 Functional analysis of DNA methylation in Physcomitrella patens
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DNA and histone modifications take part in establishing epigenetic heritable states of gene transcription, which play important roles in regulating angiosperm development. In Arabidopsis, the epigenetic state of the haploid gametophytes, have a profound influence on the embryo, endosperm and seed. However, little is known about the differences in epigenetic states that distinguish the gametophyte and sporophyte. It is unknown how widespread they are and the extent to which these differences might influence plant development. It is not feasible to address these questions in Arabidopsis because the dramatic reduction of the gametophytic generation in angiosperms renders them inaccessible. Thus the moss Physcomitrella patens, may serve as a more suitable model system to address the above questions as the gametophyte and sporophyte stages are both physically accessible. We have identified candidate homologues of DNA methyltransferase in the Physcomitrella genome, among them a homolog of the chromomethylases (CMT) gene. To study the function of the above genes we are taking a genetic approach generating knockout moss plants. Preliminary analysis of these mutants reveals alteration in their developmental program as compared with wild type plants. The above approach will enable to understand the role of DNA methylation during moss gametophyte and sporophyte development. A progress report of the above research program will be presented.

P04-030 Mosses with roots? Independent recruitment of a development switch mechanism
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The evolution and diversity of development mechanisms in multicellular organisms is partly due to the modification of underlying transcription regulatory networks. The plasticity of these regulatory networks allows a relatively small array of transcription factor families to specifically control a myriad of biological processes. The homologous basic-helix-loop-helix (bHLH) transcription factors RHD6 and RSL1 are required for the development of root hairs in the sporophyte of Arabidopsis. Their homologs in Physcomitrella are required for the development of caulonema cells and rhizoids in the gametophytic generation. This suggests that an ancient regulatory mechanism was independently recruited for two non-homologous processes in mosses and angiosperms. A second family of bHLH transcription factors, closely related to RHD6, is also specifically involved in root hair development in Arabidopsis. This family is present in Physcomitrella, suggesting that its members belong to the same ancient RHD6 regulatory network. To test this hypothesis, we are making Physcomitrella mutants that lack the function of these genes. We will then further dissect the molecular basis of this mechanism through protein-protein interaction and cross species complementation studies.

P04-031 PpRMS1, homolog of pea branching gene RMS1, regulates the branching and the morphology of colony in Physcomitrella
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Our work on the control of branching in pea is based on a large series of highly branched rammosus (rms) mutants corresponding to five genes (rms1 to rms5). We have developed an original model on which novel signals are involved in addition to auxin and cytokinin, that are classically involved in apical dominance. These genes are conserved in higher plants (MORE AXILLARY GROWTH (MAX) genes in Arabidopsis for example). Homologous sequences of RMS/ MAX genes can be identified for non vascular organisms such as moss Physcomitrella patens. We are using the moss to have a better understanding of the functional evolution of the “RMS system” in land plants and the origin of branching in vascular plants. We obtained a knockout moss mutant for the gene PpRMS1. This gene is involved in the biosynthesis of the novel carotenoid derived compound which acts as a branching inhibitor. During the early growth of colony, the Pprms1 mutant shows a dens colony due to an increase of secondary filaments development. After gametophores development, branching of filaments which extend radially is maintained in the mutant in contrast to wild type in which branching and extension of filaments stop. PpRMS1 seems to control the branching of filaments and consequently regulates the number of leafy shoots on the colony.

P04-032 Identification of homologues of the mammalian peripheral-type benzodiazepine receptor in Physcomitrella patens
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In the moss Physcomitrella patens, the gene PpTSPO1 was identified by the cloning of abiotic stress-inducible genes. PpTSPO1 encodes a protein homologous to the mammalian mitochondrial peripheral-type benzodiazepine receptor (MBR) and the bacterial tryptophan rich sensory protein (TSPO) which are integral membrane proteins involved in the transport of porphyrin intermediates. Like the mammalian homologue, PpTSPO1 is localised to mitochondria and the analysis of PpTSPO1 null mutants revealed an essential role of PpTSPO1 in the abiotic stress adaptation. The analysis of the P. patens genome indicates the presence of four additional PpTSPO homologues whereas A. thaliana contains a single TSPO gene. Based on the compartmentalised plant porphyrin biosynthesis pathway TSPO proteins may reside in mitochondrial processes.
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as well as plastidic membranes to allocate porphyrin intermediates at the sub-cellular level for the different biosynthetic branches. The N-terminal sequences of PpTSPO1, PpTSPO4 and PpTSPO5 as well as the A. thaliana homologue contain several in-frame start codons. Assuming that the N-terminal extensions may contain particular targeting information to control PpTSPO localisation we developed a series of GFP fusions to infer possible intracellular sorting of PpTSPO proteins and the A. thaliana homologue. The sub-cellular targeting and putative roles of the PpTSPO gene family will be discussed.

P04-033 Evolution of the WOX gene family: from basal functions to modern genes involved in stem cell homeostasis

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In seed plants, the family of Wuschel-like Homeobox (WOX) transcription factors is involved in stem cell homeostasis, organ formation and embryonic patterning. Genome sequence information and degenerate primer PCRs revealed that the WOX gene family was complete at the base of angiosperms. A phylogenetic analysis based on homeodomain amino acid sequences shows that the gene family consists of three major branches emerging at critical points in plant evolution: a basal clade consisting of Arabidopsis WOX13 and relatives present in all land plants, a WOX9-like clade emerging with the invention of vasculature and a modern clade containing WOX1-7 and WUS that started to evolve in gymnosperms. By examining gene function and expression patterns at crucial points in plant evolution, we address the emergence of new genes and the origin of the entire gene family. To gain knowledge about the ancestral function of WOX genes, we have chosen the moss Physcomitrella patens as a model organism and via homologous recombination have generated knock-out plants for the three PpWOX genes, all of which belong to the basal WOX13 clade. In parallel, we are analysing Arabidopsis WOX13 mutant alleles to possibly detect remnants of the ancestral function in the model species. The emergence of the modern clade of WOX genes is presently analysed in three major gymnosperm radiations. In the basal angiosperm Nymphaea caseonica a major focus resides on stem cell homeostasis in the root and shoot meristems.

P04-034 Ectopic expression of heterologous Arabidopsis and rice genes in the moss Physcomitrella patens

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Several genes isolated from Arabidopsis and rice were over-expressed in the moss Physcomitrella patens. Genes involved in abiotic stresses, male-sterility, senescence, or root hair development, which we characterized their functions in Arabidopsis and rice using Northern analyses after treating various phytohormones and stresses, as well as using over-expression and knock-out plant studies, were used for this study. We report the observed phenotypic changes and their putative roles in P. patens.

P04-035 Efficient transformation of the moss Physcomitrella patens by particle bombardment

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High rates of homologous recombination (HR) in comparison to other plants make the moss Physcomitrella patens an attractive model organism for genetic studies as well as biotechnological applications. Here we compare direct transformation of Physcomitrella protonema tissue by microcarrier bombardment and PEG mediated transformation of protoplasts. We have developed a simple protocol for the hand-held Bio-Rad Helios Gene Gun and used enhanced yellow fluorescent protein (EYFP) as a reporter gene. EYFP makes evaluation of transformation possible with a regular fluorescence stereomicroscopy without interference of cell wall autofluorescence in contrast to GFP, where confocal microscopy is needed to dissect fluorescence. The bombardment with 1 μg gold particles was approximately three times more effective than PEG transformation and yielded approximately 40 transformants per 1 μg of DNA precipitated on gold microcarriers. Half of the transformants were stable.

P04-036 The moss Physcomitrella patens - a novel tool to study mechanistic and regulatory properties of chloroplast protein import

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The majority of chloroplast proteins are synthesized as precursor proteins in the cytosol and posttranslationally imported into the organelle. Two translocation machineries at the outer and inner envelope of chloroplasts facilitate the import of precursor proteins. At present, the translocon at the inner envelope of chloroplasts (TIC complex) is thought to consist of seven proteins, whose function and structure is a subject of intensive research. Nearly all known TIC components have homologs in Physcomitrella patens, therefore we now use this organism to study protein import into chloroplasts. Physcomitrella is an excellent tool for several reasons (1) its few cell types and the simple morphology make it an ideal candidate to combine molecular and cell biological methods; (2) the life cycle is dominated by a haploid gametophytic generation; and (3) its high degree of homologous recombination in the nuclear DNA, which is several orders of magnitude higher than in any other characterized plant species. For the analysis of the structure and function of the TIC components, mutants are generated. Phenotypic and biochemical analyses are carried out using e.g. electron microscopy, 2D electrophoresis and protein import assays. Furthermore, the composition, stoichiometry and structure of the TIC complex will be
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P04-037 Acclimation of moss *Pleurozium schreberi* (Brid.) Mitt. photosynthetic characteristics between-habitat and within-canopy light gradients

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While most bryophytes are obligatory shade plants, several are able to grow over a broad range of light intensities. In vascular plants, acclimation to light involves a variety of structural and physiological modifications that occur similarly within the canopy light gradient and between habitats with varying light availability, but those mechanisms of light acclimation of mosses are yet poorly understood. We studied between-habitat and within-canopy variation in physiology and structure of the forest moss *Pleurozium schreberi* to determine the key traits responsible for light acclimation. Our results showed a reduction in pigment content with increasing light availability. Within the canopy, pigment content increased from top to 2 cm deep and then it decreased with growing age along the canopy. There was no significant change between-habitat in Chl a/b ratio, but it decreased from canopy top to bottom. Chl/carotenoid ratio decreased with increasing light along both light gradients. N was not associated with habitat light conditions, but it decreased along the canopy. While maximum quantum efficiency of Photosystem II (Fv/Fm) increased with increasing light, the maximum electron transport capacity (Jmax) was not correlated with habitat light conditions, but Jmax decreased along the canopy. These results demonstrate that most photosynthetic potentials acclimate to light gradients, but within the canopy, age-dependent changes strongly interact with light acclimation.

P04-039 Milestones towards the *Physcomitrella patens* genome v2.0

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The draft genome of the moss *Physcomitrella patens* was published in January 2008. Here we report our analyses and first results towards generation of the v2.0 genome. In order to generate the basic set of *P. patens* protein coding genes, those predicted genes overlapping with transposable elements as well as tRNA genes, miRNA precursors and gene models on contaminated scaffolds were removed. Nevertheless this will not be the final set of moss protein coding genes. For numerous loci gene models are lacking, fragmentary or wrong. We are performing a new round of gene structure prediction using the Eugène gene finder in combination with an extended model selection process to improve the quality and coverage of the gene models. A physically anchored SSR-based genetic linkage map was merged with an AFLP-based map. The resulting merged map will be used for BAC-end aided super-scaffolding. We will report our analyses of tandemly arrayed genes, in particular our comparative analysis in *P. patens* and *S. moellendorfii*. Nuclear insertions of organellar DNA (norgDNA) are a common phenomenon in animal and plant genomes. We will show evidence for nuclear mitochondrial (NUMTs) and plastid DNA (NUPTs) within the *P. patens* genome. We are grateful to the labs of A.C. Cuming, M. Hasebe, T. Nishiyama, R.S. Quatrano and Y. Van de Peer as well as to the JGI for providing data. Funding by the DFG (RE 837/10-2) is gratefully acknowledged.