Lectures

L5.1

Guarding the gates: stomatal responses to pathogens

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Pathogens exploit different infection strategies to infect plants and stomata represent a direct pathway by which microbes can enter the plant. To counter this, guard cells have evolved the ability to detect conserved microbial molecules and close stomata. In our attempts to understand the full nature of the interactions that occur between a potential pathogen and its host, we are focussing on stomatal immunity. The plasma membrane receptor FLS2 confers plant immunity through perception of bacterial flagellin (flg22), which triggers stomatal closure. Despite the importance of stomatal immunity, the pathways underlying stomatal behavior and their interaction with immunity control remain largely unknown. To address this, we determined the genetic framework of stomatal immunity. I will present our high-throughput imaging pipeline of detecting stomatal apertures, and will discuss some of our recent findings of stomatal immunity control. This includes some recent works on ESCRT-I components that are required for plant immunity at the level of stomata closure. Taken together, advanced bioimaging allows us to tackle the role of stomatal closure involved in the interaction between plants and microbes.

L5.2

Novel roles for glutathione in linking oxidative stress to phytohormone-dependent signalling

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Cellular redox state is regulated by numerous components. The thiol-disulfide compound, glutathione, is considered to be one of the most significant, owing to its antioxidant power and potential influence over protein structure and function. While signalling roles for glutathione in plants have been suggested for several years, hard proof is scarce. Our analyses of *Arabidopsis* mutants points to a role for the glutathione reductase-glutathione system in regulating defense phytohormone pathways triggered by oxidative stress. Recently, effects triggered by genetic manipulation of glutathione status in a catalase-deficient background reveal that this status is important to allow oxidative stress to activate pathogenesis-related phytohormone signalling pathways. The role of glutathione does not seem to be linked to its antioxidant capacity, and is not mediated by regulation through the known pathogenesis-related signalling protein, NPR1. Based on these data, we suggest that new glutathione-dependent components that link oxidative stress to response outputs await discovery.

Oral presentations

O5.1

ROSMETER: a bioinformatic tool for the identification of reactive oxygen species (ROS) transcriptomic imprints

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The chemical identity of ROS and their subcellular localization leaves a transcriptomic imprint, and we developed a tool, ROSMETER, which portrays it. Transcriptome data, for which the ROS type and organelle origin are known, were compiled into indices and made accessible by http://app.agri.gov.il/noa/ROSMETER.php. The ROSMETER compares any transcriptome data to theses indices and provides correlations values and their lists of genes. The ROSMETER was applied to identify the ROS signatures in transcriptomes of senescing plants and of those exposed various stresses. A mitochondrial ROS signature was identified in the early pre-symptomatic stages of leaf senescence. The ROSMETER analysis of stresses such as: salt, cold, UV, drought heat and pathogens revealed both commonalties and prominent differences. The early responses to the various abiotic stresses clustered together, independently of later responses, and exhibited negative correlations to several ROS indices. In general, the ROS transcriptome signature of abiotic stress was confined to only few indices, while that of biotic stress is common to several indices. The ROSMETER can assists in elucidating the molecular mechanisms of plant acclimation to stress.

O5.2

Natural variation in ozone induced cell death in *Arabidopsis*

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In nature plants have adapted to various climates and are likely to face particular stresses (e.g. heat or drought) depending on their habitat type. This is also true for *Arabidopsis thaliana* whose distribution ranges from Scandinavia to Northern Africa, including vast variation in climatic and day length conditions. The atmospheric pollutant ozone (O_3) can be used to study signalling activated by reactive oxygen species and treatment of plants with short, high concentrations of O_3 activates programmed cell death (PCD). The molecular responses to acute O_3 exposure are overlapping with responses to other stresses. Natural accessions of *A. thaliana* have shown in earlier studies remarkable phenotypic variation in their response to O_3 exposure. We have expanded the selection to include 350 natural *Arabidopsis* accessions and 400 multi-parent advanced generation inter-cross (MAGIC) lines to study genetics of ozone induced PCD. We have identified candidate genes by genome-wide association mapping (GWAS) and quantitative trait locus (QTL) mapping. The underlying genetic variation has proven to be complex and for different accessions different main effect QTL may prove to be responsible for O_3 triggered cell death.

O5.3

Oxidative stress responses are regulated by heat shock factor HSFA4A in *Arabidopsis*

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Heat shock factors (HSFs) are principal regulators of plant responses to environmental stress. Overexpression of the previously unknown HSFA4A in *Arabidopsis* confers tolerance to salt, as well as reduced sensitivity to heavy metals, anoxia and oxidative agents, while the *hsfa4a* T-DNA insertion mutant is hypersensitive to salt stress. HSFA4A overexpression decreases, whereas the *hsfa4a* mutation elevates hydrogen peroxide accumulation and lipid peroxidation. Genome-wide transcript profiling indicates that HSFA4A regulates the expression of a large set of genes responding to oxidative stress. HSFA4A shows dimerization in yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays, which is reduced by mutagenesis of conserved cysteine residues. Conserved serine and threonine residues of HSFA4A is phosphorylated by the mitogen-activated protein kinases MPK3 and MPK6 that interact with HSFA4A in yeast and plant cells. HSFA4A acts as a downstream regulator in MPK3/6-dependent regulatory pathways, controlling responses to different environmental stresses.

O5.4

Role of NADPH oxidase in the regulation of peroxisome dynamics

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Reactive oxygen species (ROS) play a central role in the signalling networks that regulate essential cell processes. Recently, it has been demonstrated that ROS is capable of regulating the dynamics of peroxisomes under stress conditions caused by Cd treatment (Rodríguez-Serrano et al., Free Rad Biol. Med. 2009; 47: 1632-1639), although the functioning of these changes and their regulation are still unknown. In this study, we analyze the dynamics of peroxisomes in response to short and long periods of Cd treatment by using a constitutive *Arabidopsis* line expressing CFP-SKL in peroxisomes. Time lapse analysis of peroxisomal dynamics shows changes in the morphology (peroxule formation), number and speed of peroxisomes during treatments which were regulated by ROS. To identify the source of ROS regulating these processes, we obtained *Arabidopsis* lines deficient in NADPH oxidases (C, D and F) and expressing CFP-SKL to analyse peroxisome dynamics as well as oxidative stress parameters. The role played by NADPH oxidases in regulating peroxisomal dynamics and proliferation will be discussed. Support by ERDF-cofinanced grants from the Ministry of Economy and Competitiveness BIO2008-04067 and BIO2012-36742. Research was supported by OTKA Grant K-81765, IPA project HUSRB/1002/214/036 and COST action FA0605.

O5.5

RCD1 regulation and recognition of poly(ADP-ribosyl)ation in *Arabidopsis*

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Plants are continuously exposed to abiotic and biotic stresses. A common feature of plant stress response is the excessive accumulation of reactive oxygen species (ROS). The RCD1 (Radical-induced Cell Death1) protein is an important regulator of stress and developmental responses in *Arabidopsis*. A loss-of-function mutation in RCD1 results in highly pleiotropic phenotypes including increased sensitivity to apoplastic ROS and tolerance to chloroplastic ROS. RCD1 is a multidomain protein containing the N-terminal WWE domain, the inactive PARP-like domain and the RST domain, mediating interaction of RCD1 with a large variety of transcription factors. Here we show that the RCD1 WWE domain binds poly(ADP-ribose) polymer. Poly(ADP-ribosyl)ation is involved in *Arabidopsis* stress response; however, no acceptor proteins have been described so far. RCD1 is tightly regulated in planta at the protein level via phosphorylation and proteasomal degradation. Analysis of the protein level under variety of stresses show differential response to specific stresses. These data provide novel insights into the biochemical function of RCD1, a central integrator of plant stress and developmental response.

O5.6

The role of miRNAs upon metal stress in *Arabidopsis thaliana*

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In plants, microRNAs (miRNAs) control various biological processes by negatively regulating the expression of complementary target genes post-transcriptionally by cleavage or translational inhibition. MiRNAs are main players in metal stress responses. Exposure of plants to excess metal concentrations disturbs the cellular redox balance and enhances ROS accumulation. Plants modify their gene expression by the activity of miRNAs in response to metal toxicity to regulate complexation of excess metals, defense against oxidative stress and signal transduction. In this study, miRNAs with an altered expression upon Cd and Cu toxicity are identified and the expression levels of their targets are investigated in *Arabidopsis thaliana*. Furthermore, SPL7 is an important transcription factor of several miRNAs. *Spl7* knock-out mutants revealed that SPL7 is involved in the expression of miR397, miR398 and miR857 under control conditions and after exposure to Cd and Cu. On the other hand, the expression levels of the targets, that function in ROS scavenging, sulfate assimilation and lignification, are dependent on SPL7 under control conditions, but other regulatory mechanisms are involved in their regulation upon Cd and Cu toxicity.

Posters

P5.1

Elevated CO_2 attenuates oxidative stress caused by drought and elevated temperature in four C3 plant species

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It is frequently observed that increased CO_2 protects against stress impact. However, the underlying molecular mechanisms are not well understood. Protection may be obtained by altered defense mechanisms, or reduced ROS generation (i.e. relaxation). Four C3 plant species (*Lolium perenne*, *Poa pratensis*, *Medicago lupulina* and *Lotus corniculatus*) were subjected to drought stress in ambient climate and in elevated temperature (+3 °C)and elevated CO_2 (620 ppm). Drought reduced plant growth, inhibited photosynthesis and stomatal conductance and induced oxidative stress. Elevated temperature increased drought effects, e.g. it further decreased fresh weight and photosynthesis, and increased oxidative stress and increased osmo-protectants. Elevated CO_2 protected against stress impact at the level of oxidative stress parameters. It decreased H_2O_2 and lipid peroxidation in parallel with reducing NADPH oxidase and LOX activities in most species. Elevated CO_2 also induced a reduction in proline, SOD, CAT, GPX and GR, but did not affect the ASC-GSH cycle. PCA analysis demonstrated that stress and CO_2 responses, are, to some extend, plant-family specific. ROS relaxation could, at least partially, explain the CO_2 protection effect.

P5.2

Implication of early nitric oxide and reactive oxygen species generation in potato resistance to *Phytophthora infestans*

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Both reactive nitrogen and reactive oxygen species are early activated by plant after pathogen recognition. The turnover of RNS and ROS appears to be crucial in the regulation of redox signalling leading do resistance or disease development. The kinetics and intensity of RNS and ROS generations were elucidated in the susceptible and resistant potato genotypes to *P. infestans* after inoculation. Obtained data revealed that resistant response was early linked with strong overproduction of $O_2^{\bullet -}$ what together with enhanced NO generation, immediately triggered $ONOO^-$ formation. As we found minor increase of H_2O_2 accumulation correlated with strong SOD up-regulation. Fast antioxidants and –SH abundant proteins content raised rapidly in non-compatible interaction. Above stated changes enabled plant to activate successful defense strategy through up-regulation of *PR-2* and *PR-3* activity as well as *PR-1* transcript accumulation. To sum up, presented results indicate that early synthesis of NO and ROS might play an important role in creating intracellular redox equilibrium tuned with resistance of potato to *P. infestans*. This work was supported by the Grant of National Science Centre 2011/03/N/NZ9/00290.

P5.3

Evaluation of biochemical and standard effects of acetaminophen in *Lemna minor* vs *Lemna gibba*

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Acetaminophen is one of the most widely prescribed drugs, due to its antipyretic and analgesic properties. However, it presents high toxicity when the dosage surpasses the detoxification capability of an exposed organism, with the involvement of an already described OS pathway. To address the issue of the ecotoxicity of paracetamol, we acutely exposed two aquatic plant species, *L. gibba* and *L. minor*, to acetaminophen. Chlorophyll content, number of fronds, biomass, lipid peroxidation (TBARS assay) and proline content were determined. Our results showed marked differences between the two species. Acetaminophen caused a significant decrease in the number of fronds on *L. minor* (EC50 = 446.6 mg/l) and on *L. gibba* (>1000 mg/l). No effects were reported, both for chlorophyll content and total biomass, for both species. However, the proline content in *L. gibba* was substantially reduced. The overall conclusions pointed to the occurrence of an oxidative stress event, more prominent in *L. minor* that exhibited a significant increase of TBARS. Interestingly, the mechanisms that allowed *L. gibba* to cope with acetaminophen exposure were distinct from those reported for *L. minor*, with the likely involvement of proline as antioxidant.

P5.4

ROS-associated systemic control of cucurbit scab by some novel compounds

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Several substances known to boost ROS but unknown as inducers of systemic resistance were tested towards cucurbit scab. Those were photosensitizers (dyes bengal rose and methylene blue) or mercaptopyridine N-oxide as well as inhibitor of catalase (aminotiazole) and that of superoxide dismutase (diethyl dithiocarbamate). The solutions were applied to the cucumber 1st leaf. As a result, generation of superoxide radical in drop diffusates of this leaf was stimulated. When the 2nd leaf emerged it was inoculated with *Cladosporium cucumerinum* spores. All chemical pretreatments reduced the disease severity on the 2nd leaf and (all but aminotriazole) increased O_2^- formation in its diffusate. Besides, some compounds rendered the diffusate fungitoxic. Keeping plants treated with methylene blue for the first day in the darkness reduced the effectiveness of protection as well as stimulation of O_2^- formation in the 2nd leaf. Therefore, the novel compounds controlled the disease systemically. Apparently, the localized oxidative burst caused by them in the treated tissues primed the distant leaves for resistance, which involved ROS again. Extracellular ROS might play roles in both cases in addition to intracellular events.

P5.5

In vivo localization of singlet oxygen ($^1O_2$) in *Arabidopsis* roots infected with beet cyst nematode *Heterodera schachtii*

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Reactive oxygen species (ROS), especially singlet oxygen ($^1O_2$), are produced in plants in response to biotic stress. They cause oxidative damage leading to programmed cell death and hypersensitive response nematode infection. It is also supposed that $^1O_2$ plays a potential role as a signal molecule. The aim of our research was to analyze changes in localization and abundance of $^1O_2$ during nematode migration, induction and development of feeding sites (syncytia) induced by a beet cyst nematode (*H. schachtii*) in roots of *A. thaliana*. *Arabidopsis* roots systems containing 1, 3 and 5 day-old feeding sites were in vivo incubated in Singlet Oxygen Sensor Green reagent, which is highly selective marker for $^1O_2$ molecules. Thereafter, they were in vivo examined under confocal laser scanning microscope. Fluorescent signal indicating the presence of $^1O_2$ appeared specifically in necrotized cells along nematode migration path and inside 3- and 5-day-old syncytia.

P5.6

The role of PsbS in regulation of the cellular light memory

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Plants had to develop complex acclimatory mechanisms to survive under naturally variable light conditions. To survive, they need to regulate the fate of absorbed photons during excess light (EL) or in light limiting conditions. But are plants able to physiologically memorize the excess light incident and prepare themselves for recurrence of various stresses? To test PsbS function in regulation of the cellular light memory we treated low light (LL) acclimated *Arabidopsis thaliana* wild type plants the *npq4-1* mutant deficient in PsbS protein and PsbS overexpressor with single 1 h EL incidents of different light quality and intensity. After such treatment plants returned for 24 h to ambient LL, then were exposed to 200 mJ of UV-C and returned to LL. We measured fluorescence dynamics, total cellular ion leakage and did Trypan Blue staining in order to asses cell death (CD) and photosystem II (PSII) damage. The level of CD and PSII damage after such complex treatments was strictly dependent on PsbS availability. Obtained results indicate that PsbS play an important role in regulation of the cellular light memory expressed as EL-induced UV-C cross-tolerance.

P5.7

Does the increased SOD activity reflects drought resistance of barley?

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The impact of drought on the oxidative stress level in leaves of barley seedlings subjected to 10-day drought was studied. Plants of all 8 used genotypes revealed either an increase in SOD activity (expressed as cytochrome units per g of FW), or no response. The highest increase (ca. 190% of control value) was noticed in case of plants of "Cam/B1" genotype, and the generation of $O_2^{\bullet -}$, the SOD substrate, was low. Such response resulted from oxidative stress, not from protection against ROS, as the analysis of chlorophyll a parameters revealed low F_v'/F_m', qP and phiPSII values (in comparison to the other genotypes), or their diminished ratios to the control. Moreover, when compared SOD activity in control plants, it was the lowest in "Cam/B1" specimens. Results indicate that the relationship between drought susceptibility and $O_2^{\bullet -}$ generation and scavenging is complex, but such analysis may serve as a tool accompanying physiological studies. Project Biotechnological tools for breeding cereals with increased resistance to drought (POLAPGEN-BD) is co-financed by European Union from European Regional Development Fund in a framework of the Innovative Economy Operational Programme 2007-2013.

P5.8

Lichen peroxidases have both pro- and antioxidant roles

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Recently, we discovered the presence of a heme peroxidase in the Peltigeralean "jelly lichens" *Leptogium* and *Collema*. Here we present the results of a survey of peroxidase activity in a range of lichens. In addition to the jelly lichens, strong peroxidase activity is also found within the Peltigeralean genera *Lobaria*, *Pseduocyphellaria* and *Sticta.* It seems likely that the enzymes have both pro- and antioxidant activities. The peroxidase probably acts as a classic scavenger of hydrogen peroxide. Peroxidase activity increases considerably following the rehydration of dry thalli, suggesting that ROS scavenging activities cause deactivation of the enzyme as the thallus dries. However, in gel staining indicates that the enzymes can readily produce superoxide ions in the presence of NADH. Significant peroxidase activity occurs in the cell wall. The ability of the enzymes to produce radicals, together with their cellular location, suggests that peroxidases may be involved in the degradation of complex macromolecules. Such degradation may allow the lichen some saprophytic existence. Understanding this role will be essential to evaluate the significance of lichens in carbon cycling in stressed habitats.

P5.9

Identification, phylogenetic analyses and subcellular localization of protein phosphatases 2c type (PP2Cs) in *Populus trichorapa*

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PP2Cs are a class of Ser/Thr protein phosphatases acting as negative regulators of protein kinases, including mitogen-activated protein kinases (MAPKs), which are involved in developmental regulation and stress signalling pathways. This phosphatase family is common and well-conserved in *Procaryota* and all eucaryotic kingdoms. *Arabidopsis thaliana* and *Oryza sativa* genomes contain respectively 80 and 76 PP2Cs encoding genes. Using bioinformatic analysis we were able to identify 111 putative PP2C in *Populus trichocarpa*. Phylogenetic analysis calculated by Neighbour-Joining algorithm showed similarities between these proteins sequences. We have identified protein motifs, related to *P. trichocarpa* PP2Cs and divided them into 13 subfamilies. We analyzed putative subcellular localization of these phosphatases. Two proteins from group B were initially identified as orthologs of AP2C1 phosphatase from *A. thaliana*, which interacts with MAP Kinase 4. We cloned these genes and prepared constructs with EYFP and ECFP. *Nicotiana benthamiana* leaves were transformed using Agro-infiltration technique. Confocal microscopy analysis showed that both proteins from poplar are localized in plastids and stromules as predicted.

P5.10

The role of ascorbate during cadmium-induced oxidative stress in *Arabidopsis thaliana*

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Metals, like cadmium (Cd), have the ability to induce the production of reactive oxygen species (ROS) at the cellular level. Plants can control ROS levels using their antioxidative defense system, under normal growth conditions as well as under stress conditions. Ascorbate (AsA) is a well-known and important component of the plant antioxidative system. As a primary antioxidant it can reduce ROS directly, and indirectly via ascorbate peroxidases (APx) in the ascorbate-glutathione cycle. To study the role of AsA during Cd stress, *vtc1-1* mutant (AsA deficient) Arabidopsis thaliana seedlings were exposed to Cd during 24h. This resulted in a decreased root biomass, which is more pronounced in *vtc1-1* mutants. Furthermore a strong stimulation of genes encoding oxidative signalling components (ZAT12,..) and antioxidative enzymes (GR1,..) was observed in wildtypes, in contrast to *vtc1-1* mutants in which no or only a small upregulation was observed. In the leaves of plants exposed to Cd, enzyme activities of CAT, APx and G6PDH were higher in *vtc1-1* mutants, but the activities of SPOD and GADPH were higher in the wildtypes. These results are the basis for further research into the role of AsA in metal-induced oxidative signalling.

P5.11

ZmCPK11, a positive regulator in salt stress tolerance in transgenic *Arabidopsis* plants

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ZmCPK11, a member of *Zea mays* calcium-dependent protein kinases (CDPKs) family, participates in jasmonic acid (JA)-dependent local and systemic response to wounding. However, its role in defense responses is unknown. For elucidation of the physiological function of ZmCPK11, the transgenic *Arabidopsis thaliana* plants expressing c-Myc-ZmCPK11 were exploited. Under normal conditions there were no visible phenotypic differences between control (wild-type and expressing vector alone) and the *ZmCPK11* transgenic plants, but 4-week old *ZmCPK11* transgenic plants were more tolerant to salt stress. Also, the ZmCPK11 transgenic plants showed enhanced tolerance to salinity in seeds germination and post-germination growth. Accumulation of abscisic acid (ABA) results in inhibition of seed germination and is also required for salt tolerance. Therefore, we have tested an inhibition of seeds germination and post-germination growth by ABA treatment. The obtained results show that expression of *ZmCPK11* in *Arabidopsis* increased sensitivity to exogenous ABA. Our findings indicate that ZmCPK11 may function as a positive regulator involved in ABA-dependent salt stress tolerance. Work was supported by grants no N N303 306737 and N N303 340835.

P5.12

Functional and biochemical characterization of *Arabidopsis* calcium sensor SCS a potential regulator of SnRK2 protein kinases

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The SnRK2 protein kinases are important regulators of plant response to water deficit as well as ABA-dependent plant development. Recently, we have identified and preliminary characterized a calcium binding protein, potential regulator of SnRK2 activity. The protein was name SCS (SnRK2-interacting calcium sensor). In *Arabidopsis*, there are two isoforms of this protein AtSCS-A and AtSCS-B, both encoded by the same gene. To characterize the biochemical and functional properties of both isoforms we have analyzed their affinity for calcium ions, conformational changes upon binding Ca^{2+} by using fluorescence and CD methods and studied the interactions with *Arabidopsis* SnRK2s using a yeast two-hybrid and BiFC assay. We have also tested in vitro the effect of AtSCS-A and AtSCS-B on the activity of recombinant SnRK2s. The studies show that both isoforms bind Ca^{2+} with different affinity and their conformation undergoes changes upon calcium binding. *In vitro* analysis revealed that AtSCS-A and AtSCS-B interact with SnRK2s and negatively regulate their activity in a calcium-dependent manner. Our data also indicate that SnRK2s may reciprocally regulate functions of calcium sensor by phosphorylation of its specific Ser residues.

P5.13

CRK5 as a convergence and abiotic stress node between senescence responses

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In plants, receptor-like protein kinases (RLKs) play essential roles in signal transduction by recognizing extracellular stimuli and activating the downstream signalling pathways. Cysteine-rich receptor-like kinases (CRKs) constitute a sub-family of RLKs and are distinguished by the novel C-X8-C-X2-C motif (DUF26) in the extracellular domains. In *Arabidopsis thaliana*, 44 members of CRKs family have been identified. One of them, CRK5, raises particular interest as a putative negative regulator of senescence and stress acclimation response. Functional characterization of *crk5* shows its impaired adaptation to abiotic stress – UVC radiation, as well as decreased chlorophyll content, lower biomass production and visible symptoms of premature leaf aging. Accelerated senescence of *crk5* is even more induced by external stimuli such as continuous dark and low CO_2 concentration. CRK5 gene has many W-Box cis-elements in its promoter region specifically recognized by WRKY transcription factors (TFs). Many WRKY, e.g. WRKY53 and 70 are well known regulators of both plant senescence and defence pathways. RT-PCR analysis suggests that CRK5 may overlap WRKY-dependent regulatory pathways.

P5.14

The role of nitric oxide and NADPH oxidase in short term signalling events activated in soybean seedlings by cadmium

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First reaction of plants to cadmium stress consists of signalling activation leading to the acquisition of stress tolerance. The results of previous experiments performed by the authors show that Cd causes induction of expression of several genes associated with signalling pathways. The aim of the present work is evaluation if nitric oxide (NO) and produced by NAPDH oxidase reactive oxygen species (ROS) mediate the observed Cd-dependent induction of genes expression. Treatment with NO scavenger, PTIO, reversed the observed Cd-dependent induction of expression of all investigated genes after 3 h of exposure to Cd. On the other hand, in the same time variant, DPI caused augmentation of Cd-dependent induction of genes encoding 1-aminocyclopropane-1-carboxylic acid synthase and DOF1 and MYBZ2 transcription factors. After 6 h of treatment none of the described effects was observed with the exception of elevated expression of *MYBZ2* gene in response to DPI noted in Cd-stressed seedlings. The obtained results imply that NO and generated by NADPH oxidase ROS, modulate signalling triggered by cadmium stress. The project was financed by National Science Center granted on the basis of decision number DEC-2011/03/N/ NZ9/00214.

P5.15

Involvement of carbohydrates and antioxidant enzymes in the oxidative balance during drought and recovery: the eucalyptus case

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When cellular functions are disturbed due to environmental deviations abiotic stress occurs. As the genetic variability suggests, mechanisms of damage, signalling and metabolic responses to abiotic stress differ among genotypes. Either way, plants respond to stress with increased production of ROS. Water deficit results in great losses in Eucalyptus plantations and new insights in the underlying mechanisms of recovering are required. Since drought may lead to oxidative stress due to stomatal closure and over-reduction of photosynthetic electron chain, it is important to study the oxidative balance involved during drought and recovery. Therefore, recovery of two *E. globulus* clones, watered to 18% (WS) and 80% (WW) field capacity was assessed. CO_2 assimilation (A) and MDA were used to characterize plant performance and oxidative stress. APX, SOD and CAT, as well as H_2O_2 and carbohydrates, were quantified to assess the cellular oxidative balance. Results showed the negative impact of drought by a reduction in A and higher MDA values, and an increase in sugar content and antioxidant enzymes. After stress relief, a differential readjustment occurs. Plant strategies to restore the physiological balance are suggested.

P5.16

Oxidative stress and antioxidant responses in olive tree subjected to cover crops under rainfed conditions

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Since Mediterranean basin is particularly vulnerable to present and future climate variability and climate change, olive tree will experiment some hard changes in its environment. Under adverse conditions, imbalances in metabolic processes may lead to increased accumulation of ROS, forming a potential threat of oxidative damage to cells. We propose green manure legumes to shift tillage, in order to improve soil water relationships during the drought period. The research was carried out in northeast Portugal under rainfed conditions. The treatments laid out were: ordinary tillage techniques and a mixture of eleven annual (AL) pasture self-reseeding legumes. The results obtained in summer 2011 revealed that olive in AL plot had higher concentrations of chlorophylls, carotenoids and total thiols, as well stomatal conductance, net photosynthesis, qP, F_v'/F_m', quantum yield of PSII, ETR and APX activity. Conversely, olive trees subjected to tillage had higher qN and GST activity. No significant differences were reported in electrolyte leakage, CAT activity and in TBARS and total phenols concentration. Thus, legume species may be a promising strategy contributing to the sustainable management of olive orchards.

P5.17

The role of the oxidative signal-inducible kinase (OXI1) in metal-induced molecular responses in *Arabidopsis thaliana*

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The hypothesis that mitogen-activated protein kinase (MAPK) signalling is important in plant defences against metal stress has become accepted in recent years. Metals, like cadmium (Cd) and copper (Cu), have the ability to induce the production of reactive oxygen species (ROS).To test the role of oxidative signal-inducible kinase (OXI1) in metal-induced oxidative signalling, the responses of *oxi1* knockout lines to environmentally realistic concentrations of Cu and Cd stress were compared with those of wild-type plants. Responses were measured at the level of hydrogen peroxide content, lipid peroxidation and transcript levels of genes involved in ROS homeostasis and signalling. A relationship between OXI1 and the activation of lipoxygenase-1 was observed under both stress conditions, suggesting that lipoxygenase-1 may be a downstream component of OXI1 signalling. Also the alterations in gene transcript levels of Cu/Zn superoxide dismutases in metal-exposed leaves was regulated by OXI1 in a manner that involves fluctuations in the expression of miRNA398.

P5.18

Glutathione boost with ectopic expression of bifunctional STGCL-GS enzyme in tobacco enhances host and non-host defense

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To assess the involvement of glutathione in response of plant immunity to pathogens, *Nicotiana tabacum* lines with overexpression of bifunctional GCL-GS enzyme from *Streptococcus thermophilus* for glutathione overproduction were used. The transgenic lines accumulated up to tenfold more glutathione than wild type and had strong increase in expression of Pathogenesis-related protein (PR) genes of both NPR1-dependent and independent branches of salicylic acid (SA) pathway. When infected with host *Pseudomonas syringae* pv. *tabaci* pathogen, transgenic lines accumulated more SA and storage glucoside form (SAG), which led to hypersensitive response(HR)-like symptoms and reduction of chlorotic areas around infection site. However, bacterial number in plants was only transiently reduced. Moreover, infection of tobacco lines with non-host pathogens of *Pseudomonas syringae* (pv. *maculicola* and *syringae)* showed strong reduction of bacterial number during the whole course of infection and formation of HR lesions. Additionally, transgenic lines had more callose depositions in response to non-host pathogens. Collectively, our results suggest an important role of glutathione in tobacco defense against both host and non-host pathogens.

P5.19

Antioxidant defence in maize cultivars triggered by the two-spotted spider mite attack and soil drought – a comparative study

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The aim of the present study was to establish if potential "side effects" in lepidopteran insect-resistant transgenic maize (YG), expressing the *cry1Ab* gene from *Bacillus thuringiensis* Berliner, concern the antioxidant defence mechanism of a modified plant. In maize cultivars (YG, its non-transgenic counterpart – ISO and conventional – Bosman), oxidative stress was provoked by short term feeding of the two-spotted spider mite (*Tetranychus urticae* Koch; *Acari*: *Tetranychidae*) (non-target arthropod-pest) and/or soil drought. The activity of leaf superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) was estimated. The activity of polyphenol oxidase (PPO) was also assessed. Comparative analysis demonstrates that leaves of Bosman cv. have a higher antioxidant defence capacity than leaves of YG and ISO cvs. In plants stressed by mite feeding and/or soil water deficiency the involvement of ROS-scavenging enzymes contributing to plant adjustment, differs depending on the stress, stress period and cultivar.

P5.20

The changes in non enzymatic antioxidants in halophyte *Cakile maritima* under arsenic and salt stress

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The purpose of the study was to determine the biochemical responses which were not investigated before, the combined effects of arsenic and salt stress in the leaves of *Cakile maritima* (Scop.). Therefore, the content of proline, non-protein thiol, ascorbate, dehydroascorbate and product of lipid peroxidation (MDA, malondialdehyde) were reported. The MDA content increased at 24h but it was more at 96h under salt stress alone. Otherwise the combined effect of arsenic and salt stress increased the MDA content at 24h more than salt stress alone. The proline content increased only at 96h under salt stress alone while it did not change under arsenic and salt stress. Nevertheless, the ascorbate content was not changed under all treatments. But the ratio of ascorbate/dehydroascorbate was decreased at 24h but not significantly at 96h under salinity. From these results, it can be suggested that salinity with arsenic stress induced oxidative damage more seriously than salt stress alone with unchanged non enzymatic antioxidants in the leaves of *Cakile maritima* which is a plant halophyte.

P5.21

The effects of cadmium on antioxidant capacities in leaves of *Brassica oleracea* L. var. *Acephala*

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In this study, *Brassica oleracea* L. var. *acephala* was treated with different cadmium (Cd) concentrations (100, 200 and 400 $\mu g \cdot g^{-1}$) for 15 days. At the end of the treatment, the effects of Cd pigment content, lipid peroxidation, enzymatic and non-enzymatic antioxidants were investigated in leaves. The pigment and carotenoid contents did not vary at all Cd treatments. Cd treatment caused an increase in the lipid peroxidation product (malondialdehyde) in leaves. Malondialdehyde content increased significantly at 400 $\mu g \cdot g^{-1}$. Cd stress caused changes in antioxidant enzymes activities. All these treatments depend on concentration caused decrease in superoxide dismutase activity, increase in guaiacol peroxidase activity. However, there was no significant change in catalase activity. Ascorbate peroxidase activity increased significantly at 200 $\mu g \cdot g^{-1}$. The analysis of the antioxidative enzymes activity indicated that GPOX was the most active enzyme in leaves of *B. oleracea* var. *acephala.* While proline content was decreasing, non-protein thiol groups and total ascorbate contents increased at all Cd treatment.

P5.22

The role of Kaolin and Bordeaux mixture in *Vitis vinifera* under water stress conditions: oxidative stress

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In Portugal the wine-grape sector has a crucial economic relevance, especially in Douro Region. Unfortunately, this region is often subjected to periods of drought associated with strong light and high temperature which usually induce grapevine damages. In this work, the effect of foliar application of Bordeaux mixture (BM) and Kaolin (K) as protector agents to increase the foliar reflectance capacity and consequently to increase the foliar protection against oxidative damages was tested. The study was conducted in potted *V. vinifera* plants treated with 5% K and 2% BM and subjected to different soil water deficit, 60% and 30% AWC (available water content). CAT and APX activities and the content in proline and H_2O_2 were determined. BM application was more effective in oxidative damage reduction under both 60% and 30% AWC conditions. Proline seems to plays an important role in plant stress protection. Acknowledgments - Fundação para a Ciencia e a Tecnologia, Project PTDC/AGR-ALI/110877/2009 and fellowship to CJesus (BI/UI88/6287/2012). FCT supported the fellowship of MCDias (SFRH).

P5.23

Guard cells anion channel SLAC1 is activated by a set of kinases originating from three different families

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Plants deal with drought stress via stomatal closure to prevent water loss. For initiating stomatal closure, the drought stress hormone abscisic acid (ABA) leads to the activation of S-type anion channel. Characterization of *SLAC1* expressed in *Xenopus* oocytes indicated that its activation by protein kinases was stimulated by ABA through an ABA receptor/phosphatase complex. ABA activates SLAC1 by stimulating its phosphorylation via Ca^{2+}-independent protein kinase OST1 and Ca^{2+}-dependent protein kinases of the AtCPK family. CIPKX belongs to the family of CBL-interacting protein kinases. To become active, CIPKs form a complex with certain CBLs in a calcium-dependent manner and in turn interact with their target proteins. Using the BiFC-method in the *Xenopus* oocyte expression system, we revealed protein-protein interaction between the CIPKX/CBL-complex and SLAC1. Electrophysiological studies in oocytes revealed that SLAC1 is activated by the complex between CIPKX and distinct CBLs. Furthermore, similar to OST1 and CPK21/23, CIPKX activity was prevented by ABI1. In conclusion, our findings suggest an ABA-signalling pathway with kinases of three distinct families that are capable to activate SLAC1 in parallel.

P5.24

Effect of Mn on photosynthetic parameters of C3 *Cleome spinosa* and C4 *Cleome gynandra* in conjugation with antioxidant defense

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There are a few studies that investigate the effects of Manganese (Mn) treatment on plants that differ in carboxylation pathways, especially in the same genus. For this aim, *Cleome spinosa* and *C. gynandra* plants were treated with different Mn concentrations (0, 10, 50, 100 μM) and production of reactive oxygen species (ROS) (H_2O_2 production), antioxidant defense system (superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR)) and lipid peroxidation were determined in all treatment groups. In this study, *C. gynandra* accumulated more Mn as compared to *C. spinosa*. 100 μM Mn application negatively affected all measured photosynthetic parameters (A, gs, NPQ, ETR). Excess Mn accumulation also decreased the total activity of SOD, GR, APX, DHAR and MDHAR and increased the TBARS content. These results showed that 100 μM Mn application has toxic effects on the metabolism of *C. spinosa* and *C. gynandra*. (BPD/41700/2007).

P5.25

Lipid peroxidation and photosynthetic apparatus oxidative damage in two varieties of grapevine growing in mediterranean climate

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In the Douro Wine Region (DWR), NE Portugal, grapevines are subjected to periods of severe drought accompanying with high temperature and high light stresses. Moreover, the DWR is likely to face new challenges in the coming decades, mainly critical for white cultivars, as climate models project an above average warming associated with a decrease of precipitation in SW Europe. Under these conditions, the ROS production increase with negative effects on the photosynthetic apparatus. Photosynthetic pigments, TBARS, total phenols, chlorophyll fluorescence and gas exchange of the white varieties Moscatel-Galego-Branco (syn. Muscat a Petits Grains) and Boal (syn. Semillon) were used as biomarkers, in order to understand which one is better adapted to climate conditions. Results revealed that Moscatel had lower degree of oxidative damage, mainly at the end of summer, that was related with a higher photosynyhetic performance, particularly at midday, as shown by higher values of F_v/F_m, Φ_{PSII}, qP, ETR, and net photosynthesis, due to lower stomatal and non-stomatal limitations. Conversely, Boal showed higher qN and phenols concentration. This study supports that Moscatel is a well-adapted cultivar to climate conditions of the DWR.

P5.26

Antioxidant enzymes activity affects *Burkholderia* sp. Ni tolerance

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Nickel (Ni) was previously described as a problem in soil and water contamination. Metals in high concentrations is toxic to cells generating oxidative stress due to the excessive production of reactive oxygen species (ROS) damaging lipids, proteins and DNA. Therefore this study aimed to characterize the Ni antioxidant response of two tolerant *Burkholderia* strains (one isolated from contaminated soil, SNMS32, and the other from non contaminated soil, SCMS54), measuring SOD, CAT, GR and GST activity, together with Ni accumulation and bacterial growth in the presence of Ni. Results show that both strains present differences in Ni accumulation and in the antioxidant enzymes response. Only the strain from contaminated soil (SCMS54) exhibited an increase in SOD and GST activity, and the activity staining analysis indicated that the strain SCMS54 expresses an extra isoform of all tested enzymes (SOD, CAT and GR). Moreover this strain exhibited a higher Ni accumulation when compared to the other strain. The data obtained indicated that the strain isolated from contaminated soil is more metabolic diverse being selected to be used and studied in soil and water bioremediation.

P5.27

ROS and NO production by axenically cultured olive seedling roots after interaction with a mycorrhizal or a pathogenic fungus

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Roots in contact with *G. intraradices* (mycorrhizal fungus – MF-) showed attenuated oxidative reactions, with very low peaks of $O_2^{\bullet -}$ (5 min) and ECPOX (15 min), and two peaks of ECSOD (the first between 5 and 10 min, and the second at 1 h) both later than that of $O_2^{\bullet -}$. On the contrary, the roots in contact with *V. dahliae* (pathogenic fungus – PF-) showed a maintained increase in O_2^{-} generation, ECPOX and ECSOD activities from 5 min up to 24 h (the oxidative reactions were constantly higher). After 1 h of contact phenylpropanoids and flavonoids content were not altered, but after 24 h the PF induced a strong increase in both compounds, while MF only slightly increased phenylpropanoid content. We visualized ROS and NO in the whole intact roots using fluorescence probes. ROS and NO were produced by roots 1 h in contact with MF or PF. After 24 h interaction with PF, the production of ROS and NO was much higher than that induced by MF. ROS were observed in the apoplast, but NO was observed in the apoplast of most cells and also in the cytoplasm of some of them. Our results indicated that roots attenuated the oxidative defense to allow the interaction with MF. The NO was involved in the response to the interaction with PT and MF.

P5.28

The ascorbate metabolism as a target for the breeding of stress resistant rice

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Rice provides the staple food for more than half of the world's population. However, yields are often limited by abiotic stress factors, which cause a redox imbalance in plant tissues leading to oxidative stress. We employ an interdisciplinary approach to identify traits and loci associated with oxidative stress resistance in rice. We focus on three types of stress affecting global rice yields, i.e. zinc deficiency, high tropospheric ozone, and iron toxicity. By mapping of quantitative trait loci (QTL), loci associated with stress resistance were identified. Converging evidence from transcriptional and biochemical analysis of contrasting breeding lines suggested that the ascorbate (AsA) metabolism plays a crucial role under diverse types of stress. In the case of zinc deficiency, the ability to maintain a high AsA biosynthesis under stress was strongly associated with stress resistance, while the apoplastic AsA cycle was more relevant under ozone stress. Resistance to iron toxicity was not clearly correlated with AsA level, perhaps because AsA may facilitate both ROS scavenging and ROS production via the “Fenton” reaction. In summary, the AsA metabolism may offer interesting breeding targets for crop improvement.

P5.29

Light and temperature of ripening tomato fruits interact to regulate ascorbate synthesis, oxidation and recycling

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To understand how light and temperature may affect fruit ascorbate (AsA) content, tomato fruit harvested at breaker stage were placed under different temperatures (12, 23 and 31 °C) and irradiance regimes (darkness or 150 μmol m^{-2} s^{-1}). After 56h, fruit were cut and pericarps were frozen and ground in liquid nitrogen. Changes in AsA metabolism were characterized from ascorbate and glutathione content, enzymic activities related to oxidative stress and AsA/glutathione cycle (MDHAR, DHAR, APX, CAT, GR) and the expression of genes coding for the 5 last enzymes of AsA biosynthesis pathway (GME, GDP, GPP, GalDH, GLDH). It confirms the important role of fruit microclimate to regulate fruit AsA content and reveals interaction between light and temperature: Indeed light increased AsA content in fruit pericarp up to 67% at 12 °C, but had no effect on AsA content at 31 °C. At any temperature tested, light enhanced the expression of genes coding for AsA biosynthesis, but at 12 °C, light upregulated a higher amount of genes compared to 23 °C or 31 °C. At 31 °C, reductase activities (MDHAR and GR) were significantly reduced under light indicating that enzymes of the ascorbate/glutathione cycle may be limiting to recycle AsA.

P5.30

Controlled freeze physics methodology for extra virgin olive oil (EVOO) stabilization

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Extra Virgin Olive Oil (EVOO) is considered to be the main fat ingredient specifically of the Mediterranean diet. The importance of antioxidant capacity of EVOO is mainly attributed to its high content in phenolic compounds. The phenolic charge of EVOO is very sensitive to interfacial physicochemical reactions e.g. at air-oil and oil-water interfaces. Therefore, the storage conditions of EVOO are very important to avoid or reduce the negative effects of EVOO autoxidation on the qualitative characteristics of the packaged product. The main factors affecting olive oil quality during storage are temperature and contact with atmospheric oxygen. The present project aims to develop a controlled freeze methodology for the optimal stabilization of phenolics in EVOO by studying the Quality Indexes (QI) (i) total phenolic. (ii) aromatic compounds, (iii) total antioxidant load, (iv) free radicals, and (v) lipid peroxidation. The evaluation of the aforementioned QI's for EVOO stored in the dark at temperatures −80, −20, +4 and +25 °C in the presence/absence of O_2 indicated that the phenolic charge is best stabilized for > 2years at < 4 °C and in the absence of O_2.

P5.31

Oxidative stress responses in lignin-reduced *Arabidopsis thaliana* mutants in both control and cadmium-exposed conditions

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Producing lignocellulosic biomass for biofuel production on contaminated soils can contribute to reduce the food-fuel competition for fertile land. An additional advantage is the decontamination of these soils by making use of plants. The recalcitrance of lignin polymers is a limiting factor during processing of lignocellulosic biomass. Genetic modification of the lignin-content (e.g. downregulating cinnamoyl-CoA-reductase (CCR1) or 4-coumarate-CoA-ligase (4CL1)) increases the efficiency of this process. However both the mutation and contamination can negatively affect plant growth. To better understand these effects *Arabidopsis thaliana* is used as model organism and cadmium (Cd) as model contaminant. As parameters biomass, root length chlorophyll content, lipid peroxidation (TBA), capacities of stress-related enzymes and the expression of genes responsible for pro and anti-oxidative enzymes, Cd-chelation and stress-related lignin production were investigated. The obtained results point out that both the genetic modification and Cd-exposure have an effect at all studied parameters. In future experiments I aim to counter the negative effects of both studied aspects by the use of growth-promoting bacterial endophytes.

P5.32

Mi-tomato responses to two-spotted spider mite attack

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The presence of the *Mi-1.2* gene in the tomato plants results in resistance against pests belonging to distant taxons – nematodes (*Meloidogyne incognita*, *M. javanica*, *M. arenaria*) and hemipteran insects (*Macrosiphum euphorbiae*, *Bemisia tabaci*, *Bactericerca cockerelli*). Currently, there are many Mi-tomato cultivars on the market, however there is no data available on their tolerance to pests other than nematodes and hemipterans. In the present study, intensity of photosynthesis, chlorophyll fluorescence and antioxidant capacity were monitored in tomato cultivars (Motelle – with the *Mi-1.2* gene and Moneymaker – without it) in response to the two-spotted spider mite, one of the most serious arthropod pests of greenhouse tomato to check if the above-mentioned parameters contribute to Mi-plant tolerance to mite-pests and if they are useful markers of Mi-plant tolerance to mite-pests. It has been shown that short-term mite feeding triggers highly contrasting physiological and biochemical responses in Motelle and Moneymaker leaves, some of which may be useful tolerance markers.

P5.33

NADPH oxidase C is not the main source of ROS under arsenic toxicity but regulates antioxidants and metal transporters

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Arsenic (As) is a toxic metalloid in plants, animals and humans, although its toxicity mechanisms are not well known. In this study we analyse the effect of As on the oxidative metabolism and nutrition in wild type (WT) and *Arabidopsis thaliana* (L.) plants deficient in NADPH oxidase C (AtrbohC). The content of H_2O_2 and malondialdehyde increased with As concentrations in WT and AtrbohC plants, while an opposite tendency was found for NO. Arsenic reduced catalase and increased glutathione reductase activities to a similar extent in WT and AtrbohC plants. All SOD isoforms were induced in WT plants and opposite effect was found in AtrbohC plants. Glycolate oxidase considerably increased with the treatment in both *Arabidopsis* lines. Arsenic induced the uptake and translocation of P, Cu, Zn, and Fe in WT plants, while in AtrbohC plants, an opposite trend was noted and the accumulation of As was reduced. These results suggest that As causes oxidative stress by inducing glycolate oxidase, while NADPH oxidase does not appear to participate in ROS overproduction but is critical in regulating antioxidants and nutrients uptake. Support by ERDF-cofinanced grants from the MEC BIO2008-04067 and BIO2012-36742.

P5.34

High temperature inhibition of potato tuberisation is associated with altered redox status and associated transcript profiles

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Minimising yield loss due to abiotic and biotic stress is essential for achieving food security. A key abiotic stress impacting the globally significant potato crop is elevated temperature, considered the most important uncontrollable factor affecting yields. To date, the majority of experiments investigating temperature stress in plants have focussed on heat shock responses to highly elevated temperatures. However, in temperate agriculture stresses are rarely sufficiently extreme to threaten plant survival and instead mild stress impairs crop yield. In the present study, tuberising plants were acclimated to elevated temperature prior to analysis of carbon fixation and partitioning. Elevated temperature resulted in reduced tuber yield despite increased carbon assimilation by leaves. Furthermore, the leaf glutathione pool was significantly more reduced at high temperature, associated with changes in the expression of transcripts encoding enzymes of the AsA-GSH cycle. Transcriptional profiles of a range of thioredoxins, glutaredoxins and peroxiredoxins were also impacted by temperature in both leaves and tubers. We discuss our findings in terms of redox control of carbon assimilation, partitioning and metabolism.

P5.35

EPR-spin labelling measurements of thylakoid membrane fluidity during barely leaf senescence

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Physical properties of thylakoids isolated from barley were investigated by electron paramagnetic resonance (EPR)-spin labeling. EPR spectra of stearic acid spin labels 5-SASL and 16-SASL were measured as a function of temperature in mature and senescing secondary barley leaves, and during dark induced senescence of these leaves. Oxygen transport parameter was estimated from the line widths of EPR spectra measured in the presence and absence of molecular oxygen at 25°C. Parameters of EPR spectra of both spin labels showed an increase in the thylakoids fluidity during senescence of barley, indicating that membrane fluidity increases in the headgroup area of the membrane, as well as in the interior. The oxygen transport parameter also increased with the age of barley, indicating easier diffusion of oxygen within membrane and its higher fluidity, consistent with the EPR-spin labeling data. These results were further confirmed when senescence was induced in mature secondary barley leaves by dark incubation. Such leaves showed higher membrane fluidity in comparison with leaves of the same age, grown under light conditions. Obtained results indicate that membrane fluidity increases with progress of senescence in barley leaves.

P5.36

Alteration of plasma membrane NADPH oxidase and NADP-recycling enzymes in cucumber roots stressed with cadmium

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An increase in permeability related to membrane damage is observed in plants that have been subjected to heavy metal stress. It disrupt the ionic and redox homeostasis of the cell. A key player in ROS producing enzymes is the NADPH oxidase. The NADPH is an important molecule in the redox balance of the cell. The enzymes which have the capacity to generate reducing power in the form of NADPH are: glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme and isocitrate dehydrogenase. The effect of Cd on the activities of the plasma membrane NADPH oxidase and NADP-recycling enzymes in cucumber roots was studied. Short-treatment of the cucumber seedlings with Cd decreased the NADPH oxidase activity. On the other hand, in plants treated for longer time with heavy metal increased the enzyme activity. Moreover, exposure of plants to cadmium changed also the activities of the NADP-recycling enzymes. Comparing these results with our previous studies on the role of the plasma membrane H+-ATPase under heavy metal stress, we suggest that the modification of the plasma membrane NADPH oxidase and NADP-recycling enzymes could be related to enhanced tolerance of plants to cadmium.

P5.37

Cadmium-treated *Arabidopsis thaliana* reveal a regulatory role of glutathione in superoxide dismutase

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Plants possess a common defence strategy against cadmium (Cd) stress in which glutathione (GSH) plays a central role as chelating agent, antioxidant and signalling component (Jozefczak et al., Int. J. Mol. Sci. 2012; 13(3): 3145-3175). First, a kinetic screening of Cd-treated wild type plants demonstrated time- and dose-dependent responses. On the one hand, GSH-related defence mechanisms were activated including de novo GSH biosynthesis, phytochelatin (PC) production, the ascorbate(AsA)-GSH cycle and the cellular redox balance. On the other hand, iron superoxide dismutase (Fe-SOD) activities were increased in contrast to copper(Cu)/zinc(Zn)-SOD. In order to focus on GSH and its role in Cd detoxification, a second experiment was performed with GSH-deficient mutants. Although these mutants were not able to produce high levels of GSH or PC under Cd stress, they were able to increase the activity of both SOD-isoforms. Our findings provide evidence for a new function of GSH that hasn't been suggested before: a central role in Cu/Zn-SOD regulation.

P5.39

Alternative respiration as primary defence during cadmium-induced mitochondrial oxidative challenge in *Arabidopsis thaliana*

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Pollution of soils with cadmium (Cd) severely restricts plant growth and development. Moreover, Cd can induce an oxidative challenge at organellar and cellular levels. As mitochondrial alternative respiration mediated by alternative oxidase (AOX) and alternative NAD(P)H dehydrogenases (NDs) is suggested to be crucial in plant stress acclimation, we evaluated the responses of these enzymes in *Arabidopsis thaliana* kinetically exposed to environmentally realistic Cd concentrations (5 or 10% μM). Cadmium-exposed seedlings rapidly respond to the metal-induced oxidative challenge at the mitochondrial level by increasing several AOX and ND isoform transcripts in roots and leaves. Their expression was influenced to a much higher extent as compared to changes in mitochondrial antioxidative enzymes. For AOX, the higher transcript level was realised as a transient increase in protein content. We propose a functional but condensed electron transport chain consisting of cytosolic NDs and AOX during Cd stress. Its potential importance in plant acclimation to Cd was underpinned during chronic Cd exposure, where *aox1a* knockout seedlings had a more stress-responsive phenotype than wild-type plants.

P5.40

Heat-induced susceptibility of barley to a biotrophic and a hemibiotrophic pathogen – differential changes in superoxide levels

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Recent research has shown that heat pre-treatment of barley induces susceptibility to barley powdery mildew (*Blumeria graminis* f.sp. *hordei*, Bgh), a biotrophic pathogen. Here we demonstrate that barley resistant to Bgh receiving a short (30 sec, 49 °C) heat pre-treatment 2 h before inoculation not only becomes partially susceptible but displays a significant reduction in levels of the reactive oxygen species superoxide ($O_2^{\bullet -}$), as compared to untreated plants. Reduction of $O_2^{\bullet -}$ in heat pre-treated barley leaves is visible from the first day after inoculation, when monitored by NBT (nitro blue tetrazolium) tissue staining. Infection of heat pre-treated barley with the hemibiotrophic pathogen *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) also results in increased susceptibility, i.e. elevated pathogen multiplication as assayed by real time qPCR and enhanced leaf necrosis. Interestingly, in this case increased susceptibility is correlated with enhanced, rather than reduced, levels of $O_2^{\bullet -}$. It is possible that during infection by a hemibiotrophic fungus heat pre-treatment that reduces $O_2^{\bullet -}$ levels also permits increased pathogen growth, enhanced leaf necrosis and finally overproduction of $O_2^{\bullet -}$.

P5.41

The role of MIR_{4415} in soybean response to asian soybean rust infection

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MicroRNAs are endogenous and small non-coding RNAs that regulate gene at the post-transcriptional level. Plant miRNAs use to regulate transcripts by single and highly complementary target sites. The majority of annotated *MIRNA* are family- or species-specific. Using 5′RACE and RT-qPCR we investigated candidate targets for soybean-specific MIR_{4415}, previously detected by our group using high-throughput sequencing. MIR_{4415} expression profile varied between different soybean genotypes submitted to water-deficit and after Asian soybean rust inoculation. In the present study, ascorbate oxidase (AO) mRNA was shown to be regulated by MIR_{4415}. AO is an apoplastic enzyme that catalyses the oxidation of ascorbic acid (AA), playing a major role in controlling the redox state of the apoplast. AO was induced in both genotypes after *P. pachyrhizi* infection, showing the relevance of the oxidative stress pathway in studies of soybean-rust interaction.

P5.42

Interplay between ROS and RNS in tobacco response to cryptogein

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Cryptogein is a 10 kDa protein secreted by *Phytophthora cryptogea* triggering a systemic acquired resistance as well as a hypersensitive response in tobacco. The signalling events induced by cryptogein have been studied intensively and include, production of reactive oxygen species and nitric oxide. While the origin for NO production is unknown, NtRBOHD has been reported as the primary source for superoxide anion and derived H_2O_2. In this study, we investigated the cross-talks between NO and ROS in cryptogein signalling. Using antisense tobacco suspension cells impaired in *NtRBOHD* expression and a pharmacological-based approach, we showed that NO synthesis is partly regulated through a ROS-dependent mechanism. Conversely, scavenging of NO leads to a slight increase of ROS accumulation, suggesting that NO might regulate its pool. We further defined NO as a major determinant of cryptogein-induced cell death. To better assess the role of NO in tobacco response to cryptogein, a search for NO-responsive genes was performed. Fifteen NO-dependent genes of interest were selected. Expression of some of them was regulated by H_2O_2 either, reinforcing the hypothesis of shared signalling pathways for these two reactive species.

P5.43

Powdery mildew resistance graft-transmitted from cherry pepper to sweet pepper correlates with superoxide accumulation

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Susceptible sweet pepper plants become resistant to pepper powdery mildew (*Leveillula taurica*) upon grafting on resistant cherry pepper (*Capsicum annuum* var. *cerasiforme*) rootstocks. Furthermore, susceptible pepper grafted on resistant rootstocks display accumulation of the reactive oxygen species superoxide (O_2^-) due to the effect of an unknown, graft-transmitted signal. Non-grafted susceptible sweet pepper contains very low amounts of O_2^-, while high amounts are detectable in leaves of resistant cherry pepper. O_2^- was detected by the NBT (nitro blue tetrazolium) tissue staining method. Activity of NADPH oxidase, the enzyme mainly responsible for pathogenesis-related superoxide generation correlates well with superoxide accumulation. Changes in levels of salicylic acid (SA), a central molecule of plant disease resistance do not seem to be associated with powdery mildew resistance. SA levels were assayed by high performance liquid chromatography (HPLC). The direct biochemical cause of graft-transmitted pepper powdery mildew resistance seems to be the enhanced accumulation of superoxide. To our knowledge this is the first demonstration of graft-transmission of a pathogen-specific form of disease resistance.

P5.45

Ascorbate-glutathione cycle and other bioactives variability in two kale genotypes in relation to soil type and N-level

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Genotype and the environment are the two main factors influencing growth and development of horticultural plant before harvest, and finally their quali-quantitative yield attributes after harvest and during storage. The interrelationship between an increasing N supply tested on the lessive and mud soils and efficiency/fluctuation of the antioxidant machinery acting in leaves of two kale genotypes, Redbor F_1 and Winterbor F_1 varying in their antioxidant potential was the topic of this study. The increasing N-level, up to 200 $mg \cdot dm^{-3}$, not only did not cause any negative impact, but even intensified accumulation of some tested antioxidants and biomass production of kale plants. Plants growing on the lessive soil expressed greater N-impact on the content/activity and/or fluctuations of ascorbate-glutathione cycle than those growing on the mud soil. There were no clear responses of phenolics and anthocyanins contents in relation to N-level. N-fertility did not affect the total antioxidant ability. Genotypes differed considerably in both, antioxidant potential and their response to N-level, especially on the lessive soil.

P5.46

Reactive oxygen species and DNA methylation changes in wounded maize leaves

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Reactive oxygen species (ROS) can damage DNA, RNA, proteins and lipids, resulting in cell death when the level of ROS exceeds an organism's detoxification and repair capabilities. The methylation pattern of genomic DNA dynamically changes upon stresses in plants. We analyzed the amount of 5-methylcytosine (m^5C) in DNA from normal and wounded maize tissues using post-labeling with [g^{32}-P]ATP of DNA hydrolyzate and Enzyme-Linked Immunosorbent Assay (ELISA). The results show that wounding leads to a decrease of the m^5C level in DNA within 1 h after treatment. This indicates a relatively quick reaction to stress, leading to induction of gene expression crucial for the stress response. The decrease of the m^5C level was transient and returned to initial level 3-4 hours after wounding. Such rapid reversible methylation changes suggest involvement of non-enzymatic reactions caused by ROS. We have shown that wounding of maize leaves induces two-step oxidative stress, first (very fast) at 1 min after wounding and the second two hours later. Our results suggest that the oxidative stress caused by cell injury leads to the transient changes of the cytosine methylation level. Supported by grant NN303 306737.

P5.47

UV-B induced oxidative stress and protective effects of NO under myo-inositol-deficient background in *Arabidopsis*

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The influence of ultraviolet B (UV-B) overexposure on oxidative stress development and its mediation by NO in plant cells were studied. Experiments were performed using *Arabidopsis thaliana* (Columbia 0) wild-type and mutant on myo-inositol-1-phosphate synthase (AtIPS1) transformed with gene encoding fused glutaredoxin and roGFP2 to determine dynamic redox changes using CLSM. Five day old seedlings were irradiated by UV-B up to 81 kJ/m^2. Plants pretreated with 10-100 μM SNP as NO donor revealed lower oxidative stress level that varied in roots and underground tissues. In mutant plants this protective effect was more expressed and manifested in the tolerance to higher UV-B dose that were confirmed in growth tests. Thus, the pretreatment of wild-type with 10 μM SNP before irradiation (81 kJ/m^2) was not significantly influenced by changes in the length of roots and hypocotyls. On the other hand, the negative effects of UV-B exposure were in 1.9 and 1.5 times less pronounced for roots and hypocotyls of mutants under the same conditions. Obtained data indicate the relationship of NO signalling and metabolism of myo-inositols in the mediation of UV-B induced oxidative stress in plant cells.

P5.48

High light inducible leaf antioxidants improve UV tolerance in greenhouse grown tobacco

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The effect of high light (HL) acclimation on tolerating supplemental ultraviolet (UV) irradiation was studied in greenhouse grown tobacco plants. Plants grown at 200 μmol photon m^{-2} s^{-1} photosynthetically active radiation (PAR) were exposed to 1000 μmol m^{-2} s^{-1} PAR at 6-leaves stage for 5 days. Afterwards, both HL pretreated and control (LL) plants were irradiated with 8.95 kJ m^{-2} d^{-1} biologically effective UV dose (290-400 nm) supplemental to growth light. After 6 days, total and ROS specific antioxidant capacities were measured in leaf extracts. HL pretreatment increased leaf antioxidant capacities (Trolox equivalent antioxidant capacity) and the amount of UV-B absorbing compounds. HL plants showed no loss in photosynthetic activity, while LL plants had 60% lower photosynthesis by the end of UV treatment, although the latter showed increases in leaf antioxidant capacities (ferric reducing antioxidant power and superoxide radical scavenging activity). Our results show the importance of HL induced antioxidants in tolerating supplemental UV radiation and emphasize the role of UV-B absorbing compounds as antioxidants contributing to this cross-tolerance. Supported by the Hungarian Scientific Research Fund OTKA NN85349.

P5.49

Environmental factors-induced stomatal signalling

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Balanced stomatal opening and timely stomatal closure are crucial for plant survival during drought and air pollution episodes. To address the role of signalling through PP2C phosphatases and PYR/RCARs receptors for whole-plant stomatal conductance and stomatal closure induced by elevated CO_2, reduction of air humidity, darkness and ozone, we used a set of *Arabidopsis* mutants defective in ABA metabolism and signal transduction. We show that basal ABA signalling through PYR/RCAR receptors plays a fundamental role in controlling whole-plant water loss. PYR/RCAR-dependent inhibition of PP2Cs was required for rapid stomatal regulation in response to darkness, reduced air humidity and ozone. Furthermore, PYR/RCAR proteins seem to function in a dose-dependent manner and there is a functional diversity among them. Inhibition of CO_2-induced stomatal closure was less clear and bicarbonate-induced activation of S-type anion channel currents was reduced but functional in dominant active PP2C mutants, *abi1-1* and *abi2-1*. Thus we show that PYR/RCAR receptors play a fundamental role for the whole-plant stomatal adjustments and responses to low humidity, darkness and ozone and are involved in responses to elevated CO_2.

P5.50

Oxidative stress induced autophagic cell death in plants

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Autophagy is a self-digestion process that degrades intracellular structures in specialized double membrane vesicles autophagosomes during development and stresses. In response to oxidative stress plants use autophagy to degrade and recycle oxidized and damaged intracellular components. In wheat roots, exogenous application of prooxidants (paraquat, salicylic acid, mitochondrial poisons) and the disruption of membrane integrity by nystatin and gramicidin S induced ROS accumulation, formation of autophagosomes and decrease in cell viability. Using transmission electron microscopy, numerous autophagosomes containing cytoplasmic fragments and organelles were detected. Autophagosomes were also visualized by laser confocal microscopy using fluorescent dye Lyso Tracker Red. Autophagic processes are controlled by numerous *ATG* genes. RT-PCR analysis showed that treatment of roots with prooxidants, mitochondrial poisons and channel-forming agents highly up-regulates the expression of autophagic genes *TaATG4* and *TaATG8*. In conclusion, autophagy is an important response of plant cells to the disruption of redox metabolism and can control both pro-survival and pro-death consequences.

P5.51

Ethylene mediates in the early oxidative stress induced by mercury

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Exposure of plants to mercury (Hg), as a result of several anthropogenic activities or naturally occurring pollution (i.e. in Almadén, Spain), causes a rapid inhibition of root elongation, imbalance of the cellular redox homeostasis and reactive oxygen species (ROS) accumulation. A transcirptomics analysis revealed the participation of the phytohormone ethylene in the early responses of alfalfa (*Medicago sativa*) seedlings to moderate Hg stress (3 μM $HgCl_2$). The expression of several ethylene responsive genes such as ERF1 and AP2 transcription factors or 1-aminocyclopropane carboxylic acid synthase (ACCS) was further investigated in ethylene insensitive plants, either alfalfa seedlings treated with the ethylene inhibitor 1-methylcyclopropene (1-MCP) or *Arabidopsis thaliana ein2-5* mutant defective in ethylene signalling. All early stress symptoms associated with Hg were blocked in ethylene insensitive seedlings, including ROS generation. Thus, extracellular H_2O_2 generation (using AmplexRed fluorescence) depended in ethylene at moderate doses of Hg. We hypothesise that Hg induces ROS production via plasma membrane NADPH-oxidase activation, activity that is maintained at control levels in ethylene insensitive seedlings.

P5.52

Antioxidant activity responses under freezing stress in two potato cultivars with different sensibility

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Cultivated plants have a limited capacity to survive temperatures below freezing. Considerable attention has focused on improving the biotic and abiotic stress tolerance of crops by increasing their antioxidant capacity. Seed tuber production is normally limited by freezing tolerance and it is correlated with inherent antioxidant responses. The aim was to investigate if antioxidant enzyme activities are linked to freezing sensibility in two cultivars with different response to freezing stress used in seed production programs. Microplants in soil were exposed to freezing conditions ($-6 \pm 1\,^{\circ}C$) in darkness for 4 h. Sensitive cultivar showed more electrolyte leakage than the tolerant one after freezing treatment. POX activity and H_2O_2 content increased in both cultivars after freezing treatment, and CAT activity decreased. The tolerant cultivar showed more antioxidant enzyme activity and H_2O_2 content than the sensitive before and after freezing treatment. Differential isoenzymes activities of POX were observed between sensitive and tolerant cultivars. Antioxidant system could be a useful tool for selection of freezing tolerance in potato cultivars.

P5.53

Arabidopsis thaliana MKKK18 is involved in abscisic acid signalling

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Current findings have demonstrated that MAP kinases play an important role in the transmission of the ABA signal. The critical outstanding question is: which MAPKs are involved in the ABA signal transduction. Our project involves the identification and functional analysis of the MAPKKK18 in relation to ABA signalling. To verify the ABA responsiveness of MKKK18 a 1.6kb promoter region and its serial deletions fused to the GUS and the GFP reporter were generated. The full-length ProMKKK18D1:GUS promoter activity revealed strong ABA-induced expression in the flowers, leaves and root tissue. Truncated promoters drove strong ABA-induced expression only in vascular tissues and roots but not in flowers and guard cells. The *Arabidopsis* MKKK18 ORF fused either to the GFP gene or YFP reporter was localized to the nucleus and the cytoplasm. Moreover, an ABA signalling mutant (*abi1td*) had an effect on MKKK18 localization. Finally, at the level of germination MKKK18 knockout plants were hypersensitive to ABA. Overall, our results suggest that MKKK18 is a negative regulator of ABA signalling and response.

P5.54

Biophoton emission as a tool to study interactions of pathogen elicitors with plant cells

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Elicitins are small extracellular proteins secreted by oomycetes and fungi, which cause hypersensitive reaction in members of *Solanaceae* and *Brassicaceae*. Elicitins enter into plant cells by specific receptors, activate MAP-kinases and induce ROS production. Biophoton are emitted by metastable excited compounds formed during oxidative reaction and their increased emission in plants is connected with oxidative stress and lipid peroxidation. Biophoton emission was recorded by sensitive CCD camera in leaves and cell cultures of *N. tabacum* cv. *xanthi* after the addition of oligandrin, cryptogein and its mutants (L41F, V84F, V84F/L41F, L80F/V84F) with different capacity to induce ROS production, HR and necrosis. Emission was increased locally in tobacco leaves already 6h in sites of infiltration in case of cryptogein and V84F and L80F/V84F mutants with high capacity of ROS induction. This was associated with high level of lipid peroxidation and extensive necrosis. ROS scavengers and inhibitors of SOD, NADPH-ox and POX were used to evaluate their role in ROS production and catabolism. We demonstrated biophoton emission as a valuable tool for in vivo plant pathogenesis studies. Supported by Czech Grant Agency (P501/12/0590).

P5.55

Constitutively active guard cell plasma membrane H^+-ATPase impairs ozone – elevated CO_2^- and darkness-induced stomatal closure

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Ozone acts as an air pollutant in the lower levels of the atmosphere, and has negative effects on crop yield and overall carbon fixation by vegetation. Ozone degrades in the apoplastic space rapidly into reactive oxygen species (ROS), and has been therefore used as a tool to study ROS-induced processes. Application of ozone triggers a rapid transient decrease in stomatal conductance (RTD) which coincides with the burst of ROS in guard cells. RTD is abolished in plants that have impaired guard cell S-type anion channel activity. In order to study what is the role of guard cell plasma membrane H^+-ATPase deactivation for ozone-induced RTD, we used dominant *ost2* mutants that have constitutive H^+-ATPase activity. This revealed that both *ost2-1D* (Ler bg) and *ost2-2D* (Col-0 bg) did not have RTD in response to ozone. The mutants also did not respond to elevation of CO_2 concentration and application of darkness. It was found that S-type anion channel activity was intact in *ost2-2D* mutants. These results suggest that ozone/ROS-, CO_2^- and darkness-induced stomatal closure requires deactivation of H^+-ATPase.

P5.56

Redox regulation of root glucose-6-phosphate dehydrogenase by thioredoxins in the context of salt stress

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In non-photosynthetic cells, like in roots, NADPH is mainly produced from glucose by the oxidative pentose phosphate pathway (OPPP). Glucose-6-phosphate dehydrogenases (G6PDH) catalyse the first and committed step of the OPPP, and plastidial isoforms are oxidatively activated by thioredoxins (TRX). TRX are small ubiquitous thiol oxidoreductases with a large number of important cellular functions. Plant TRX display a notable diversity and biochemical data obtained *in vitro* revealed that plastidial isoforms can have specific functions. Recent data obtained *in planta* validated the functional specificities of some plastidial isoforms and their role in response to stresses in leaves. Previous studies have shown a relationship in plants between the OPPP and the response to different stresses and a central role has been assigned to G6PDHs in response to salt stress, including in roots. Here, we studied the TRX-dependent redox regulation of *Arabidopsis* G6PDH P2 isoforms (G6PDH2 and G6PDH3) that are preferentially expressed in non-photosynthetic plastids. We show that m-type TRX who are the best activators of G6PDH2 and G6PDH3 *in vitro* consistently regulate G6PDH activity in *Arabidopsis* roots upon salt stress conditions.

P5.57

Modification of ROS level by *m*-tyrosine during tomato (*Lycopersicon esculentum*) root growth

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Allelopathy is an ability of some species to influence growth of neighboring plants by chemicals released into the environment. The allelopathic compound *m*-tyrosine is an non-protein amino acid isolated from fescue root exudates. Growth of root of wide range of plants is affected by *m*-tyrosine. Toxicity of *m*-tyrosine may be due to its incorporation into proteins, leading to alterations in theirs structure and function. Increase of *m*-tyrosine content was observed during oxidative stress in animal cells as a result of non-enzymatic oxidation of phenyloalanine. Therefore, *m*-tyrosine can be a marker of oxidative stress. The aim of this work was to investigate modifications of the level of ROS in tomato (*Lycopersicon esculentum*) roots subjected to *m*-tyrosine. Growth of tomato roots was inhibited by *m*-tyrosine in dose-depended manner. *m*-Tyrosine led to enhanced membrane deterioration and outflow of H_2O_2 from the roots to the surrounding medium. It was accompanied by time-dependent increase of ROS (H_2O_2 $O_2^{\bullet -}$) concentration. Alteration in ROS content correlated with modification of cellular antioxidant enzymatic system: superoxide dismutase (SOD), catalase (CAT) and substrate non-specific peroxidase (POx).

P5.58

Cis-, *trans*-, transcriptional regulation of the *Arabidopsis thaliana* gene *AtTrxo1* and its response upon germination and to salt

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Plants contain several genes encoding thioredoxins (Trxs), small proteins involved in redox regulation of many enzymes in different cell compartments. Among them, mitochondrial *Trxo* has been described to have a response in plants grown under salinity but there is scarce information about its functional role in abiotic stress or its gene regulation. In this work, the transcriptional regulation of the mitochondrial *AtTrxo1* gene has been studied for the first time, by identifying functionally relevant *cis*- elements in its promoter: two conserved motives were found as positive and one as negative regulators. Using them as baits for the screening of an arrayed yeast library containing *Arabidopsis* Transcription Factors (TF) ORFs, two TFs were selected that are now being validated at the molecular level. We have also studied the response of T-DNA insertion mutant plants for *AtTrxo1* to salt stress. The K.O. *AtTrxo1* mutants presented several phenotypic changes including the time required to reach 50% germination under salinity, without affecting the final germination percentage. (*equal authors). This work was supported by MICINN (BFU2011-28716) and Seneca Foundation, Murcia (04553/GERM/06), Spain.

P5.59

Protein oxidation and degradation in mitochondria and chloroplasts of *Arabidopsis thaliana* under sulphur deficiency

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Sulphur (S) deficiency in plants may lead to higher production of reactive oxygen species (ROS) that cause an irreversible carbonylation of proteins. In the leaves of S-deficient *A. thaliana* the histochemical localization showed higher levels of H_2O_2 and $O_2^{\bullet -}$ as compared to control. Oxidized proteins and the activity of proteases involved in degradation of the damaged proteins were analyzed in leaf and root tissue extracts, isolated mitochondria or chloroplasts. S-deficient plants were characterized by over 20% higher carbonyl concentration and protease activity in leaf tissue extracts only; similar carbonyl concentration in mitochondria and about 30% higher activity of mitochondrial proteases; 40% higher level of oxidized proteins and the activity of proteases similar to control in chloroplasts. These results show that higher ROS production in the leaf tissues of S-deficient *A. thaliana* increases the level of protein carbonylation. Chloroplasts and mitochondria have developed individual strategies to cope with the oxidative damage of proteins. Funded by grant N N303 800240 from the NCN given to IMJ and the intramural grant DSM 501/86-102342 from MSHE through the Faculty of Biology, University of Warsaw, given to MO.

P5.60

Profiling of plasma membrane bound peroxidases in maize (*Zea mays* L.)

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Heavy metal stress is known to be toxic to plants, triggering physiological responses, specifically for cadmium that even causes genotoxic effects. Cadmium is one of the most prevalent heavy metal in our environment. It has become clear that at least parts of the metal-induced phytotoxicity can be attributed to oxidative stress. Reactive oxygen species (ROS) play not only an important role in the response of plants to biotic and abiotic stress, but also in plant cell growth, stomatal opening, hormone signalling, programmed cell death and regulation of gene expression. Peroxidases, which are involved in production and detoxification of ROS, can control the level of ROS together with other antioxidant systems, sensitively. This Project focusses on the plasma membrane bound peroxidases ZmPrx01, ZmPrx66, ZmPrx70 in roots. These three peroxidases belong the class III peroxidases (secretory pathway). There have been at least 143 distinct peroxidases found in *Zea mays*, yet. The expression profile under cadmium exposure of each peroxidase was estimated by real time PCR in a short term and long term trial. Further the necessity of each peroxidase is going to be assessed by RNAi.

P5.61

The effect of trace metal pretreatment on plant fungi interaction

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Plants are exposed to numerous biotic and abiotic stresses during their growth, these factors may affect physiological conditions, development and reproduction. Most common abiotic stress can be caused by factors like trace metals, cold, salt whereas biotic stress is caused by pathogens, bacteria or fungi. ROS have a central role in mediation between both stress responses although function differently in each. In biotic stress the pathogen attack will trigger the production of ROS, their role is to induce damage to the pathogen, to reinforce the plant cell wall and to act as a secondary messenger. In abiotic stress presence of stress factors also leads to increased ROS amount, but their presence is extremly harmful and removal is essential for survival of the plant. To minimize damage plants produce antioxidants and ROS-scavening enzymes. As stresses often occur in combination, the relationship between ROS signalling mechanism in different stress responses is complex. The presence of an abiotic factors can have the effect of reducing or enhancing susceptibility of plants to a biotic factors, and *vice versa*.

P5.62

Effect of drought and re-watering on the metabolomic profile and antioxidant response of eucalyptus plants

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Effects of water stress on plants are well-documented. However, the particular responses of re-watering after drought and the underlying mechanisms remain relatively unknown. *Eucalyptus globulus* expansion is restricted by its drought sensitivity. Acclimation of plants to drought is often associated with increased levels of reactive oxygen species (ROS) and antioxidative systems to remove them. Metabolomics is becoming a key tool in understanding cellular responses to stress. Here we describe temporal alterations in the physiology, cell status and metabolomic profile of *E. globulus*, resultant from water deficit and subsequently re-watering. Physiological processes and cell damages were monitored by determining MDA levels, further supported by antioxidant balance of SOD, CAT and APX measurements, stomatal conductance and ABA content. The recovery of *E. globulus* clones watered to 18% FC was assessed. Results showed the negative impact of drought by a reduction in gs and higher MDA levels, and an increase in antioxidant enzymes. An important part in drought relief characterization in *E. globulus* is deciphered, as well as the importance of plant metabolomics in characterizing plant´s metabolic responses are highlighted.

P5.63

The influence of mitochondrial Complex I impairment on ROS metabolism under ammonium supply

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Ammonium, when present in excess is deleterious for many plant species (named "ammonium toxicity"). A first visible symptom of ammonium toxicity is stunted growth. It was shown that *frostbite1* (*fro1*) plants carry a point mutation affecting the 18-kDa Fe-S subunit of Complex I in the mitochondrial electron transfer chain (mtETC). Complex I is the major entry point of electrons into mtETC therefore its dysfunction may alter cellular oxidation-reduction state and energy metabolism. Surprisingly, biomass production of *fro1* plants is increased when grown on ammonium. To understand this phenomenon we focused on (intra-)cellular changes in ROS metabolism. Enzymatic (enzyme capacities, protein and transcript level) and non-enzymatic antioxidant systems together with ROS concentration and subcellular localization were determined in *fro1* leaf tissue under nitrate and ammonium supply. Our investigations demonstrate that adaptations/changes brought about by Complex I impairment alleviate ammonium toxicity. We are grateful to Prof Jian-Kang Zhou for kindly donating mutant seeds. This work was partially supported by grant N N303 401536 given to B. Sz. and grant N NZ3 02953 given to A. P.

P5.64

ROS metabolism in arabidopsis leaves under ammonium supply

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When ammonium is supplied as an exclusive nitrogen source, most plant species show symptoms of toxicity ("ammonium syndrome"). The stress symptoms observed under sole ammonium nutrition can be related to a combination of several metabolic events including oxidative stress. It was proposed that mitochondrial ROS (mtROS) production under ammonium supply may be enhanced. We have shown that ammonium nutrition causes changes in mitochondrial enzymatic antioxidant systems and leads to injuries of mitochondrial components. Further we measured mtROS production and subcellular localization of ROS under ammonium supply. Our results clearly demonstrate that ammonium nutrition is associated with altered mtROS metabolism. The role of mitochondria in regulation of the cellular oxidation-reduction state under ammonium supply is discussed. This work was partially supported by grant N N303 401536 given to B. Sz. and grant N NZ3 02953 given to A. P.

P5.65

Elements of signalling pathways in the response of *Solanum* species to *Phytophthora infestans*

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Among components of plant signal transduction pathways, protein kinases and reactive oxygen species (ROS) play a pivotal role in pathogen defence responses. Mitogen-activated protein kinases (MAPKs) cascades and calcium-dependent protein kinases (CDPKs) activities as well as their expression profile and ROS production were investigated in *Solanum* species representing: non-host resistance – *Solanum nigrum* var. *gigantea*, field resistance – *S. tuberosum* cv Bzura, susceptibility – *S. tuberosum* clone H-8105 in the interaction with pathogen *P. infestans*. After elicitor treatment, the H-8105 showed the highest increase in the ROS production in comparison with *S. nigrum* var. *gigantea* and Bzura. MAPK and CDPK activities increased in response to elicitor treatment, were positively correlated with the level of plant resistance, however varied with respect to intensity and timing. We have demonstrated that transcripts of MAPKs and CDPKs are present in all studied *Solanum* species, although only transcript level of CDPKs increased after elicitor treatment. The obtained results widen the knowledge about signalling pathways occurring in *Solanum* species in response to *P. infestans*. Supported by grant 2012/05/B/NZ3/00911

P5.66

Zn-induced ROS and RNS metabolism alterations in plants with modulated senescence *via* cytokinin level

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Different kind of stress can induce premature senescence at any stage of plant life. Plants with delayed senescence are more tolerant against various stresses including abiotic stress. Gan and Amasino (1995) developed transgenic tobacco plants with SAG12 promoter fused with ipt gene synthesising cytokinins. At the onset of senescence, this promoter is activated and cytokinin level is increased due to a higher isopenthenyl transferase activity. Their life span is longer and they also display higher stress tolerance. We used this transgenic tobacco (*Nicotiana tabacum* L.) cv. Wisconsin 38 (SAG plants), as a control we used the wild type plants (WT plants). Plants were grown for 11 weeks in soil without Zn contamination or with two rates of Zn (Zn1 = 250, Zn2 = 750 mg kg^{-1}). At Zn2, SAG plants showed higher chlorophyll content and photochemical efficiency. WT plants showed lower antioxidant protection and higher nitrotyrosine content. Both plant types did not differ in their nitric oxide content. Our results show that SAG plants were damaged to lesser extent and seem to be better equipped to cope with high Zn content. Acknowledgements: This research was supported by Grant Agency of the Czech Republic, Grant No. P501/11/1239.

P5.67

Assessment on negative effects of Sb ions on selected lettuce cultivars

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In this study, an attempt was made to evaluate the effects of Sb^{3+} on selected cultivars of lettuce (*Lactuca sativa* L.) i.e. "Satine" (red leafed) and "Locarno" (green leafed). Plants were grown in hydroponic culture and treated with Sb in concentrations of 5, 10 and 25 mg/dm^3. Control plants were not treated with Sb. Parameters describing efficiency of photosynthetic apparatus and transpiration rates were determined during plants growth. At harvest data on biomass accumulation, levels of reactive oxygen species, activity of enzymes of anti-oxidative system and selected ions' content were recorded. Biomass accumulation in aboveground parts and roots was negatively affected by Sb, what corresponds well with decreased intensity of photosynthesis, stomatal conductance and transpiration rate. Sb influence on total chlorophyll content and parameters of chlorophyll a fluorescence was ambiguous, depending on cultivar and measured parameter. Plants exposed to Sb had increased levels of anion-radical and hydroxyl radical with simultaneous slightly higher activity of catalase and ascorbate peroxidise. Evaluation of selected ions' content is in progress.

P5.68

Regulation of cytochrome b-561 protein family in maize (*Zea mays* L.) roots by iron and cadmium stress

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During their life, plants have to cope with a variety of abiotic stresses. Although iron is an essential nutrient for plants, its accumulation within cells can be toxic. A recent proteomic study of our group suggested alterations in protein abundance for AIR12 under iron stress conditions. Besides AIR12, further members of the cytb-561 protein family have been identified in corn root plasma membranes on the protein level. Additionally, biochemical characteristics of recombinant cytb-561 (Zmcyt b561-1) have been published. So far functions in iron reduction and/or regulation of the apoplastic redox state were postulated for the cytb-561 protein family. Besides iron excess, cadmium is highly toxic to plants. Due to its easy water solubility, cadmium is readily taken up in tissues by iron transporters and affects the redox state of the cell. In the present study gene expression of known members of the cytb-561 protein family (cytb-561, AIR12, DoH and two DoH-cytb-561) have been investigated on the transcriptional level by quantitative real-time PCR in maize roots. Circadian rhythm of these genes and their regulation by iron and cadmium stress has been characterized for short-term and long-term conditions.

P5.69

In vivo quantification of diatom redox metabolism provides insights into sensing nitrogen stress in the marine environment

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Diatoms are ubiquitous marine photosynthetic eukaryotes that are responsible for about 20% of global photosynthesis and therefore are an important component of the large biogeochemical cycles in the ocean. Here we explored diatom mechanisms of oxidative stress perception by combining in vivo imaging of subcellular redox potential with quantification of the whole redox proteome (redoxome) in the model diatom *Phaeodactylum tricornutum*. Using highly sensitive and quantitative mass spectrometry-based approach, we quantify the level of oxidation of around 4000 cysteines in *P. tricornutum* proteome and identify around 300 redox-sensitive proteins. Intriguingly, redox-sensitive thiols were found in numerous enzymes participate in nitrogen assimilation and in the diatom peculiar urea cycle. Using the roGFP sensor, we revealed differential alterations in the redox potential of the chloroplast and mitochondria during nitrogen starvation and by resupply of different nitrogen sources. We propose that accurate sensing of nitrogen status by organelle-specific oxidation patterns and redox sensitive enzymes may provide diatoms with important machinery for rapid and reversible responses to fluctuated nitrogen availability.

P5.70

Plant hemoglobins can be maintained in active form by reduced flavins and confer tolerance to nitro-oxidative stress

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Genomic analyses have unveiled an ample distribution of Hbs in all kingdoms of life, where they perform diverse functions such as transport and scavenging of oxygen and detoxification of nitric oxide. We have characterized the nonsymbiotic Hbs (two class 1 and one class 2) and truncated Hbs (two class 3) of the model legume *Lotus japonicus*. The five globins can be maintained in their active state by free flavins, which act as electron carrier intermediates between NAD(P)H and the heme iron. Class 1 Hbs were reduced at very fast rates preferably with FAD, class 2 Hbs at slower rates with both FMN and FAD, and class 3 Hbs at intermediate rates preferably with FMN. The three globin classes were immunolocalized predominantly in the plastids and nuclei. In the chloroplasts, class 1 and 2 Hbs were more associated with starch grains and class 3 Hbs with the thylakoids. Expression of the proteins in a yeast mutant deficient in flavohemoglobin conferred tolerance to oxidative stress induced by methyl viologen, copper and low temperature. Flavins were quantified in plant tissues, nodule cell nuclei and yeast cells, and their concentrations were sufficient to sustain the activity of plant globins.

P5.71

Cyclic AMP deficiency simulates a stress condition in tobacco BY-2 cells

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Although the existence of cyclic AMP in higher plants has been widely debated, nowadays its involvement in several physiological processes has been definitively demonstrated. To better investigate the role of cAMP in plants, tobacco BY-2 cells have been transformed with the cAMP-sponge (cAS) a non invasive tool, able to selectively reduce cAMP concentration. The cAS is composed of two high-affinity cAMP binding domains of the regulatory subunits Ib of human PKA that specifically bind cAMP and not cGMP. The cAS, under the control of the 35S CaMV promoter, in frame with the reporter gene mCherry, was transferred in tobacco BY-2 cells via *A. tumefaciens* -mediated transformation. After the assessment of transgene integration and expression, the growth parameters of transformed BY-2 cells were characterized. The obtained results show that low levels of cAMP negatively affect growth of tobacco BY-2 cells during the exponential phase. In particular, the transgenic BY-2 cells slow down cell cycle progression and contemporary enhance antioxidants, suggesting that cAMP deficiency is sensed by plant cells as a stress condition.

P5.72

NADPH oxidase mediated ROS control susceptibility of *Arabidopsis* plants to a parasite

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Reactive oxygen species (ROS) produced in response to pathogen infection can mediate programmed cell death following successful recognition of certain pathogens by plants. These ROS in plants are mainly generated by plasma membrane-located NADPH oxidases closely related to those present in mammalian neutrophils. Mutation in NADPH oxidase genes can result in a compromised immune response in plants and animals. However, the function, if any, of host ROS production during successful infections in compatible plant-pathogen interactions remains largely unknown. Here we provide evidence that plant NADPH oxidase mediated ROS is induced by sedentary plant parasitic nematodes, and that this suppresses activation of host defense responses at and around infection sites. As aggregation of nematodes inside plant roots is reduced in the absence of ROS, they also seem to serve as signals for nematode orientation inside plant tissue. Our work establishes a framework for understanding how ROS help a sedentary nematode that causes significant cell damage during root invasion to establish and maintain a highly active and specific feeding cell system in the root, rather than killing host cells during the infection process.

P5.73

Functional characterisation of tissue specific acclimation to oxidative stress

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Biotic and abiotic stress-conditions are responsible for massive losses of yield in plant agriculture. Reactive oxygen species are known to be important players in stress sensitivity and -response. Photorespiration is a major source of hydrogen peroxide (H_2O_2) and catalase is an important scavenger of it. *Arabidopsis thaliana* catalase knock-out plants displayed a tissue specific adaptation in newly developed leaves under photorespiratory stress conditions. Older leaves, developed before stress treatment, display areas of clear cell death and in some cases the entire leaf is dead. In contrast, young leaves, developed post-stress treatment, are darker green, free of chlorosis and slightly serrated. We employed these phenotypic changes to study the role of H_2O_2 in stress acclimation, contributing further to the knowledge portfolio of genetic regulatory networks during oxidative stress. A microarray-based transcriptome analysis of plants grown under different oxidative stress conditions, resulted in the selection of 63 genes that were specifically down or up regulated, when non-adaptive and adaptive leaves were compared. Loss-of-function knockout mutants are currently tested in oxidative stress-related bio-assays.

P5.74

Are carbonylated proteins involved in plant – mite-pest interactions?

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Comparative experiments have been carried out on the protein carbonylation in non-transgenic insect-sensitive (ISO) and transgenic insect-resistant (YG) maize cultivars subjected to two-spotted spider mite attack. In response to 4-day mite feeding, carbonylated protein (CP) content increased (ISO) or did not change (YG), yet decreased in 6-day mite-damaged leaves. The response of non-infested leaves (above mite-infested leaves) differed: after 4 days – CP content decreased in YG and remained unchanged in ISO. Feeding prolongation decreased CP content in ISO and increased in YG leaves, reaching control level. Immunoblotting showed that regardless of the cultivar, after 4 days of mite feeding, the abundance of bands for oxidized proteins increased only slightly, whereas longer infestation increased the number of 25-35 kDa protein bands. At the same time, the increase of 25-35 kDa and ~15 kDa protein bands was more pronounced in non-infested YG than ISO leaves, whereas 2 days later, the opposite response was recorded. This suggests a time-dependent contribution of low molecular carbonylated proteins in local and systemic response of both cultivars to mite attack.

P5.75

ABA-independent SnRK2 kinases are involved in plant response to salinity

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Sucrose non-fermenting 1-Related Protein Kinases type 2 family (known as SnRK2) are important group of kinases present in all tested plant species. It is known that all SnRK2 (excluding SnRK2.9) are activated in response to salinity and hyperosmolarity in *Arabidopsis thaliana* protoplasts after short time of treatment. In plants, they are involved in the early responses to drought and salinity. Recently published data indicate that SnRK2.4 plays important role in the maintenance of root system architecture during salt stress and in response to cadmium treatment. It was also suggested that in roots facing cadmium treatment, SnRK2.4 controls ROS accumulation leading to alteration of stress tolerance. Here, we studied the involvement of ABA-independent SnRK2 kinases in ROS accumulation and scavenging during salt stress in *A. thaliana* leaves by investigating the level of glutathione and hydrogen peroxide in insertion mutants of SnRK2.4 and SnRK2.10. We also established the role of kinases studied as regulators of plant tolerance to salt stress by testing chlorophyll degradation in KO lines.

P5.76

Uranium exposure induced reactive oxygen species and nitric oxide generation in *Arabidopsis thaliana*

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The objective of this work was to look at the role of reactive oxygen species (ROS) and nitric oxide (NO) in the uranium induced stress response. Exposing *Arabidopsis thaliana* roots to uranium, resulted in a significant induction of cell death. Considering the ability of NO to form complexes with metals and alleviation of heavy metal toxicity, we examined the induction of NO following exposure to uranium. *Arabidopsis* roots and leaves exposed to uranium showed an increase in nitrite concentration indicating the generation of NO. We further confirmed NO production in the roots by DAF-2DA staining in the presence and absence of NO scavenger (methylene blue) and NO synthase inhibitor (L-NAME). DAF fluorescence was weakened in the presence of methylene blue and L-NAME. In addition to NO, the production of hydrogen peroxide in uranium exposed roots was indicated by an increase in DCF fluorescence and measurement as H_2O_2: $TiCl_4$ complex as well. To further examine the induction of ROS by uranium, the expressions of different genes (*LOX* and *RBOH*) involved in ROS generation in *Arabidopsis thaliana* are being followed. Our observations suggest that uranium induces ROS and NO generation possibly leading to observed root cell death.

P5.77

Response to cadmium depends on iron-status

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The aim of the research was to evaluate the usefulness of transforming tomato with *AhNAS2* to enhance a plant performance under low Fe and modify Cd metabolism. We have used tomato plants expressing *AhNAS2* from *A. halleri* encoding nicotianamine synthase (NAS), which is involved in NA biosynthesis. Transgenic and wild-type plants were exposed to control and Fe-deficient conditions without and with 0.1 μM Cd. On 13th day in *AhNAS2*-expressing plants Fe-deficiency symptoms have receded and their leaves turned to green. Higher tolerance of transformed plants to low Fe was accompanied by higher Fe level in leaves and expression of *LeIRT1* and *LeFRO1* genes. Expresion of *AhNAS2* and *LeNAS* in transformants under Fe-deficiency was higher in roots than in leaves. Transgenic plants exhibited higher tolerance to Fe-deficiency conditions with 0.1 μM Cd. This was confirmed by a higher level of Fe and lower level of Cd in leaves of transformed plants and diverse accumulation of H_2O_2 between transgenic and wild-type plants. Results indicate that *AhNAS2* expression in tomato is developmentally and organ-specifically regulated, and suggest the role of NA not only in Fe-deficiency tolerance but also in reducing Cd root-to-shoot translocation.

P5.78

Induction of tolerance to low temperatures in S. *tuberosum* by treatments of salicylic acid and hydrogen peroxide

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In previous reports salicylic acid (SA) induced freezing tolerance even though it does not always leads to hydrogen peroxide (H_2O_2) accumulation; meanwhile H_2O_2 induced freezing tolerance just in cold sensitive *in vitro* cultivars. The potential of SA and H_2O_2 in the induction of freezing tolerance as a short (ST) and long term (LT) effects in plants raised from tubers was studied. ST treatments: Under glass house conditions, plants 15 days old were sprayed twice a week with H_2O_2 and SA solutions up to 1.5 and 4 months. LT treatments: Tubers were immersed in H_2O_2 and SA before planting under glass house. At 1.5 and 4 months old (MO), both ST and LT treatments, were exposed to -6°C for 4 h. In ST treatments: Plants 1.5 MO, SA and H_2O_2 enhanced survival 2.91 and 4.16 fold respectively in contrast to the control; there were no significant differences in H_2O_2 content. In 4 MO, survival enhanced 3 and 4 fold respectively; higher survival was associated to higher H_2O_2 content and reduced CAT activity. LT treatments increased survival rate in both ages. H_2O_2 content increased only in 1.5 MO, CAT activity was not correlated. Depending on the age, H_2O_2 and SA induced freezing tolerance in LT and ST.

P5.79

Influence of ascorbate-recycling, light and temperature during tomato fruit ripening on ascorbate pool and ascorbate-degradation

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Ascorbate is a powerful antioxidant in plants. Ascorbate concentration depends on its biosynthesis, recycling and degradation; these are under genetic control and closely related to environmental conditions. Ascorbate recycling, controlled by the enzymes monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR), ensures a high-rate turnover of the ascorbate pool. Transgenic lines silenced for an MDHAR gene in a cherry tomato cultivar have been generated. The objective of this study was to determine whether limiting turnover of the ascorbate pool may affect levels of its reduced form and its degradation products. Degradation occurs via DHA to assayed candidate compounds: oxalate, tartrate and threonate. In order to investigate the impact of the reduction in MDHAR activity under different conditions of light and temperature on ascorbate concentration and ascorbate-degradation products, we used off-vine tomato fruits. Mature green fruits were harvested and placed in environmental chambers at 12°C, 23°C or 32°C, in darkness or light. Results are discussed and shed light on the stability of the ascobate pool in fruits under different environmental conditions.

P5.80

The role of ascorbate oxidase in ozone stress in rice

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The concentration of tropospheric ozone is increasing due to anthropogenic gas emissions, and causes adverse effects on crop yields and quality. Ozone enters plants through the stomata and is degraded to ROS in the apoplastic space, thereby causing oxidative stress. Previous QTL and microarray studies suggested that a gene annotated as an ascorbate oxidase (AO, Os09g0365900) might be a candidate gene determining ozone stress tolerance in rice (M. Frei et al., J. Exp. Bot. 2010; 61: 1405-1417). Paradoxically the expression of this gene is strongly induced under ozone stress, which supposedly facilitates the oxidation of ascorbate, although reduced apoplastic ascorbate is required to detoxify ROS. To analyze the role of this putative AO gene under ozone stress, knockout and over-expression lines were obtained. The knockout line showed less oxidative damage under ozone stress compared to the over-expression and wild-type line, as indicated by lower MDA level and H_2O_2 production. There were also clear genotypic differences in growth parameters. Ongoing analyses aim at elucidating in detail the mechanisms underlying the phenotypic differences between these three lines.

P5.81

Study of oxidative stress in crops treated with glyphosate herbicide

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We investigated the effect of the herbicide "Uragan Forte" (glyphosate 500 g/l) at different concentrations on lipid peroxidation and accumulation of hydrogen peroxide in wheat, oat, barley, alfalfa, by treatment soil and plant leaves. In a 1-2 weeks after treatment of soil by herbicide in the shoots and leafs of wheat, oats and barley roots an increased of lipid peroxidation in 1.5-2 times compared to control only when used the most high concentration of herbicide. The H_2O_2 content no change at all concentrations used in seedlings of wheat and barley, and increased in oat and alfalfa. We observed the significant increase the content H_2O_2 in 3-5 times higher than the control at used concentrations of herbicide above normal. After treatment of leaves 7-day-old seedlings was found that in 6-24 hours after treatment plants do not have a strong oxidative stress. After 48 hours only in seedlings of oats H_2O_2 content and lipid peroxidation increased 1.5-2 times with increasing of herbicide concentrations. Therefore, glyphosate treatment at concentrations recommended for use in the fields, does not cause significant accumulation of ROS and only high concentrations of herbicide to increased the oxidative stress.

P5.82

The role of 24-epibrassinolide induced antioxidant defence during germination under salinity in chickpea

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Reactive oxygen species (ROS) are key components of plant development. However,. researches on the positive effects of ROS production to seed germination are rather limited. ROS are produced due to physiological processes in cells and but they are highly reactive and can easily damage the proteins, nucleic acids and lipids. Beside the their harmful effects, they are also involving in signalling cascades to control growth and development, response to environmental stresses and programmed cell death. After the imbibition, respiration begins and cell redox state is changed which causes extremely high production of ROS. In this respect, how ROS production effects seed germination and ROS induced antioxidant system in this period were investigated in chickpea. Seed germination efficiency effected by induced ROS content was also enlightened. Activities of antioxidant defence enzymes were also reported during seed germination. 24-epibrassinolide which take roles in seed germination applied to seeds with stress factor (200 mM NaCl) to understand the differences in antioxidant defence system. The role of antioxidant system during seed germination by induced ROS production was also established.

P5.83

The profiling of osmotic adjustment and water status in *Apera intermedia* exposed to salinity

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The effects of different NaCl concentrations (0, 150, 300 and 600 mM) on growth, relative growth rate (RGR), relative water content (RWC), osmotic potential (Ψ_{Π}), PSII photochemistry [maximal efficiency of PSII photochemistry (F_v/F_m), the actual efficiency of PSII, the coefficients of photochemical quenching and non-photochemical quenching], osmo-protectant [proline (Pro), choline (Cho) and glycine betaine (GB)] and thiobarbituric acid reactive substances (TBARS) evaluated in *Apera intermedia* for 7 and 14 days. After exposure to 300 mM, the significant reduction in RWC began after the first day of stress. RGR and Ψ_{Π} decreased at 300-600 mM. After 150 and 300 mM during the experimental period, stress did not change in F_v/F_m and qP but, the increase in NPQ began after 300 mM. Salinity caused an increase in Pro, GB and Cho as from the first day of stress. 150 mM had not perceived in toxic levels because of (i) maintaining internal water balance, (ii) better osmotic adjustment to salt stress through higher concentrations of osmo-protectant, (iii) have lower Ψ_{Π} allowing them to absorb water from the soil (iv) more protection in photosynthetic apparatus (v) the lower accumulation of TBARS.

P5.84

The effect of spermine on regulation of antioxidant defense system under salinity in *Arabidopsis thaliana*

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Salinity is one of the major abiotic stress factor that decreases plant growth and development. Osmotic and ionic effects of salt stress induce the production of reactive oxygen species (ROS) in plants due to loss of redox homeostasis. Previously, spermine (Spm) was shown to be vital for salinity tolerance in *Arabidopsis*. However, still physiological and biochemical properties and mechanisms of this polyamine is unknown. In this study, the effect of Spm on antioxidant defense under salt stress was investigated in Spm deficient (*spms*) and *acl5/spms* double knockout *Arabidopsis* mutants, *Arabidopsis* over-producing Spm (*OX1*) and wild type (Col.). Hydroponically grown 12d old *Arabidopsis* genotypes were subjected to 50 mM and 100 mM NaCl for 4d. After the salt stress, hydrogen peroxide (H_2O_2) content, lipid peroxidation, activities of key antioxidant enzymes such as peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD) were determined. In addition to these, NADPH oxidase activity was also determined for elucidating the effect of Spm on ROS signalling.

P5.85

Elevated CO_2 mitigates the impact of a combination of heat wave and drought in *Arabidopsis thaliana*

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Recent studies have shown that elevated CO_2 (eCO_2) mitigates the stress impact, but the underlying mechanisms are unclear. To get insight into the stress mitigating CO_2-effect mechanisms, we investigated growth, physiological, biochemical and genome-wide transcriptional responses of *Arabidopsis thaliana* to a combination of heat wave and drought stress in ambient (380 μmol CO_2 mol^{-1}) and eCO2 (730 μmol CO_2 mol^{-1}). Stress combination reduced growth, photosynthesis and F_v/F_m, whereas eCO_2 tended to mitigate these detrimental effects. Microarray analysis revealed that photosynthesis genes were most adversely affected by stress (all genes were down-regulated) in both the CO_2 levels. However, under eCO_2 less down-regulation of gene expression was observed. We expected this CO_2-protective effect to be related with up-regulated antioxidant defense metabolism, but no clear patterns were found in ASC and GSH, or in the activities and expression levels of antioxidant enzymes. In contrast, under eCO_2 less H_2O_2 was produced due to the less photorespiration (Gly/Ser). We therefore conclude that eCO_2 mitigates the stress impact, possibly by altering the antioxidant defense mechanism, but surely by reducing H_2O_2 production.