Lectures

L10.1

Plant immunology: a sleigh ride through the SNO

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Changes in redox status are a conspicuous feature of immune responses in a variety of eukaryotes, but the associated signalling mechanisms are not well understood. In plants, attempted microbial infection triggers the rapid synthesis of nitric oxide (NO) and a parallel accumulation of reactive oxygen intermediates (ROIs), the latter of which is generated by NADPH oxidases related to those responsible for the pathogen-activated respiratory burst in phagocytes. Both NO and ROIs have been implicated in immune signalling and the control of the hypersensitive response (HR), a programmed execution of plant cells at sites of attempted infection. Our findings suggest that S-nitrosylation, the addition of an NO moiety to a protein cysteine thiol to form an S-nitrosothiol, is a key regulator of the plant defence response, controlling ROI synthesis, the accumulation of the immune activator, salicylic acid (SA) and cognate SA signalling. We are employing a variety of complementary approaches, including: forward and reverse genetics, Solexa-based gene expression profiling and novel proteomics strategies, to uncover the molecular landscape of S-nitrosylation during plant immune function.

L10.2

Cryptogein signalling in tobacco: in search for nitric oxide targets

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Nitric oxide (NO) triggers various physiological responses in plants. Notably, NO is recognized to account for the response to biotic stresses. We previously reported that NO is produced in tobacco cells exposed to cryptogein, a 10 kDa elicitor secreted by the oomycete *Phytophthora cryptogea*. To decipher the role of NO, we identified and characterized S-nitrosylated proteins in tobacco cell suspensions elicited by cryptogein. Several candidates were identified including the chaperone-like AAA+ATPase CDC48 and a calmodulin isoform (CaM). Interestingly, the Cys residue undergoing S-nitrosylation in CaM is located in the first Ca^{2+} binding EF hand and is not or poorly conserved in other organisms. The possibility that NO regulates CaM at a post-translational level further confirms our previous finding that NO and Ca^{2+} works together in plant immune signalling. To complete our investigation, we undertook a microarray analysis in order to identify cryptogein-modulated genes regulated through a NO-dependent process. Part of the genes of interest were also reactive oxygen species (ROS)-dependent, highlighting the occurrence of a cross-talk between NO and ROS. The mechanisms underlying this cross-talk will be discussed.

Oral presentations

O10.1

New insights into functional roles of nitric oxide in roots

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Nitric oxide (NO) is an important signal molecule in plants. Nitrite, is produced by cytosolic nitrate reductase (NR) and then converted to NO by the same enzyme. The mitochondrial electron transport chain is another site for nitrite to NO reduction. Using plant mitochondria and roots, we show that nitrite reduction to NO increases as oxygen availability decreases. The reaction is linked to proton pumping and therefore to ATP synthesis. Some of the NO diffuses from the mitochondria to the cytosol where it can be converted to nitrate by the hypoxically-induced hemoglobin. We present experimental data indicating the importance of all these reactions and showing that under low oxygen, the plant mitochondrion serves as a nitrite: NO reductase and becomes a major component in anoxic nitrogen cycling where it directly contributes to a decrease of cell reduction level and to limited ATP synthesis. We also investigated the role of NO in the induction of alternative oxidase (AOX). We found that NO inhibits aconitase in mitochondria and increases citrate which then acts as a potent inducer of AOX expression. Citrate increase leads to a shift of plant metabolism towards amino acid biosynthesis via 2-oxoglutarate.

O10.2

Cold-evoked NO controls cold-responsive gene expression and modulates protein S-nitrosylation status in *Arabidopsis*

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We previously reported that the exposure of *Arabidopsis thaliana* to low temperature triggers a rapid production of NO required for the proper regulation of cold-responsive gene markers. To identify the network of genes targeted by NO during cold response, we carried out a global transcriptomic analysis of *Arabidopsis* leaves impaired for NO formation by cPTIO infiltration. Out of the 3665 genes modulated after 1 and/or 6 h cold treatment, 1270 were deregulated in cPTIO-infiltrated leaves. Analysis of *cis*-element motif distribution in the promoter region of NO target genes identified a range of overrepresented elements including W-box and DREB-related motifs. Genes belonging to lipid metabolism and microRNA functional classes were over represented, as well as genes involved in DNA modification. To get further insight into NO-based mechanisms operating in plant response to cold, we performed a screen for modulations of protein S-nitrosylation status using a iCAT-coupled biotin switch method. It allowed us to identify 11 proteins that underwent modifications of their S-nitrosylation level under cold stress. A possible link between one of the S-nitrosylated targets and cold-responsive gene expression will be discussed.

Posters

P10.1

Nitric oxide trascriptional players during seed dormancy and germination

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Seed dormancy and germination are complex traits regulated by the interaction of phytohormone abscisic acid (ABA) and plant growth regulators as nitric oxide (NO). The molecular basis of the ABA and NO crosstalk are currently unknown. The identification of the elements that participate in this response is essential to understand the NO perception and signalling in plants. We have characterized several mutants encoding transcriptional factors (TFs) showing ABA- and cPTIO- (2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) insensitive phenotypes in the transition from dormancy to germination. These TFs expression was localized in seeds and in the meristem zone of emerging roots during seed germination, and was highly induced after ABA treatment in all the emerging organs. Microarray expression analysis revealed several hierarchical clusters with different function in redox status during germination, highlighting a putative role of this TFs in the ABA and NO crosstalk. Insights in the posttranslational redox modifications of these TFs will be presented together with their physiological relevance. Acknowledgements: EcoSeed-311840; MICINN (BIO2011-26940); MEC, CONSOLIDER Programa 28317. (CSD2007-00057).

P10.2

NO modifies ABA- and GA-related gene expression in germinating apple (*Malus domestica* Borkh.) embryos

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NO pre-treatment eliminates symptoms of delayed germination and morphological abnormalities, typical for dormant apple embryos. Seed dormancy is maintained by ABA, while germination is controlled by GA. NO-induced dormancy removal correlates with low ABA concentration in embryonic axes and reduced embryo sensitivity to ABA. We analyzed the expression of genes encoding key enzymes of ABA degradation (*CYP707A*) and elements of ABA transduction pathway (*PYL*, *RCAR*, *ABI*, *PP2C*, *AREB*, *ABF*). A role of GAs in regulation of germination led us to investigate expression of genes encoding enzymes of GAs biosynthesis (*GA20ox*, *GA3ox*), degradation (*GA2ox*) and transduction pathway (*GID1*, *GID2*, *DELLA*). The expression profiles of genes mentioned above was done in embryonic axes isolated from dormant or NO pre-treated embryos. The analyzed genes were differentially regulated during dormancy alleviation and "sensu stricto" germination, the main modifications in transcription level were detected for *CYP707A*, *PP2C*, *ABI2*, *ABF*, *DELLA* and *GID1*. It seems that regulatory role of NO in removal of seed dormancy is associated with stimulation of ABA degradation and the increase in GA synthesis as well as induction of this hormone signalling pathway.

P10.3

Localization of nitric oxide (NO) in effective and partially effective *Medicago truncatula* root nodules

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Root nodules are plant organs responsible for N_2 fixation. They are colonized by bacteroids which are symbiotic forms of free living bacteria. *Medicago truncatula* plants were inoculated with *Sinorhizobium medicae* WSM 419 strain or *Sinorhizobium meliloti* 1021 strain respectively, to produce fully effective or partially effective nodules. The reason for diminished nitrogen fixation effectiveness for the second strain was not established. NO plays a role in earlier steps of symbiosis interaction especially in nodule development. NO was detected on fresh nodule sections after its reaction with DAF-DA, by confocal microscopy. The differences between two types of nodules were established by diverse NO localization. Effective nodules displayed stronger fluorescence in infection threads and infected cells in comparison to partially effective ones. Obtained results suggest that NO may be crucial for bacteria release and establishment of effective symbiose.

P10.4

Differential transcriptomic analysis by RNA-seq of GSNO-responsive genes between *Arabidopsis* roots and leaves

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S-nitrosoglutathione (GSNO), a low molecular-weight nitrosothiol generated by the reaction of nitric oxide (NO) with reduced glutathione (GSH), has been detected in different plant organs under physiological and stress conditions (Corpas F.J. et al. Front Plant Sci. 2013; 4: 126). GSNO modulates gene expression and it also can regulate protein function by S-transnitrosylation mechanism (Astier J. et al. Plant Sci. 2011; 181: 527-533; Begara-Morales et al. BMC Plant Biol 2013; 13: 61). Using *Arabidopsis* plants exposed to exogenous GSNO via roots, Illumina-RNA seq technology allowed us to identify a total of 3,263 genes modulated by GSNO. Most of these genes were associated with the protection mechanism against stress situations, identifying new genes that were not previously described as GSNO-targets. Furthermore, 1,945 genes expressed differently between roots and leaves and 114 genes with organ-specific response were identified. Taking all together, RNA-seq extends our knowledge of GSNO-signalling mechanism between different organs in plants under non-stress conditions. Supported by ERDF-confinanced grant BIO2012-33904, Ministry of Science and Innovation, Spain.

P10.5

Dual regulation of cytosolic ascorbate peroxide (APX) by tyrosine nitration and S-nitrosylation

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Post-translational modifications (PTMs) mediated by nitric oxide (NO)-derived molecules can modulate the function of target proteins (Corpas F.J. et al. Plant Sci. 2011; 181: 604-611). Proteomic data have shown that ascorbate peroxidase (APX) is a target of these PTMs (Lozano-Juste J. et al. J. Exp. Bot. 2011; 62: 3501-3517; Fares A. et al. Biochem. Biophys. Res. Commun. 2011; 416, 331-336). Using recombinant pea cytosolic APX, we have analyzed the impact of tyrosine nitration and S-nitrosylation processes, identifying the residues involved in these NO-related PTMs. Results showed that nitration by peroxynitrite inhibits and S-nitrosylation by GSNO enhances APX activity. Moreover, pea plants grown under salinity stress showed an enhancement of the APX activity as well as an increase of H_2O_2, NO and S-nitrosothiols (SNOs) content that can justify the induction of the APX activity. Our results provide a new insight into the molecular mechanism of the regulation of APX which can be both inactivated by irreversible nitration and activated by reversible S-nitrosylation. Supported by ERDF-confinanced grant BIO2012-33904, Ministry of Science and Innovation, Spain.

P10.6

De-etiolation of cucumber cotyledons under different light sources: effect of nitric oxide

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The effect of two NO donors on photosynthetic pigment content and modulated chlorophyll fluorescence were studied in *Cucumis sativus* cotyledons. Measurements were carried out 11 days after sown in agar pots at 22 °C in a dark chamber. Pairs of cotyledons excised with hypocotyl hooks were incubated for 72 h in Petri dishes containing NO donors and/or NO scavenger under fluorescent continuous white (WL) or red light (RL). After different incubation periods, the cotyledons were assayed for fresh weight, area, chlorophylls and carotenoids content and chlorophyll fluorescence parameters. The incubation media was analyzed for nitrates. Cotyledon weight and area was significantly changed both by NO donors and light quality. NO donors enhanced the size and weight of hooked cotyledons. Excised cotyledons incubated under monochromatic RL, as compared with WL, showed a reduction in growth. The F_V/F_M ratio reflecting the optimal quantum yield in dark-adapted samples and the F_V/F_M, the effective quantum yield in illuminated samples, were slightly affected by NO donors. However, cotyledons incubated with the NO scavenger (cPTIO) showed an important decrease in both fluorescence parameters after 24 h continuous WL.

P10.7

ROS and RNS are required for polyamines-dependent germination of apple (*Malus domestica* Borkh.) embryos

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Polyamines (PAs) in complex with phytohormones affect seed dormancy and germination. Apple (*Malus domestica* Borkh.) seeds are characterized by deep dormancy, which may be removed after nitric oxide (NO) treatment. Similar reaction occurres after embryos imbibition in Put, Spd and their precursor Arg. In plants beside reductive NO_2- dependent NO biosynthetic pathway, NO may be formed from Arg in NOS-like, or PAs-mediated reaction. PAs catabolism by polyamine oxidase (PAO) leads to H_2O_2 formation. ROS and RNS are required for seed transition from dormancy status to germination. The aim of our work was to investigate the effects of PAs and Arg on *sensu stricto* germination of apple embryos by determination of ROS and RNS level. Stimulation of embryos germination by PAs and NO was associated with overproduction ROS. At the stage of *sensu stricto* germination effect of Put, Spd and Arg was accompanied by enhanced RNS emission. Positive action of PAs and Arg was removed by NO scavenging. Activity of PAO in embryo axes was enhanced by embryo fumigation with NO. Our data demonstrate interplay of RNS/ROS with PAs and point on NO action as integrator of signals activating germination. Work financed by grant NN303821840.

P10.8

S-nitrosylation promotes functional and structural changes in the mitochondrial psPrxII F protein

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Peroxiredoxins (Prxs) have emerged as important factors that link ROS metabolism to redox-dependent signalling events. Together with ROS, nitric oxide is an essential part of the signal transduction through post-translational modifications proposed to regulate protein function as S-nitrosylation and nitration. We have recently identified mitochondrial PrxII F as target of S-nitrosylation under salt stress, but no information is available about the regulation of the function of this protein by NO. In this work, we have studied the S-nitrosylation of recombinant PrxII F *in vitro* induced by GSNO and SNP, observing a switch from the peroxidatic to a chaperone activity. The S-nitrosylation provoked a conformational change in the PrxII F protein as deduced from the intrinsic Trp fluorescence spectral and changes in the fluorescence spectra of bis-ANS. Results showed by the first time, the acquisition of chaperone activity by PrxII F through its modification by NO. This regulation could define the function of the protein and NO would also exhibit its signalling action through the cross-talk with H_2O_2. (Supported by MICINN (BFU2011-28716) and Seneca Foundation (04553/GERM/06), Spain).

P10.9

Identification and characterization of S-nitrosylated nuclear proteins in *Arabidopsis thaliana*

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Nitric oxide (NO) is an important signalling molecule, which regulates many different physiological processes in plants by modifying protein/enzyme functions or gene expression. The most important regulatory mechanism of NO is S-nitrosylation, is a selective and reversible protein post-translational modification that plays an important role in plant defense response. We isolated defense-related nuclear proteins, which are targets for protein S-nitrosylation. *Arabidopsis* cell cultures were infected with avirulent *Pseudomonas syringae* for 2 and 13 hours. Afterwards nuclear proteins were isolated, subjected directly to the biotin switch assay (± S-nitrosoglutathione). After affinity chromatography on a Neutravidin-matrix purified proteins were identified by mass spectrometry. In this way we generated a map of proteins which are potential candidates for S-nitrosylation. After 2 hours of treatment with *P. syringae* 71 S-nitrosylated nuclear proteins could be detected, whereas after 13 hours of infection only 10 S-nitrosylated nuclear proteins could be identified. These results will provide new knowledge to understand the regulatory function of S-nitrosylation on nuclear proteins especially during plant defense response. This research was supported by a Marie Curie Intra-European Fellowship within the 7th European Community Framework Programme (FP7-PEOPLE-2011-IEF).

P10.10

Redox-regulation of S-nitrosoglutathione reductase

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In recent years nitric oxide (NO) has emerged as a signalling molecule in plants being involved in diverse physiological processes like germination, root growth, stomata closing and response to biotic and abiotic stress. S-nitrosoglutathione (GSNO) seems to have a very important function in NO signalling since it can transfer its NO moiety to other proteins (trans-nitrosylation). Such trans-nitrosylation reactions are equilibrium reactions and depend on GSNO level. The breakdown of GSNO and thus the decrease in S-nitrosylated protein is regulated by GSNO-reductase (GSNOR). In this way, this enzyme controls the level of S-nitrosylated proteins and seems to play a balancing role in fine-tuning NO signalling. Interestingly, GSNOR activity is reversibly inhibited by H_2O_2 implying a direct cross-talk between ROS- and RNS-signalling. We demonstrated that inhibition of GSNOR results in enhanced NO/SNO followed by enhanced glutathione levels. Moreover, the activities of glutathione-dependent enzymes were increased. Especially the NO-induced glutathione biosynthesis seems to be a general redox signalling mechanism which is also part of the NPR1-dependent defense pathway.

P10.11

The role of alternative oxidase in hypoxic metabolism of nitric oxide

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Alternative oxidase (AOX), contrary to cytochrome c oxidase (COX), is not inhibited by nitric oxide (NO), therefore it can maintain respiration in its presence. NO is intensively accumulated under hypoxia when AOX operation is expected to be limited because of its low affinity to oxygen as compared to COX. We used *A. thaliana* lines with low NO production and *N. tabacum* lines with modified AOX expression to determine possible operation of AOX under low oxygen. The hypoxic rates of NO production in tobacco were significantly higher both in AOX knockdown and overexpressing lines as compared to the wild type. This will be discussed in relation to the contribution of AOX to either the avoidance or generation of NO, depending upon O_2 concentration. In *Arabidopsis*, NO produced in roots under hypoxia inhibits aconitase which in turn leads to a marked increase in citrate levels. The accumulating citrate triggers the AOX1A gene expression and also shifts metabolism towards formation of amino acids. The hypoxic induction of NO metabolism along with AOX expression has likely impact on nitrosylation, cell signalling and ROS scavenging. It is concluded that AOX is actively involved in NO metabolism under low oxygen conditions.

P10.12

A screening strategy to identify candidate genes involved in NO signalling during hypersensitive response cell death

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The hypersensitive response (HR) is a plant defense mechanism against pathogens characterized by the formation of necrotic lesions at the attempted sites of infection, aiming to restrict pathogen growth and spread. The reactive nitrogen specie nitric oxide (NO) is known to play a key role in this defense mechanism, working synergistically with hydrogen peroxide to trigger the activation of the cell death program. However, the molecular mechanism through which NO acts is still unclear. To gain further insights into NO signalling network underlying the activation of HR-cell death, we established an NO fumigation system and identified conditions of NO treatment activating a cell death program in four weeks old plants of *Arabidopsis thaliana*. By using this facility we screened 25,600 M_2 mutagenized *Arabidopsis* plants and identified more than 80 mutants impaired in NO response. Fifteen lines presenting a consistent impaired NO response phenotype and higher levels of insensitivity to NO were selected and checked for alteration in HR-cell death. This way, nine mutant lines impaired in HR-cell death have been identified and the putative alteration in NO signalling during HR in these lines is currently being further enquired.

P10.13

Cytokinin overproducing *ipt-161 Arabidopsis* shows altered NO generation and insensitivity to selenite

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Selenite causes developmental alterations in plants through e.g. modulation of endogenous hormonal status and signalling. Both cytokinins (CKs) and nitric oxide (NO) play roles in many aspects of plant development. In this study, four-day-old wild-type (WT) and *ipt-161 Arabidopsis* possessing increased zeatin content were used. Compared to the WT, untreated *ipt-161* plants had significantly smaller cotyledons and root system. The cotyledon area and root length did not show reduction in selenite-exposed *ipt-161*. In case of selenite exposure, cell viability decreased in WT organs, but it did not change in *ipt-161*. Under control conditions, CK overproducer mutants possessed significantly reduced NO content in their cotyledons than the WT. Lower selenite concentrations resulted in NO accumulation in WT cotyledons, while in case of *ipt-161* only more serious selenite excess caused NO production. Based on these, CK overproduction can result in selenite-insensitivity and altered NO generation. This work was supported by the European Union and Hungary in the frame of "National Excellence Program" (TÁMOP 4.2.4.A/2-11-1-2012-0001) and the Hungarian Scientific Research Fund (OTKA PD100504).

P10.14

Function of S-nitrosoglutathione in *NPR1*-dependent defence response

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Nitric oxide (NO) is a reactive free radical with pleiotropic functions that participates in diverse biological processes in plants, such as germination, root development, stomatal closing, flowering, abiotic stress and defence responses. To get a deeper insight into the regulatory function of NO in plant defence signalling, we characterize the effect of S-nitrosoglutathione (GSNO), as an NO donor, on the redox sensitive *NPR1* (nonexpressor of *PR* genes 1) protein. We have shown that GSNO induced the nuclear localization of *NPR1*, as well as the induction of pathogenesis related genes after 20 hours treatment. Interestingly, NO fumigation of WT plants resulted in increased level of glutathione (GSH), which is a central component involved in regulating redox balance in plant cells. Studies on nuclear translocation of GFP-labelled *NPR1* in GSH-deficient *Arabidopsis* mutant showed that GSNO-induced nuclear accumulation of *NPR1* is GSH dependent. Surprisingly, all these redox reactions seem to be upstream of salicylic acid. In sum, these data support a cross talk between GSNO/NO and GSH which is crucial component of the *NPR1*-dependent defence pathway.

P10.15

Molecular studies on plant S-nitrosoglutathione reductases

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S-nitrosoglutathione reductase (GSNOR) plays a crucial role in the homeostasis of reactive nitrogen species, S-nitrosoglutathione (GSNO) and protein S-nitrosylation. We investigated GSNOR from *Brassica*, *Lactuca* and *Solanum* spp. Corresponding genes (*BoGSNOR*, *LsGSNOR*, *SlGSNOR*) were sequenced, cloned and expressed in *E. coli* cells. Recombinant enzymes were purified and their identity verified by MALDI-TOF. GSNOR, a zinc-containing cytosolic enzyme, occurs in native form as a dimer and exhibits high thermal stability. GSNOR gene showed higher expression in roots and stems compared to leaves of young plants. GSNOR preferentially catalyses the reduction of GSNO, while it can oxidize S-hydroxymethylglutathione, alcohols and omega-hydroxyfatty acids. In comparison to human GSNOR, plant enzymes exhibit different composition of the anion-binding pocket, which negatively influences the affinity for hydroxyfatty acids. N6022, potent pyrrole-based inhibitor of human GSNOR, inhibits plant GSNOR at concentrations of 10^{-7} M and thus represents an attractive tool for plant *in vivo* studies. Supported by Czech Grant Agency (P501/12/0590), Ministry of Education of Czech Republic (LH11013) and Palacký University in Olomouc (PrF_2013_037).

P10.16

Redox and nitric oxide (NO) homeostasis differentially regulated in tomato roots and leaves under salinity stress

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Glutathione (reduced, oxidized and S-nitrosylated forms) and nicotinamide adenine dinucleotide phosphate (reduced and oxidized forms) are important molecules in the redox homeostasis of plant cells. Using tomato (*Solanum lycopersicum*) plant grown in the presence of 120 mM NaCl, we studied the redox state (NADPH and GSH), ascorbate, nitric oxide (NO) and S-nitrosoglutathione (GSNO) content as well the activity of the main enzymes involved in the metabolism of these molecules in both roots and leaves. Salinity causes a differential readjustment of NADPH/$NADP^+$, GSH/GSSG and GSH/GSNO ratios and NO content in both organs. Similar differential behavior was observed in the enzymatic activities of the main NADPH-generating dehydrogenases, S-nitrosoglutathione reductase and glutathione peroxidase, in both organs under salinity conditions. However, catalase and gluthahione reductase activities as well as ascorbate content showed similar patterns in both organs. These data demonstrate that redox state and NO content could be excellent parameters to evaluate the physiological condition of the plants under adverse stress conditions.

P10.17

Plant peroxidases and nitrogen metabolism: interactions with nitrates and nitrites

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Accumulation of nitrogen (N_2) compounds and the products of their metabolism is highly toxic for plants and animals. Together with N_2-specific enzymes, oxidoreductases peroxidases can utilize N_2 compounds resulting in the formation of nitro-derivates of phenolic compounds. In present study we demonstrated that apoplastic peroxidases of wheat roots are involved in the metabolism of N_2 compounds by the effective reduction of NO_3 to NO_2 and also the co-oxidation of NO_2 with phenols. Using HPLC and NMR, the product of co-oxidation of p-coumaric acid and NO_2 by partially purified apoplastic peroxidase was identified as 4-hydroxy-3-nitrocinnamic acid. In the absence of peroxidases this product was not detected. Considering that the formation of nitrophenols is mediated by phenoxyl radicals, the generation of primary and secondary phenoxyl radicals by peroxidases was examined by EPR. During oxidation of chlorogenic acid by apoplastic peroxidase, primary phenoxyl radicals were gradually converted to secondary radicals, while following NO_2 addition the immediate formation of secondary phenoxyl radicals occurred. This study provides strong evidence that wheat peroxidases may play an important role in nitrogen metabolism.

P10.18

Localization of nitric oxide (NO) in Al-treated pea (*Pisum sativum*) root nodules

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The present study examined the localization of nitric oxide (NO) in pea root nodules growth under aluminium (Al) stress. Pea plants were inoculated with *Rhizobium leguminosarum* bv. *viciae* 248 to produce effective root nodules. 2-weeks old plants were exposed to 50μM $AlCl_3$, for 2 and 24h. NO plays a role in all steps of nodule development from initiation to senescence. Al induced changes in nodule apoplast and caused premature senescence of bacteroidal tissue. The histolocalization of NO in nodules using diaminofluorescein diacetate (DAF-2DA) and confocal microscopy revealed that Al-induced levels of NO. Al-treated nodules displayed stronger fluorescence in infected cells, enlarged infection threads and degenerated infected cells compared with control ones. Obtained results suggest that NO may be crucial for infection process and premature senescence of nodules under Al stress.

P10.19

Involvement of S-nitrosoglutathione reductase in *B. lactucae* and *O. neolycopersici* pathogenesis in *Lactuca* spp.

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Reactive nitrogen species (RNS) play an important role in the interactions of plants and fungal pathogens. Production of nitric oxide (NO) has been reported both in the host plants and the pathogens. Accumulation of NO was previously observed in infectious structures of the tomato powdery mildew *Oidium neolycopersici* and oomycete *Bremia lactucae*, whereas NO production in infected cells of resistant plants suggests its role in the initiation of hypersensitive reaction. The role of S-nitrosoglutathione reductase (GSNOR) in the plant defence was studied using two pathosystems: *B. lactucae* x *Lactuca* spp. (host plants) and *O. neolycopersici* x *Lactuca* spp. (non-host plants). Parameters of RNS metabolism were studied in control and infected lettuce leaves 0-6-24-48-72 and 168 hours post inoculation. Expression profile, enzyme activity and protein levels of GSNOR, tyrosine nitration and nitrosothiol level were analysed. Our results confirmed GSNOR involvement in the regulation of RNS levels after infection and suggest GSNOR plays important role in the plant-pathogen interaction. Supported by Czech Grant Agency (P501/12/0590), Ministry of Education of Czech Republic (LH11013) and Palacký University (PrF_2013_037).

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Changes of S-nitrosylation pattern in grey poplar (*Populus* x *canescens*) leaves after ozone treatment

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Protein S-nitrosylation, the covalent binding of nitric oxide (NO) to protein cysteines, is one of the main mechanisms of NO signalling. De-nitrosylation represents a less described aspect of NO signalling. Poplars are receiving enormous attention due to the increasing demand for renewable bioenergy, and therefore became a scientific model to analyse stress responses. We identified endogenously S-nitrosylated proteins in poplar and quantified the change in S-nitrosylation/de-nitrosylation after exposure to acute ozone. Using the biotin-switch assay in conjunction with LC-MS/MS 188 proteins were found to be S-nitrosylated including 109 new targets of S-nitrosylation. In 32 proteins, ozone fumigation caused changes in the S-nitrosylation pattern. We observed both: an increase and decrease in the S-nitrosylation level of proteins. S-nitrosylation increased in enzymes involved in cell redox status and protein folding (i.e. catalase, protein disulfide isomerase, HSP70). De-nitrosylation occurred among enzymes involved in polyphenol biosynthesis (i.e. phenylalanine ammonia-lyase, chalcone synthase). In sum, our quantitative analysis gives insight into *in vivo* S-nitrosylation/de-nitrosylation processes during oxidative stress.