Lectures

L9.1

Oxidative signalling in seed germination and dormancy

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Seed dormancy is a block to the completion of germination of an intact viable seed under apparently favorable conditions. It is a poorly understood phenomenon, influenced by the genetic background of the species, the environmental conditions and the balance of abscisic acid (ABA) and gibberellins. In addition, the role of reactive oxygen species (ROS) in the regulation of this process is currently emerging. We investigated the role of ROS in the regulation of seed germination during the early phases of imbibition using seeds from 3 different species (*Arabidopsis*, barley and sunflower). Increased generation of ROS seems to be a common feature of the so-called germination *sensu stricto* phase which is the critical step of the process since it involves the activation of a regulatory system controlled by intrinsic and extrinsic factors. By combining ROS measurement, *in vivo* ROS imaging, mutant phenotyping and identification of downstream ROS targets at the proteomic and transcriptomic levels, we show that ROS are likely to play a key role in the transduction of hormone signals and that there exists a crosstalk between hormones involved in seed dormancy (ethylene, ABA, gibberellins) and ROS.

L9.2

ROS production and redox control in response to abiotic stress in seeds and seedlings

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Factors that contribute to the control of the intracellular redox environment affect seed germination and early seedling development. A lag in germination of aged seeds may be linked to delayed recruitment of glutathione in the nucleus. Post-translational oxidative modification of proteins, and regulation of genes related to oxidative stress and programmed cell death also contribute to viability loss upon ageing. These intracellular events may be coupled to the extracellular environment, where many enzymes produce reactive oxygen species (ROS), whose abundance increases as seedlings develop, enabling ROS-dependent processes needed for growth and development, and in response to stress factors. This is particularly apparent for rapidly growing tissues of seedlings, which are arguably the most sensitive stage in the plant life cycle. Root meristems may be killed upon desiccation, and if parts of the tissues survive, enzymatically regulated, extracellular production of the superoxide anion radical occurs in the region of subsequent secondary root growth, reinforcing the importance of ROS related to signalling events associated with establishing new meristems.

Oral presentations

O9.1

HD-Zip class III transcription factors control root development through the modulation of ROS levels

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In the *Arabidopsis* root the distinct spatial distribution of two ROS species, hydrogen peroxide and superoxide, controls a dynamic balance between cell division and cell differentiation. Superoxide accumulates in the root meristem, while hydrogen peroxide accumulates in the elongation and differentiation zone. The balance between both species controls cell proliferation. Mutants defective in the developmental control regulators encoded by Class III homeodomain-leucine zipper (HD-Zip III) genes display alterations both in root growth rates and levels of ROS species. A gain-of-function mutant of the PHB HD-Zip III gene presents a short root length and a small meristem size, while the multiple loss of HD-Zip III genes functionality results in a bigger root meristem size. Additionally, gain- and loss-of-function mutants present contrasting ROS balances. Our analyses of transcriptome changes upon depleting HD-Zip III levels suggest that the HD-Zip III transcription factors act upstream of ROS-related genetic pathways controlling meristem size and growth rate. Thus, the HD-Zip III transcription factors may act to control root growth by modulation of ROS levels.

O9.2

Redox state modulation in rice and its implications for plant development and stress responses

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The reactive oxygen species (ROS) detoxification in plants is essential to protect plant cells against their toxic effect. The differences in subcellular localization and biochemical properties of antioxidant enzymes result in a versatile antioxidant system. To understand how different ROS-scavenging enzymes contribute to the maintenance of these system we have investigated the effect of the knockdown of the isoforms of two different classes of antioxidant enzymes in rice plants, ascorbate peroxidases (APX) and glutathione peroxidases (GPX). Recently, an additional group of sequences has been characterized and named ascorbate peroxidase-related (APx-R). The knockdown of different APXs and GPXs in rice resulted in different phenotypes. GPX knockdown produced altered shoot and root development, suggesting that silencing of OsGPX3 lead to a stress induced morphogenic response. The silencing of chloroplastic APXs produced plants with altered response to stress and delayed development. The knockdown of other members of the APX family resulted in delayed senescence. The implication of the endogenous production of H_2O_2 in different subcellular compartments to plant development and stress responses will be discussed.

Posters

P9.1

Characterization of nitrated proteins from pepper (*Capsicum annuum* L.) fruits

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Nitration is a post-translational modification promoted by RNS which alters the protein activity and has been associated to nitro-oxidative stress (Ischiropoulos H., Gow A. Toxicology 2005; 208: 299-303; Corpas et al. Front. Plant Sci. 2013; 4: 29). Additionally, nitrated proteins can participate in the modulation of cell signalling as a ROS intermediary in several processes (Ischiropoulos H., Gow A. Toxicology 2005; 208: 299-303). Pepper (*Capsicum annuum* L.) fruits are the second worldwide consumable vegetables and excellent sources of essential nutrients for humans. During pepper fruit ripening a number of metabolic changes occurs (Palma et al. J. Proteomics 2011; 74: 1230-1243; Mateos et al. Int. J. Mol. Sci. 2013; 14: 9556-9580), including a higher protein nitration. In this work, using peppers from California type, the characterization of nitrated proteins was investigated in green and red mature fruits by proteomic analysis combining 2-D electrophoresis and western blotting with an antibody against nitrotyrosine. A total of 12 nitrated proteins were identified and the effect of nitration in the enzyme activity of some of them was studied. Supported by the ERDF-cofinanced grant AGL2011-26044, Ministry of Economy and Competitiveness, Spain.

P9.2

Involvement of ROS and its scavenging enzymes in the response of dormant *Avena fatua* L. caryopses to karrikinolide (KAR1)

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Smoke derived from burning plant material, smoke saturated-water and their active components such as karrikins has been shown to promote the germination of seeds of plant species from many ecosystems, as well as agricultural and horticultural plants. *Avena fatua* L. (wild oat) is a persistent weed in many cereal growing regions of the world, including Poland. Caryopses from florets of *A. fatua* L. were primarily dormant and did not germinate at 5 and above 20°C. KAR1 applied at 15 and 20°C caused complete caryopses germination. Stimulatory effect of KAR1 at 20°C was associated with increasing amylases and dehydrogenases activities before visible germination occurred. Treatment with methylviologen or menadione and hydrogen peroxide markedly stimulated germination of dormant caryopses. Histochemical *in situ* localization of ROS shows its accumulation in scutellum and close to radicle tip after imbibition in the presence of KAR1. DPI decreased the stimulatory effect of KAR1. It was found that KAR1 increased level of ROS and activities of SOD, CAT and APX during imbibition of dormant caryopses. This work was supported by the Ministry of Science and Higher Education Grant NN 310 726340.

P9.3

Involvement of *Arabidopsis* p23 in hormone regulation through regulation of reactive nitrogen species

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Homologues of the p23 co-chaperone of HSP90 have been found in all eukaryotes, suggesting conserved functions for this protein throughout evolution. While p23 has been well studied in animals, little is known about its function in plants. *Arabidopsis* owns two isoforms of p23 and their expression pattern was analysed *in planta*. The expression profile of the two isoforms was characterized and redundant functions in the analysed pathway identified. In order to determine the function of the two p23 genes, knockout insertional mutant lines and overexpressing transgenic lines for both genes were selected. The analysis of knockout mutants and overexpressing lines showed these proteins as involved in nitric oxide production both in physiological and in stress-induced conditions. All these lines showed alterations in root growth parameters. The results allow us to suggest an involvement of p23 in hormone regulation through regulation of reactive nitrogen species.

P9.4

Antioxidant response in natural senescence of *Sempervivum tectorum* L. leaves

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Sempervivum tectorum L. (*Crassulaceae*) is a perennial herb that occurs in the arid and semiarid habitats, and is an important medicinal plant. To examine the relationships between oxidative stress and aging/senescence we studied changes in the activity of several antioxidant enzymes, and the content of phenolics and pigments in leaves of different age. Leaf exudates was analyzed for sugar, phenolics and ascorbate contents and correlated to intracellular levels. Dry weight of leaves, soluble protein and phenolics content decreased progressively with ageing, while chlorophyll, carotenoids and ascorbic acid (ASA) content increased in 1st and 2nd leaf level and decreased in subsequent older leaves. Superoxide dismutase, Class III peroxidase and catalase activities, however, were the highest in the third leaf level and decreased with senescence. On contrary, the antioxidative enzymes activities revealed that naturally senescing leaves (3rd leaf level) decreased. The results presented above suggested that aging process begun in the third leaf level indicated by progressive decrease in the protein, pigment, phenolics and ASA contents.

P9.5

Redox changes affect growth and gene expression in maize

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The aim of the present experiments was the pharmacological modification of redox environment, and the study of its effect on growth and gene expression in maize. Thiol-dependent changes in redox environment were monitored. Total glutathione levels were increased in roots and decreased in the shoots and the redox potential of the glutathione/glutathione disulphide (GSH/GSSG) was not affected in the roots and increased in the shoots after the most treatments. The concentration and redox potential of the GSH precursors were also changed by the applied compounds. The fresh weight of the roots and shoots was reduced by all used reductants, but not by H_2O_2. The development of lateral roots was also inhibited except for H_2O_2. The expression of several redox-responsive genes was measured after the treatments and the transcript level of thioredoxin5 was decreased by the reductants. The present experimental system was appropriate for the modification of the redox environments in shoots and roots which resulted in reduced growth and alterations in the gene expression. This work was supported by the EU, the National Development Agency and the Hungarian Scientific Research Fund (TÁMOP-4.2.2/B-10/1-2010-0025 and OTKA K83642).

P9.6

Sensitivity and response to hydrogen peroxide of nuclei of sunflower seeds

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Reactive oxygen species (ROS) have been shown to be toxic but also function as signalling molecules in a process called redox-signalling. Cellular signalling transduction pathways enable organisms to receive signals and respond in specific manner. In seeds, ROS are produced at different developmental stages including dormancy release and germination. Recent data suggests that the nucleus *via* its effectors and transducers can also generate its own signalling of ROS. This data led us to focus on the determination if ROS formation can be generated directly by the nuclei in dormant and non-dormant seeds of the sunflower. Enriched nuclei fraction isolated from embryonic axes during imbibition was tested for the ability to produce ROS as a response to exogenously applied hydrogen peroxide. Dormant and non-dormant seeds differed in the amounts of ROS formed after 50 μM hydrogen peroxide treatment, though higher amounts were obtained in each variant of non-dormant seeds comparing to the dormant ones. The increase of ROS production was reversed by GSH and decreased or inhibited by diphenyleneiodonium – the inhibitor of flavoenzymes. We discuss the possibility of nuclear ROS signalling in dormant and non-dormant seeds.

P9.7

Redox control of gene expression during the vegetative/generative transition in wheat

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Winter cereals require vernalization for their vegetative/generative transition which is fulfilled by growth at low temperature. Redox changes were monitored by measurement of glutathione and H_2O_2 levels during this process. Both the size of glutathione pool and the redox potential of the glutathione/glutathione disulphide couple showed characteristic alterations at the individual phases of the vegetative/generative transition. Major regulators of the vernalization are the Vrn genes. Vrn1 and Vrn3 genes were induced and Vrn 2 was repressed during the transition, but the speed of these changes depended on level of the vernalization sensitivity of studied genotypes. The possible target genes of Vrn1 during vernalization were selected by transcriptome analysis using *vrn1* deletion wheat mutants. Genes encoding the CBF transcription factor, ferritin and a heat shock protein were found to be affected by Vrn1. A relationship was found between the changes in redox environment and in expression of several genes during the vegetative/generative transition in wheat. This work was supported by the EU, the National Development Agency and the Hungarian Scientific Research Fund (TÁMOP-4.2.2/B-10/1-2010-0025,OTKA K83642, CNK80781).

P9.8

Long term effects of salicylic acid and hydrogen peroxide on tuber sprouting associated with catalase activity

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Potato tuber dormancy and sprouting have a pivotal role in post-harvest physiology for farmers and industry. Previously Salicylic Acid (SA) and Hydrogen Peroxide (H_2O_2) induced tuberization and inhibited stolon growth. The effect of SA and H_2O_2 on tuber sprouting at 2 storage temperatures was studied. Experimental conditions were: 1) tubers from sprayed plants were stored at 8°C (S8) and 18°C (S18), 2) as in 1), but immersed in SA or H_2O_2 and stored at 8°C (SI8) and 18°C (SI18). In both conditions S8 and SI18, SA inhibited sprout elongation (SE) significantly whereas H_2O_2 1mM enhanced SE significantly. Significant higher number of sprouts was produced by SA in S8 and SI8 treatments. Spray-Immersed treatments induced higher percentage of tuber sprouting. Both molecules rose CAT activity in tubers at harvest without changes in H_2O_2 levels. CAT 2 was involved in tuber sprouting whereas CAT 1 was absent in the process. At 8°C, reduced CAT activity by H_2O_2 was related with higher SE. Higher CAT activity by SA was related with reduced SE rising sprouting percentage. SA and H_2O_2 induced long term effects on CAT and tuber sprouting. The potential application of both molecules in control of tuber sprouting is discussed.

P9.9

Seed priming improves salt stress tolerance during germination by modulation of antioxidative capacity

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Salinity is one of the major abiotic stresses that may cause inhibition or delay of seed germination. Under salt stress elevated level of ROS and activation of antioxidant systems are observed. Seed priming is one of the techniques adapted to improve tolerance to stresses. Primed seeds exhibit faster imbibition and revival of metabolism thus provide greater germination rate, also in salt stress condition. In this study we focused on activation of antioxidative metabolism due to seed priming. We observed higher level of H_2O_2 accumulation in primed seed during imbibition in water and 100 mM NaCl. However, higher levels of H_2O_2 were accompanied by decreased level of lipid peroxidation. Moreover, we stated enhanced activities of APX, CAT and SOD in primed seeds, which were correlated with increased expression rate of *APX*, *CAT* and *SOD* genes. APX activity increased in response to salt stress. CAT activity was at the same level in primed seeds germinated in both water and 100 mM NaCl, whereas in unprimed seeds was lower and decreased under salt stress. We conclude that there is a correlation between the activation of antioxidant metabolism in primed seeds and increased tolerance to salt stress during germination.

P9.10

Pollen tube NAD(P)H oxidases modify specific cell wall components to maintain cellular integrity during polarized cell growth

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Reactive oxygen species (ROS) produced by NAD(P)H oxidases regulate diverse biological processes in plants, including pathogen defense, abiotic stress response and plant development. Here we show that ROS produced by NAD(P)H oxidases are required for the maintenance of cellular integrity in *Arabidopsis thaliana* pollen tubes. Functional loss of the pollen expressed NAD(P)H oxidases RBOHH and RBOHJ in homozygous double-resulted in severely reduced fertility caused by male deficiency. Superoxide production was reduced, in double mutant pollen tubes. *In vitro* analysis of pollen tube growth showed that a large fraction of double mutant pollen tubes spontaneously burst. Subcellular localization experiments involving XFP-fusion proteins revealed a polar localization of both enzymes at the subapical shank of growing *Arabidopsis* pollen tubes. The polar localization of NAD(P)H oxidases at the subapical shank coincided with the site of the pollen tube collapse indicating that extracellular ROS modify specific cell wall components within this region. Using immunocytochemical staining, we show that intrinsic properties of cell wall arabinogalactan proteins (AGPs) are altered in *rbohh rbohj* mutant pollen tubes.

P9.11

Role of spruce respiratory burst oxidase homolog (RBOH) during lignin formation in Norway spruce

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H_2O_2 is required for extracellular lignin production in Norway spruce tissue culture as scavenging of H_2O_2 with KI repressed extracellular lignin formation. This suggests that peroxidases activate monolignols for lignin polymerisation. One possible source for apoplastic H_2O_2 are NADPH oxidases (respiratory burst oxidase homologs, RBOHs). RBOHs reduce O_2 to superoxide that dismutates to H_2O_2. We isolated a full-length sequence of PaRBOH1 from the extracellular lignin-producing tissue culture and from developing xylem of spruce. Ca^{2+} binding, phosphorylation and protein-protein interactions are important in regulation of RBOH activity. *In silico* analysis showed that PaRBOH1 has several putative phosphorylation sites in its N-terminal region. We used a heterologously expressed N-terminal peptide of PaRBOH1 as a substrate in *in vitro* kinase assays with spruce protein extracts as kinase sources. The results with [^{33}P]ATP indicate that the peptide was subjected to phosphorylation(s). Next we aim to resolve the phosphorylation number and sites in the peptide by mass spectrometry analysis. Also yeast-two-hybrid assays have been conducted to study.

P9.12

The influence of algaminoplant and route on rhizogenesis of stem cuttings of *Cotinus coggygria* "Royal Purple" and "Young Lady"

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The UE-imposed restrictions on the manufacture and use of plant protection chemicals impose on the nurseryman the need to screen for new substances that are environmentally friendly and yet effective in the production of plant material. Biopreparations may constitute such a group as they contain substances that have little environmental impact. In this study biopreparations AlgaminoPlant and Route were evaluated for their effects on rooting of stem cuttings in smoke tree (*Cotinus coggygria*) "Royal Purple" and "Young Lady". The experiment was carried out in 2011 in the commercial nursery in Wola Prażmowska. During rooting (8 weeks), cuttings were sprayed once, twice or three times with the water solutions of 0,2% AlgaminoPlant and 0,1% Route; after the third spraying leaves were sampled from the cuttings for determination of several organic compounds. Biostimulants positively affected rhizogenesis and increased content of chlorophyll, free amino acids and phenolic compounds in leaves of cuttings where they also stimulated the activities of polyphenol oxidase, peroxydase and catalase, and decreased the content of hydrogen peroxide. protein-protein interactions as a possible way to regulate the enzyme activity.

P9.13

Does senescence correlate with oxidative damage in tobacco leaves?

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Senescence is the final step of leaf development which precedes its death. Most typically, during this time the intensive remobilization of nutrients take place. It is supposed that accumulation of reactive oxygen species (ROS) and/or oxidative damage may trigger the senescence program. However, a precise determination of malonodialdehyde (MDA) level at different stages of tobacco leaf development did not reveal any increase in lipid peroxidation. Therefore, a goal of this study was to verify the involvement of ROS and oxidative damage at the onset of leaf senescence. The level of oxidative stress was analyzed in WT and transgenic tobacco (SAG12::IPT) plants with longer lifespan due to senescence-induced autoregulated synthesis of cytokinins. Senescence was confirmed by decline in chlorophyll and Rubisco contents, and by induction of senescence-associated genes: *CP1* and *CAT3*. Levels of MDA were not significantly different between wild type and SAG12::IPT leaves. The activity of superoxide dismutase (SOD, EC 1.15.1.) was higher in WT in comparison with SAG12::IPT, but only in the interveinal parts of leaves. We hypothesize that prevention against oxidative damage is indispensable part of the senescence program.

P9.14

The role of *Arabidopsis thaliana* aladin-related protein in the regulation of plant growth

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Cell proliferation in the meristem is the basis of plant growth. The tripeptide thiol glutathione (GSH) is indispensable for the maintenance of root meristem. Similarly, plants impaired in both GSH/thioredoxin are unable to maintain a floral meristem. GSH accumulates in the nucleus early in the cell cycle in plant and mammalian cells, severe GSH deficiency causing an arrest of the cell cycle at G_1. The WD-repeat protein ALADIN, which is a component of the nuclear pore complex (NPC), is encoded by the *AAAS* gene in mammalian cells. Defects in ALADIN promote adrenal disorders and lead to the triple A (Achalasia-Addisonianism-Alacrimia) syndrome in humans as well as infertility in female mice. The deficiency in ALADIN in proliferating human cells causes arrest of the cell cycle at G_1. We therefore investigated the role of the ALADIN-related protein in *Arabidopsis* by characterising the growth and development of an ALADIN-knockout line in order to test the hypothesis that this protein might be involved in the transport of GSH into the nucleus during the cell cycle. The ALADIN-knockout mutants show a retarded growth phenotype in shoots and roots compared to the *Arabidopsis* wild type.

P9.15

GrxS17, a multidomain glutaredoxin acting in meristems

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Glutaredoxins (Grx) are ubiquitous oxidoreductases. In plants, they form a large family (more than 40 members in *A. thaliana*) subdivided in 5 classes (Meyer Y. et al. Biochim. Biophys. Acta 2008; 1783: 589-600). In class II (4 members), the glutaredoxin S17 (GrxS17) is unique due to its multidomain structure. Previous studies have already shown the role of GrxS17 in temperature-dependent postembryonic growth (Cheng N.-H. et al. J. Biol. Chem. 2011; 286: 20398-20406) and presumably in iron-sulfur cluster biogenesis (Bandyopadhyay S. et al. EMBO 2008; 27(7): 1122-1133). Our study reveals a more complex role of GrxS17, with a function in apical meristem development under high light condition and in root meristem development in normal growth condition. In both cases, meristem size and cell division are affected. A complementation strategy with different truncated parts of GrxS17 protein has shown the role of the different domains and of the active site for protein functioning.

P9.16

Iron-superoxide dismutase from pepper: biochemical characterization, cell localization and tissue distribution

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Iron-superoxide dismutases (Fe-SODs) in plants are homodimeric enzymes with variable native molecular masses, mainly located in the stroma, thylakoids, peroxisomes and mitochondria (Palma et al., Physiol. Plant 1998; 104: 720-726). Although the involvement of Fe-SODs in plant senescence and certain stress conditions has been reported (Asensio et al., J. Plant Physiol. 2012; 169: 1253-1260), little information is still available on their physiological functions. Pepper (*Capsicum annuum* L.) is a crop species with high nutritional and economic relevance worldwide. Pepper plants contain two CuZn-SODs, one Mn-SOD and one Fe-SOD (Mateos et al., Int. J. Mol. Sci. 2013; 14: 9556-9580; Airaki et al., Plant Cell Environ. 2012; 35: 281-294). In this species, Fe-SOD shows distinct activity patterns in roots, shoots, leaves, flowers and fruits. By western blot, a monomer size of 23 kDa was found for Fe-SOD. In fruits, the proteomic analysis of isolated peroxisomes reported the presence of an Fe-SOD in these organelles. The involvement of Fe-SOD in fruit ripening and stress situations was also studied. Supported by ERDF-cofinanced grants AGL2011-26044 and AGL2008-00834, Ministry of Economy and Competitiveness, Spain.

P9.17

Oxidation of short-chain isoprenoids

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Short-chain isoprenoid alcohol molecules consist of several isoprene residues, with a hydroxyl group located at the α-end. Due to their molecular structure, i.e. numerous double bonds, they seem a perfect target for oxidation. Thus polyisoprenoids might serve as a shield against reactive oxygen species, generated either by UV light or by chemicals. Moreover ROS are formed continuously in mitochondria, chloroplasts and peroxisomes of plants. Therefore polyisoprenoids might concur with other components of a complex array of cellular components for the control of production and removal of oxygen species. In this study β-citronellol, geraniol, nerol were subjected to the thermo-oxidation; microwave assisted oxidation; oxidation with usage of sodium molibdate and hydrogen peroxide; moreover oxidation with porphyrin and oxygen access under halogen lamp irradiation was conducted. Various reaction conditions were tested (solvents, time, and temperature). Oxidation products were monitored by UV, TLC, GC-FID, GC-MS and RP-HPLC/UV techniques; additionally some of products were separated and analyzed by NMR and MS. Different types of oxidation reaction led to formation of wide range of products.

P9.18

Arabidopsis lipocalins AtCHL and AtTIL have overlapping functions essential for lipid protection and seed longevity

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Lipocalins are an ancient family of proteins which transport small hydrophobic molecules and also known to be implicated into protection against oxidative stress. They are widely distributed in animals but little is known about plant lipocalins. Considering their expression under environmental stress, it is important to survey the plant genome for stress-regulated lipocalin proteins. Plants possess two true lipocalins, CHL (chloroplastic lipocalin) and TIL (temperature induced lipocalins). Each lipocalin appeared to be specialized in the responses to specific stress conditions in *Arabidopsis thaliana*., AtCHL playing a protective role against photooxidative stress while AtTIL protects against heat stress. A double mutant AtCHL ko x AtTIL ko was more sensitive to high light stress than the single mutants, exhibiting intense lipid peroxidation. AtCHL deficiency enhanced the photosensivity of mutants (*vte1* and *npq1*) affected in lipid protection mechanisms (tochopherols, zeaxanthin), confirming the crucial role of lipocalins in the prevention of lipid oxidation. Seeds of AtCHL ko x AtTIL ko double mutant were very sensitive to natural and artificial aging, this phenomenon was associated with oxidation of polyunsaturated lipids.

P9.19

Reactive oxygen and nitrogen species as products of polyamines catabolism in dark-induced senesence of barley leaves

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Intracellular titers of polyamines must be strictly regulated in each biological process. One way is their downregulation by oxidative deamination. Hydrogen peroxide and nitric oxide are *inter alia* the molecules produced through polyamine catabolism. Our study was aimed to analyze whether polyamines catabolism is a factor initiating, regulating or control dark-induced leaf senescence. The reduction of polyamine titer, preceded by polyamines intensified synthesis, have been shown already at the beginning of the process. A feature of this was an increase in oxidases expression and activity. The reduction of polyamine level was correlated with H_2O_2 and NO production. When the leaves where incubated with polyamine oxidases inhibitors H_2O_2 and NO production was also inhibited. We can conclude that the activation of PA catabolism through the generation of H_2O_2 and NO or increases cellular oxidative stress, and induces the death of mesophyll cells or H_2O_2 and/or NO could exert signalling effects on the process. Results suggest that PAs catabolism is rather the process inducer than their regulator. This work was supported by NSC research grant no N N303 418236.

P9.20

MPK4 regulates photosynthesis and ROS homeostasis in *Populus tremula* x *tremuloides*

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Mitogen activated protein kinases (MAPK or MPK) are involved in the transduction of different signals in plant cells. Three independent transgenic lines of *P. tremula* x *tremuloides* with different decreased levels of *MPK4* expression were obtained. The line *mpk4-7*, with the most reduced expression of *MPK4*, displayed the strongest deregulation of reactive oxygen species (ROS) metabolism manifested by significantly increased content of foliar ROS (H_2O_2), higher activities of superoxide dismutase (SOD) and catalase (CAT) in comparison to wild type plants. Retarded leaf growth and lower transpiration rate correlated with changes of the fast phase of chlorophyll *a* fluorescence parameters (OJIP-test) from photosystem II (PSII) in all transgenic lines. Moreover, transgenic poplar line *mpk4-7* showed strongly reduced expression of several genes encoding an important components of PSII. The obtained results showed that MPK4 plays a role in optimization of photosynthetic electron transport efficiency, energy flux regulation in PSII and transpiration, leading to optimal growth responses in poplar. Our results showed strong MPK4 functional similarities between *Arabidopsis* and poplar trees.

P9.21

Transcriptome analysis of enzyme activities regulating ROS metabolism in olive (*Olea europaea* L.) reproductive tissues

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Transcriptome analysis of the olive stigma and pollen grain was carried out at different developmental stages and during in vitro pollen germination, respectively. Isolated mRNA was used to build cDNA libraries, which were subjected to 454+ sequencing. Readings were combined with sequences obtained from subtractive libraries, in order to produce a de novo assembly of the olive reproductive transcriptome. The presence of transcripts corresponding to major enzymes regulating ROS metabolism was assessed. Sequences corresponding to catalases, superoxide dismutase, dehydroascorbate reductase, GSNO reductase, NADPH oxidase, lipoxygenase and thioredoxins were retrieved and analysed using basic descriptive tools. Expression of transcripts in pollen and pistil was analysed by semiquantitative RT-PCR. Cellular localization of several enzymes was also carried out by using immunocytochemistry. We discuss how these enzyme activities may be involved in the olive pollen metabolism through germination, in the olive stigma over the periods before, after and during receptivity, and how they may contribute to the interaction pollen-stigma. Work supported by ERDF cofunded grants BFU2011-22779, AGL2011-24428, P2010-AGR6274, P2011-CVI7487.

P9.22

Enzyme activities regulating ROS metabolism in olive (*Olea europaea* L.) seeds

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Enzyme activities regulating ROS metabolism in the olive seeds are relatively unknown, in spite of the economical importance of this tree and the increasing interest of these seeds for multiple purposes. The aim of this work was to provide a comprehensive analysis of the genes encoding major antioxidant enzymes in mature seed. For this purpose, we have used transcriptomic sequence information available through our own experiments and in specific databases (Olea EST). Numerous sequences have been retrieved and analysed by using basic descriptive tools. Expression of transcripts encoding several of these enzymes has been carried out. These include catalases, superoxide dismutase, dehydroascorbate reductase, GSNO reductase, NADPH oxidase, lipoxygenase and thioredoxins. Cellular localization of several of these enzymes and histochemical detection of enzyme activities have been also carried out. We discuss how these enzyme activities may be involved in the olive seed metabolism through germination, and how they may contribute to the antioxidant capacity, stability and organoleptic properties of olive oils. This work was supported by ERDF cofunded grants BFU2011-22779, AGL2011-24428, P2010-AGR6274, P2011-CVI7487.