Session 9. Plant Cell Biology and Biochemistry

Lectures

L9.1

EXPO, exocyst recruitment, and unconventional protein secretion

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Exocyst is an evolutionary conserved multisubunit tethering factor composed of 8 proteins: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. It was originally described as a protein complex which captures and guides secretory vesicles to the plasma membrane (PM) prior to cognate SNARE-mediated membrane fusion. Exocyst homologs have also been found in plants, but in contrast to yeast and animals, the Arabidopsis genome contains 23 paralogs of Exo70 (AtExo70). It has been proposed that plant cells do not have a single exocyst complex and that different exocyst complexes participate in different physiological processes. Using AtExo70E2-GFP as a probe, we have recently identified a novel double-membrane organelle termed EXPO (exocyst-positive organelle) that mediates an unconventional protein secretion in plant cells. EXPO can also be recognized in normal cells by immunofluorescence and immunogold electron microscopy using Exo70E2 antibodies. EXPO are present as discrete structures in the cytoplasm but have also been visualized fusing to the PM. Structurally, EXPO is morphologically similar to the double-membrane autophagosome However, under normal conditions, immunofluorescent labelling with the EXPO marker anti-AtExo70E2 and the autophagosomal markers anti-ATG8e or anti-SH3P2 in transgenic *Arabidopsis* cell lines expressing either GFP-tagged EXPOs or autophagosomes shows that autophagosomes and EXPO are distinct organelles. Nevertheless, upon autophagy induction via starvation in Arabidopsis cells expressing GFP-tagged EXPO or autophagosomal markers, EXPO signals are found to colocalize with autophagic bodies in the vacuoles.
By performing transient expression in Arabidopsis protoplasts we have established that a number of exocyst subunits (especially the members of the Sec family) are unable to be recruited to EXPO in the absence of AtExo70E2. The paralog AtExo70A1 is unable to substitute for AtExo70E2 in this regard. FRET and BiFC analyses have confirmed the interaction between AtExo70E2 and Sec6 and Sec10. AtExo70E2, but not its yeast counterpart, is also capable of inducing EXPO formation in an animal cell line (HEK293A cells). Electron microscopy confirmed the presence of double membraned EXPO-like structures in HEK293A cells expressing AtExo70E2. Inversely, neither human nor yeast Exo70 homologs are able to cause the formation of EXPO in Arabidopsis protoplasts. These results point to a specific and crucial role for AtExo70E2 in EXPO formation.

L9.2

Ribosomal regulation of plant mitochondrial genome expression

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The ribosome filter hypothesis postulates that ribosomes are not simple non-selective translation machines but may also function as regulatory elements in protein synthesis. Recent data supporting ribosomal filtering come from plant mitochondria where it has been shown that translation of certain groups of mitochondrial transcripts can be differentially affected by alterations in mitoribosomes.
Silencing of a gene encoding a small-subunit protein of the mitochondrial ribosomes in *Arabidopsis thaliana* led to the generation of unique, heterogeneous population of mitoribosomes. As a consequence, the mitochondrial OXPHOS and ribosomal transcripts were both upregulated, but only the ribosomal proteins were oversynthesized, while the OXPHOS subunits were actually depleted. Most probably the mechanism of this regulation is connected with altered recycling phase of translation/termination of translation. It is tempting to speculate that the heterogeneity of plant mitoribosomes found *in vivo* could contribute to the functional selectivity of translation in response to environmental or developmental signals. Despite the importance of translation in plant mitochondrial gene expression, it seems that the coordination of expression of the mitochondrial and nuclear genomes occurs at the level of protein complex assembly, probably with an involvement of mitochondrial ATP-dependent proteases. The significance of FtsH proteases, one of ATP-dependent proteases, for complex I and V assembly/stability was documented using mutants lacking theses proteases.

L9.3

Regulation of photosynthetic antenna activity in plants at the level of a single pigment-protein complex

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Effective and fluent photosynthesis in plants is possible owing to the presence of the antenna network of pigment-protein complexes which absorb light and transfer electronic excitations towards the reaction centers. Light-harvesting pigment-protein complex of Photosystem II (LHCII) is the most abundant membrane protein in the biosphere and comprises half of chlorophyll molecules on Earth. LHCII serves as the major photosynthetic antenna complex and it covers a pronounced fraction of the thylakoid membrane surface. Under light stress (overexcitation) conditions light-harvesting activity of the complex can be associated with generation of reactive oxygen species destructive for the entire photosynthetic apparatus. Therefore, quenching of the excessive excitations in LHCII seems to be a vital regulatory process, operating to protect the photosynthetic apparatus against photo-degradation. During the talk the results of the research will be presented, carried out recently in our laboratory to uncover molecular mechanisms responsible for regulation of the photosynthetic antenna activity at the molecular level. Among the mechanisms discussed will be light-driven LHCII trimer to monomer transition and light-induced formation of LHCII supramolecular structures.

Oral presentations

O9.1

Proteomic approach reveals essential functions of prohibitins in *Arabidopsis* mitochondria

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Prohibitins (PHBs) are highly conserved proteins present in the mitochondria of all eukaryotes. PHB-depleted plants show severe growth inhibition, abnormalities in organ development, mitochondrial morphology and phytohormone signalling. However, molecular functions of prohibitins in plant mitochondria are still unknown. We used proteomic approach to look for the roles of PHBs in these organelles. We investigated changes in the protein composition of the mitochondria obtained from *Arabidopsis* plants with knockdown of two *PHB* genes: *AtPHB2* and *AtPHB6*. Our results indicate that the central function of prohibitins in *Arabidopsis* mitochondria is stabilization of mitochondrial acyl carrier proteins (mACPs). mACPs are responsible for biosynthesis of lipoic acid – cofactor of several multienzyme complexes crucial for mitochondrial metabolism. Loss of AtPHB2 and AtPHB6 results in decreased steady-state levels of mACPs and a set of subunits of glycine decarboxylase, pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase – complexes having lipoic acid as a cofactor. In *phb2/phb6* mitochondria we also observe activation of protein quality control system defined by increased steady-state levels of chaperones, AAA-type ATPases and AAA proteases. This observation indicates that PHBs in plant mitochondria are involved in protein folding/complex assembly activities. We also identified proteins interacting with plant prohibitins. Among them we found two Stomatin-like proteins (AtSLPs). This result indicates that prohibitins are involved in formation of lipid microdomains in mitochondrial inner membrane. All in all, our findings show that plant prohibitins play important roles in coordination of mitochondrial metabolism through lipoic acid and in organization of the mitochondrial inner membrane structure through formation of microdomains and protein folding/complex assembly.

O9.2

Dicer-like proteins from *Medicago truncatula*

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The biogenesis of both miRNAs and siRNAs in plants depends on a specific group of ribonucleases known as Dicer-like (DCL) proteins. Based on functional analysis of DCL proteins (DCL1-4) identified in *Arabidopsis thaliana*, four functional DCL types were distinguished in plants. DCL1-type ribonucleases mainly produce 21 nt long miRNAs. The products generated by DCL2-, DCL3-, and DCL4-type ribonucleases belong to various classes of siRNAs. DCL2-type enzymes are involved in the biogenesis of siRNAs from natural antisense transcripsts (nat-siRNA), DCL3-type ribonucleases produce an abundant class of heterochromatic small interfering RNAs (hc-siRNA) and DCL4-type proteins are mainly involved in trans-acting siRNAs (ta-siRNA) production. In addition, DCL2-, 3- and 4-type enzymes contribute to the plant defense against a diverse range of pathogens. In many plants duplications of the genes encoding DCL proteins have been observed. For example, the rice genome encodes two DCL2- and two DCL3-type proteins whereas in soybean two genes encoding DCL1-, DCL2- and DCL4-type ribonucleases were identified.
Medicago truncatula is a model legume plant closely related to many economically important cultivable species. In order to increase our knowledge on miRNA and siRNA biogenesis in *Medicago* we have screened the current genome assembly available from *Medicago truncatula* Genome Project in search for DCL-coding genes. In addition to MtDCL1, 2 and 3 characterized in the previous studies we identified three other DCL genes: MtDCL4 and two new MtDCL2 homologs. We found that one of the newly identified MtDCL2 genes codes for a truncated version of DCL2 protein. Using droplet digital PCR (ddPCR) we confirmed the existence of all MtDCL transcripts in the total RNA fractions extracted from *Medicago* plants. Additionally, we identified an alternative splicing variant of MtDCL1 mRNA. Translation of the latter may result in the formation of the truncated DCL1 protein. Finally, we determined the expression profiles of the six MtDCL genes in different parts of the plant at various developmental stages. The mRNA abundance for all assayed DCL mRNAs was significantly increased in the nodule, compared to root and other plant organs which may suggest the important role of MtDCL genes in nodule function.

Acknowledgements

This work is co-financed by the European Regional Development Fund through the Operational Programme Innovative Economy, Innovation grants.
This work was partially supported by the European Union Regional Development Fund within the PARENT-BRIDGE Programme of Foundation for Polish Science (Pomost/2011-3/5 to A.K.-K).

O9.3

3D-visualization of plastid internal membranes during chloroplast biogenesis

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Chloroplast biogenesis is one of the main process taking place during the ontogenesis of plants. Development of chloroplasts through the stage of etioplasts often takes place when the first stages of seed germination and seedlings growth proceed without light under the ground. Etioplasts contain prolamellar bodies (PLBs) which have tubules joined together in a regular network of a special paracrystalline symmetry. Together with prothylakoids (PTs) – flattened porous membranes, PLBs are precursors of the chloroplast thylakoid membranes. It is a standard assumption that the Pchlide:LPOR:NADPH complex is the main factor governing the formation and maintenance of the paracrystalline PLB structure although the role of this complex is still far from understanding. Upon illumination the photomorphogenic process called greening takes place and etioplasts develop into chloroplasts. Wihout full 3D information it is not possible to understand the process of the structural membrane transformation during the etioplast – chloroplast transition. We present in 3D the membrane changes in early stages of bean *(Phaseolus coccineus* L*)* chloroplast development. We reconstructed the paracrystalline structure and the real membrane connections within PLB and the gradual transformation from the tubular arrangement to the linear one during the greening process on light. The reconstructed spatial structure of the internal plastid membrane, using electron tomography, and in later stages also with the help of confocal laser scanning microscopy (CLSM) enables visualization and thus understanding of the real membrane connections during the key stages of chloroplast biogenesis. Morover, taking into consideration our results focused on the interactions between all PLB components, we correlated the 3D structure of PLB and of the developing bean chloroplast with the photosynthetic complex formation. Spatial models (3D) of selected stages of chloroplast biogenesis and subsequently correlation of each structural stage with appearance of functional pigment-protein complexes will allow to understand better process of biogenesis of chloroplasts.

O9.4

Effect of different phytohormones and stress conditions on expression of 1-*O*-(indole-3-acetyl)-β-D-glucose : *myo*-inositol acyltransferase (IAInos synthase) from rice (*Oryza sativa*)

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Indole-3-acetic acid (IAA) is responsible for regulation of many growth and developmental processes in plants. Hence, precise control of concentration of this phytohormone is extremely important. The conjugation of IAA is a part of the mechanism regulating free auxin level. Some cereal grains are rich sources of IAA-ester conjugates, although only enzymes involved in their synthesis from *Zea mays* have been thoroughly characterized so far. 1-*O*-(indole-3-acetyl)-β-D-glucose (1-*O*-IAGlc) : *myo*-inositol acyltransferase (IAInos synthase) is an enzyme catalyzing the second reaction of IAA-esters conjugates synthesis pathway. This enzyme is responsible for transfer of the IAA moiety from 1-*O*-IAGlc to *myo*-inositol which results in formation of indole-3-acetyl-*myo*-inositol (IAInos) according the following reaction:

1-*O*-IAGlc + *myo*-inositol → IAInos + glucose

Previous amino acid sequence analysis revealed that IAInos synthase from maize endosperm belongs to the serine carboxypeptidase-like acyltransferase family (SCPL), homologous to hydrolases of the serine carboxypeptidases. SCPL acyltransferases participate in wide range of plant secondary metabolism pathways. Unlike more abundant BAHD acyltransferases which use coenzyme A thioesters as acyl donors, SCPL acyltransferases utilize energy-rich 1-*O*-β-glucose esters as alternative substrates.
Our previous studies identified the enzymatic activity that transfers IAA moiety from 1-*O*-IAGlc to *myo*-inositol in rice seedling extracts. Moreover, it has been previously reported that some phytohormones affect expression of enzymes involved in synthesis of IAA conjugates. Thus, in our current studies we decided to analyse the effect of different phytohormones (abscisic acid, salicylic acid and auxinic herbicides: 2,4-dichlorophenoxyacetic acid, Dicamba, Picloram) and stress conditions (drought, osmotic stress) on expression level of IAInos synthase gene in 6-days-old rice seedlings. The expression of IAInos synthase was analyzed by Western blot using polyclonal anti-Zm IAInos synthase antibodies and semi-quantitative RT-PCR method using specific primers.

O9.5

Capability of *Populus tremula x P. tremuloides* and its transgenic line indicating the increase of *PttPME1* activity in phytoremediation of Pb contaminated soils

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Scientist are still looking for "perfect plant", which could take up, accumulate and tolerate more trace metals and as the result would be more effective in phytoremediation. Cell wall (CW) is one of the plant cell compartment which accumulate especially high amounts of trace metals. It has been regarded for many years that the essential capacity of the CWs for binding metal ions depends on the amount of low-methylesterified pectins (LMPs) to which trace metals, in particular Pb, indicate high affinity.
Therefore, the main aim of the study was to show if it is possible to increase plant efficiency in metal accumulation by increase the LMPs level in prospects of use them for phytoremediation.
The experiments were divided on two stages. In the first one the goal was to show if LMPs may be in fact regarded as one of the key player in Pb accumulation and in the second to check if transgenic poplar, indicating the increase activity of *PttPME1* (the gene encoding the enzyme responsible for LMPs formation in CWs), showed higher Pb accumulation capability.
The objects of the study were root tips of *Populus tremula x P. tremuloides* wild type (T89) and transgenic line(7B) treated with $PbCl_2$, (1000 μMPb; 4h) and H_2O, 4h — control. LMPs (up to 40%) were identified by JIM5 antibody in confocal and transmission electron microscopy (TEM) and; Pb as electron-dense deposits in TEM and by X-ray microalanalysis connected with TEM *PttPME1* activity profile was analyzed by qRT PCR.
In root tip tissues we detected close relationship between the distribution and the level of LMPs and Pb accumulation. The regions especially abundant in LMPs, as CWs contact sites of neighboring cells, CWs adjacent to intercellular spaces indicated especially high Pb accumulation. Furthermore, Pb deposits were often surrounded by gold particles identifying LMPs - thus the two compounds showed close colocalization. What's more in response to Pb root tips formed cell wall thickenings (CWTs) especially abundant in LMPs which also accumulated high level of this metal.
In the 7B line we detected similar rules of Pb accumulation as inT89, but 7B accumulated more Pb. Furthermore, Pb was present mostly in CWs and its CWTs. Little Pb was detected in the protoplast.
The qRT PCR showed that the expression profile of *PttPME1* decreased after Pb exposure in both T89 and 7B lines. However, transgenic line showed markedly higher expression profile of *PttPME1* under Pb treatment than wild type.
Thus, we can conclude that LMPs, almost certainly, are the key player in Pb accumulation by poplar root tip cells. The transgenic plants, indicated higher *PttPME1* activity, accumulated more Pb probably because of higher LMPs level in

their CWs. Hence, such modification seems to be is a good direction the studies which focuses on the plant efficiency increase for trace metals accumulation — in prospect of use them for phytoremediation.

Acknowledgement

Studies supported by grant MNiSW NN 303 801 940.

O9.6

ABC transporters and phytoalexin biosynthesis in *Medicago truncatula*

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Production and secretion of secondary metabolites with antimicrobial activity is one of defence strategies that protect plants against various invaders. In leguminous this function is fulfilled by isoflavonoid derivatives which distribution in plant is still poorly recognised. Previously we have shown that ABCG10 from *Medicago truncatula* is implicated in the modulation of (iso)flavonoids level during the defence reaction, associated with *de novo* synthesis of phytoalexin-medicarpin. Expression of *MtABCG10* goes along with genes encoding pivotal enzymes from the (iso) flavonoid biosynthetic pathway upon elicitation. Moreover, *MtABCG10* silencing resulted in lower accumulation of chalcone isoliquiritigenin and its derivatives from isoflavonoid branch including medicarpin. The presence of MtABCG10 in vascular bundles, plasma membrane localisation as well as observed silencing effect, suggest its participation in long distance movement of early intermediates of medicarpin biosynthesis (Banasiak *et al.*, 2013). Loading experiment conducted with *MtABCG10* silenced hairy roots and exogenously applied isoliquiritigenin resulted in the medicarpin biosynthesis restoration. These data suggest that full size ABCG10 protein from *M. truncatula* is isoliquiritigenin transporter.

Reference:

Banasiak J *et al* (2013) J Exp Bot **64**: 1005–1015.

Posters

P9.1

Mechanism of nanoparticles toxicity in macrophytes

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Water environment is indicated as one of the most exposed to the influence of toxic nanoparticles (NPs). The main aim of the studies is to explain how the most popular nanoparticles (TiO_2-NPs and Ag-NPs) affect the aquatic plants (macrophytes): *Salvinia minima* and *Limnobium laevigatum* and recognize the mechanisms involved in nanoparticles accumulation and toxicity to plant cells.
To investigate the mechanisms of NPs accumulation and interaction with the cells, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were applied. Additionally photosynthetic pigments level was investigated. Inside the cells of *S. minima* and *L. laevigatum* agglomerates of TiO_2-NPs were found. The place of deposition and agglomerates size depend on the applied concentration of NPs and differ between investigated species. Additionally some pathological changes of cell morphology were found: plasmolysis, destruction of the cell membrane, changes or degradation of the chloroplasts. Obtained results indicate toxicity of titanium dioxide nanoparticles to plants. In case of Ag-NPs endocytosis is a process of silver accumulation, NPs are transported into the cell as endosomes. The mechanisms of silver transport through the cell membrane differ between Ag nanoparticles and ions. During the studies the photosynthetic pigments degradation was found, what indicates a damage of the photosystem.
It is assumed that nanoparticles i) adsorb on the cell surfaces and disturb the cell functions or ii) penetrate the cells, release ions and the ions disturb the functionality of the important organelles.
A scheme illustrating various stages of NPs toxicity to aquatic plants was proposed.

P9.2

Why seeds of different *Brassica oleracea* forms respond differentially to smoke?

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Seeds of various species respond to active substances of smoke, karrikins (KAR), with faster or slower germination (Kępczyński *et al*., 2013; Long *et al*., 2011a, b). In a comparative experiment, seeds of red cabbage (*Brassica oleracea* var. *capitata* f. rubra) revealed greater sensitivity to smoke, smoke water and curing smoke than seeds of white cabbage (*Brassica oleracea* var. *capitata* f. alba). Before visible germination, sugars were released more intensively (up to 2-fold) in smoke-treated seeds than in the control ones, and it was accompanied with enhanced seedling occurrence in soil experiment. As the active substances of smoke have oxidative character, activation of antioxidant system in seeds of the two *Brassica* forms is studied. The interplay of KAR with phytohormones is also considered.

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Kępczyński J *et al* (2013) *Acta Physiol Plant* DOI 10.1007/s11738-012-1080–1081.
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Long R *et al* (2011b) *Aust J Bot* **59**: 609–619.

Acknowledgements

The study was supported by the DS3113 programme of Faculty of Agriculture and Economics, University of Agriculture in Cracow.

P9.3

The role of RAF1 protein in cyanobacterial Rubisco assembly

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyzes carbon fixation in the Calvin-Benson pathway, requires specific chaperones for biosynthesis. Recently, novel polypeptide – Rubisco Accumulation Factor 1 (RAF1) has been reported as a factor necessary for formation of the active enzyme in maize cells. However, due to lack of efficient procedure to analyze biosynthesis of higher plant Rubisco, its function and mode of action have not yet been determined. Genes encoding homologs of RAF1 has been identified in other plants, green algae and cyanobacteria. Our results show that prokaryotic homolog of RAF1 from *Thermosynechococcus elongatus* is expressed in cyanobacterial cells and interacts with the large Rubisco subunit. Co-expression of Rubisco-encoding genes with genes of putative chaperones in *Escherichia coli* demonstrates that RAF1 is able to support Rubisco assembly in these cells. Moreover, when co-expressed with RbcL alone, novel RbcL-RAF1 assembly-intermediate is formed. Its molecular mass determined by gel filtration coupled with Multi-Angle Light Scattering correspond to molar mass of RbcL dimer and two RAF1 molecules. The addition of RbcS into purified RbcL-RAF1 complex leads to its dissociation and formation of the active holoenzyme. On the other hand, addition of RAF1 to the RbcL octamer cause its disassembly and formation of RbcL-RAF1 intermediate. The results of our experiments contribute to the explanation of the role of cyanobacterial Rubisco Accumulation Factor 1 in the Rubisco biosynthesis process.

P9.4

ABC transporters and nodulation in *Medicago truncatula*

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Explaining mutually beneficial interaction between Rhizobium and leguminous plants remains one of the most important problems for evolutionary biology. This intimate association results in the formation of root nodules that provide an environment necessary for nitrogen fixation. A key component of nodule organogenesis is cytokinin, acting as a mobile signal. Activation of the cytokinin signaling pathway in the root cortex leads to the cell division and consequently formation of the nodule primordium (Oldroyd *et al*., 2011). However, questions about molecular and genetic basis of nodulation, especially role of plants transporters in this process, need to be answered. A major function fulfilled by ATP-binding cassette (ABC) proteins is transmembrane translocation of great variety of molecules. Mounting evidence suggests that full-size ABCG transporters could be responsible for transport of signaling molecules, crucial for successful symbiosis between legumes and rhizobia (Sugiyama *et al*., 2007).
Previously, we have identified and classified 19 full-size ABC transporters from the G subfamily in *Medicago truncatula* (Jasinski *et al*., 2009). Here we present a novel, root expressed full-size *MtABCG* gene and we address a question about its putative role in the modulation ofnitrogen-fixing symbiosis. The conducted sqRT-PCR analysis revealed that the expression of this transporter gene is strongly up-regulated during inoculation with *Sinorhizobium meliloti* as well as cytokinin treatment. We hope that the presented data provide a foundation for further studies on the role of *Medicago* ABCG transporters in symbiotic interactions. Understanding role of ABC transporters in nodulation process might contribute to improving existing symbioses and extending them to nonlegumes.

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P9.5

Drought responsive proteins in potato (*Solanum tuberosum* L.)

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Drought is one of the major abiotic stresses affecting plant growth, development and productivity. Potato (*Solanum tuberosum* L.) is moderately drought sensitive crop (Schafleitner *et al*., 2007) whose yield is drastically reduced by dehydration. Recent evidence indicates that the reprogramming of gene expression results in the reorganization of plant metabolism under unfavourable environmental conditions. Since variations in drought tolerance have been observed among different potato cultivars in the present experiments the identification of drought-related proteins was carried out in order to establish the molecular markers of drought. Therefore, three weeks after tuberisation, potato plants were subjected to soil water shortage for 14 days. Proteins were extracted from mature non-senescent leaves and separated by 2-DE. The images were de-noised in the wavelet domain, background was removed using the ALS approach, warping was performed based on the fuzzy warping algorithm and images were standardized using the robust orthogonal least squares method (Zerzucha *et al*., 2012). Protein spots were analysed by LC-MS/MS using mass spectrometry Orbitrap (Thermo) at the Institute of Biochemistry and Biophysics of Polish Academy of Sciences. Proteins were identified by searching NCBI non-redundant database using the MASCOT program (http://www.matrixscience.com). Sequence length, gene name and also protein functions were identified by searching Swiss-Prot/TrEMBL database using UniProtKB (http://www.uniprot.org).

In potato leaves of susceptible cultivar the most identified proteins up-regulated upon drought are involved in photosynthesis, energy metabolism and Reactive Oxygen Species scavenging proteins. Among them, one indicating an identity or an extensive homology with the highest probability is chloroplastic Fe-superoxide dismutase and other chloroplastic enzyme i.e. carbonic anhydrase. Among the protein markers of drought tolerance were RNA-binding proteins responsible for the proper translation and protein biosynthesis, ATP-binding proteins responsible for the energy metabolism, chaperons as well as proteins responsible for control of proper responses to environmental stress conditions.

The obtained results supported our earlier findings that tolerant genotypes could to counteract the accumulation of ROS (Boguszewska *et al*., 2010) and continue photosynthesis under soil drought (Boguszewska *et al*., 2011).

P9.6

Influence of diadinoxanthin and diatoxanthin on the fluidity of the thylakoid membranesof *Pheodactylum tricornutum* adapted to low and moderate temperature

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Diatoms are the dominant phytoplankton species in the marine cold environment. While photosynthetic organisms possess several mechanisms to adapt to low temperature to ensure optimal growth, there are only few data on the cold acclimation of diatoms. The most important adaptation strategy of plant and algae is to maintain the optimal membrane fluidity and compensate the reduced chemical reaction rates at low temperature by increasing enzyme concentration.

The aim of our research was to study the correlation between degree of diadinoxanthin de-epoxidation and the fluidity of thylakoid membranes isolated from *Pheodactylum tricornutum* adapted to low and moderate temperatures.

Thylakoid membranes were isolated from 5 or 8 day old cultures of *P. tricornutum* cultivated at 12 and 20°C, respectively. Diadinoxanthin de-epoxidation was studied in the thylakoid membranes incubated in the reaction medium buffered at pH 5.5 at room temperature (20°C). It was started by the addition of ascorbate (sodium salt), the co-substrate of diadinoxanthin de-epoxidase. The levels of diadinoxanthin cycle pigments (diadinoxanthin and diatoxanthin) were analyzed by HPLC at 0, 10, 30, 60 and 120 min of de-epoxidation reaction. The changes in fluidity of *Ph. tricornutum* thylakoid membranes during de-epoxidation were investigated in the temperature range of 0 to 45°C by EPR spectroscopy using two spin labels: 5-SASL and 16-SASL.

It was found that apart from thylakoid membranes isolated on the 5th day of the culture of *Ph. tricornutum* adapted to 12°C, in all other samples diatoxanthin was present, even without de-epoxidation. The level of Dtx/(Dtx+Ddx) after reaction in thylakoids of diatoms adapted to 12°C and isolated on 5th and 8th day of inoculation was about 25% higher than that in thylakoids of *Ph. tricornutum* adapted to 20°C.

Order parameter (S) determined in our EPR studies of *Ph. tricornutum* thylakoid membranes by the use of 5-SASL was independent on Dtx/(Dtx+Ddx) level and growth temperature. On the other hand, the S values obtained for membranes labeled with 16-SASL indicated strong rigidifying effect of diatoxanthin in broad range of temperatures, for thylakoids isolated from cells adapted to 20 and 12°C respectively, when 70–90% of diadinoxanthin was converted to diatoxanthin. For control samples, the order parameter decreased with the increase of temperature. The obtained results showed that fluidity of the membrane hydrophobic interior could be stabilized by high diatoxanthin concentration.

Acknowledgements

This work was supported by project No. 2011/01/M/NZ1/01170.

P9.7

Involvement of enzymatic antioxidants and salicylic acid in response of cucumber to salt stress and bacterial infection applied separately and in combination

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Plants are constantly exposed to a combination of environmental stress factors. Among stressors, salinity and pathogens are the most important factors limiting plant growth and productivity. Understanding plant response to stresses mediated by signaling molecules, metabolites and phytohormones is important for breeding plants and producing crops varieties with enhanced tolerance to changing environmental conditions.
We exposed cucumber plants to short-term salt stress and test their response to subsequent *Pseudomonas syringae* pv *lachrymans* (Psl) infection. Plant growth (leaf surface, shoot length fresh/dry weight), leaf cell damage recognized by Evans blue staining, and chlorophyll content were measured. Moreover, automated image analysis for quantification the accumulation of $O_2^{\cdot-}$ and H_2O_2 as well as infection development was performed by a feed-forward type, two layer neural network. We also assayed changes in activities of enzymatic antioxidants (ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione reductase) as well as contents of salicylic acid (SA) and its glucosylated conjugates (SAGC). Abiotic stress impaired the defense response to Psl as shown by more severe angular leaf spot disease symptoms and enhanced leaf cell damage when Psl infection was combined with salinity. The reduced performance of salinized plants under biotic stress could be related to salt stress-induced plant growth inhibition with leaf expansion being the most sensitive to salinity, decreased chlorophyll content and prolonged H_2O_2 and $O_2^{\cdot-}$ accumulation in leaves implying perturbations in redox homeostasis. The response of NaCl-treated and non-treated plants to bacterial infection differed in terms of H_2O_2 and $O_2^{\cdot-}$ generation. Our results showed that antioxidant enzymes are involved in cucumber response to stresses and their activities vary between single stress and combination of two stressors. In cucumber leaves SA occurred mostly as SAGC. Bacterial infection induced a significant increase in SA and SAGC concentrations, however the intensity and dynamics of SA and SAGC accumulation differed in leaves of NaCl-treated and non-treated plants. This study may provide new insights into how response to a combination of stress factors is regulated and how the environment can modulate plant-pathogen interactions.

Acknowledgements

This work was partly supported by grant No 2012/07/N/NZ9/00041.

P9.8

Identification of new Arabidopsis lines carrying T-DNA-insertion in the *AtTOR* gene

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TOR kinase is an evolutionary conserved serine-treonine kinase, that is responsible for integrating signals connected with environmental stress, cell energy status and availability of amino acids. Homologues of TOR kinases were also identified in plants. Due to embryolethality of Arabidopsis *tor (attor)* mutants, the current knowledge about functioning and regulation of plant TOR kinase is very limited. We therefore screened the Koncz collection using PCR- based technique and identified four new *Arabidopsis thaliana* lines carrying T-DNA insertions in the *AtTOR* gene. Subsequent phenotypic analysis revealed that three of these lines exhibited embryolethal phenotype. By contrast, a single line carrying T-DNA insertion in the body of *AtTOR*, close its 3'- end, segregated viable homozygous *attor* mutant plants. Here, we report detailed phenotypic and molecular analysis of the new T-DNA lines carrying insertions in the *AtTOR* gene.

Acknowledgements

Financial Support: DI2012 021842 'Diamond Grant' programme by the Polish Ministry of Science and Higher Education.

P9.9

The use of 1,3-dipolar cycloaddition in the synthesis of AZT-systemin conjugate and it's movement throughout tomato plant

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Click chemistry is a newer approach to the synthesis of drug-like molecules that may accelerate the drug discovery process by utilizing a few practical and reliable reactions. Click reaction is wide in scope and easy to perform, uses only readily available reagents, and is insensitive to oxygen and water. In fact, in several instances water is the ideal reaction solvent, providing the best yields and highest rates. Reaction work-up and purification uses benign solvents and avoids chromatography [1].
Systemin is 18-aa peptide defense hormone released in response to plant (tomato, tobacco) damage or pathogen attack. We examinated whether systemin's fast movement through plant tissues could be used for cargo (AZT) transport. AZT (3'-azido-2'3'-dideoxythymidine), a modified nucleoside used in antiretroviral therapy (AIDS) and peptide plant hormone – systemin were used as substrates of 1,3-dipolar cycloaddition which leads to 1,4-disubstitiuted triazole ring [2, 3].
AZT-systemin conjugate has been synthesized by click chemistry, using systemin modified at N-terminus with propiolic group and AZT. The conjugation was catalyzed by Cu(I). The reaction was fast, efficient and regioselective. Its progress was easily monitored by capillary electrophoresis (CE).
CE was also applied for characterization of systemin and AZT-systemin stability and movement throughout tomato leaf and stem. Despite the fact that systemin moves rapidly through tomato tissues, our calorimetric (ITC) studies showed that the peptide does not interact with liposomes–cell membrane model.

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Acknowledgements

This work is supported by the European Social Funds (LiSMIDoS) and Polish National Science Centre grant no N N204 355540.

P9.10

Molecular cloning and expression analysis of selected ABA biosynthetic genes during maturation and germination of triticale seeds

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Abscisic acid (ABA) is a plant hormone that regulates many important aspects of plant biology, including embryogenesis, dormancy, germination, flowering, fruit ripening and stress response. ABA plays a central role in the acquisition of primary dormancy during seed maturation as well as in maintaining dormancy in imbibed seeds. The local concentration of active ABA is a result of a balance between its biosynthesis and catabolism. Enhanced seed dormancy or delay of germination is presumably a result of increased ABA biosynthesis. Oxidative cleavage of *cis*-violaxanthin and *cis*-neoxanthin to xanthoxin, which is catalyzed by 9-*cis*-epoxycarotenoid dioxygenase (NCED), is considered as a rate-limiting step in *de novo* ABA biosynthesis. However, in non-photosynthetic tissues such as seeds and roots, zeaxantin epoxidase (ZEP) might also play an important role.
Two triticale 9-*cis*-epoxycarotenoid dioxygenase genes were cloned and designated as *TsNCED1* and *TsNCED2*. Fragment of gene encoding zeaxantin epoxidase were also cloned and designated as *TsZEP*. The highest transcript level of *TsNCED1* and *TsNCED2* was observed after halfway during triticale seed development, which is a period of expected peak of ABA accumulation. Expression of both *TsNCEDs* was higher in embryos of cultivar more resistant to pre-harvest sprouting (Fredro) than of cultivar more susceptible to pre-harvest sprouting (Leontino). There where no differences in expression of *TsZEP* in maturing triticale seeds of these cultivars. When mature triticale seeds were subjected to imbibition, the expression of *TsNCED1* was higher in embryos of Fredro cultivar than in Leontino cultivar, while there were no significant differences in expression of *TsNCED2* between these cultivars.The expression of *TsZEP* was observed at slightly higher level in Fredro cultivar. The obtained results might suggest, that enhanced expression of the ABA biosynthetic genes, may contribute to higher resistance to pre-harvest sprouting.

P9.11

Light-dependent protochlorophyllide oxidoreductase: subunit properties and structure of the oligomer contribute to efficiency of the enzymatic reaction

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Light-dependent protochlorophyllide oxidoreductase (POR, EC:1.3.1.33) catalyzes one of the terminal reactions of chlorophyll biosynthesis, the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) with NADPH as a cofactor. Interestingly, it is the only enzyme of *Eucaryota* that requires light to decrease the activation energy of the reaction. Although the molecular mechanism of this phenomenon is still not fully elucidated, we focused our research on comparative study of photophysical properties of reconstituted NADPH:Pchlide:POR complexes and the efficiency of product accumulation for different enzyme isoforms. Using recombinant POR A, B and C from *Arabidopsis thaliana*, we have shown that incubation time of POR, Pchlide and NADPH in the dark, before starting the reaction by light, plays the key role in the efficiency of substrate-to-product conversion. We measured progress of the reaction by both room temperature and low-temperature fluorescence measurements. The rate of the reaction was similar for all the examined isoforms when the measurements were started immediately after mixing of the substrates. However, small but significant differences could be observed after a longer incubation time. Taking into consideration these findings and our earlier conclusions suggesting that POR functions as an oligomeric complex, we could distinguish two factors contributing to the efficiency of the reaction: (1) the ability of a single subunit to perform the reaction, that is similar among all the examined isoforms, and (2) interactions between subunits within an oligomer that the increase probability of substrate-to-product conversion.
Our finding will help to understand the requirement of POR oligomerizaiton *in vivo* and the mechanisms of possible ways of regulation of the enzyme activity in cell.

Acknowledgements

This work was supported by Grant 2011/01/B/NZ1/00119 from the National Center of Science of Poland (NCN).

P9.12

Changes in cellular redox state and oxidative modification of proteins upon dehydration of wheat seedlings

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Wheat (*Triticum aestivum* L.) seedlings are a perfect plant model to study dehydration tolerance due to the transition from dehydration tolerant to intolerant state about 5th day of germination when seedlings are still in heterotrophic phase of growth. Therefore, experiments were done on 4-day-old seedlings, tolerant to dehydration, and 6-day-old seedlings, which are drought-sensitive.
The redox state of seedlings has been evaluated on the basis of the ratio of reduced (AsA) to oxidized ascorbate (DHA) and reduced (GSH) to oxidized glutathione (GSSG). It has been shown that the ratio of AsA/DHA in tolerant seedlings was lower than in sensitive ones due to lower ascorbate content. On the contrary, the content of GSH and GSSG was higher in fully turgid tolerant seedlings and remains higher upon dehydration. Activities of ascorbate peroxidase and glutathione reductase increased in both tolerant and sensitive seedlings but dehydration induced new activity bands with the molecular mass between 115 -160 kDa only in tolerant seedlings. Despite the higher activity of investigated enzymes in dehydrated sensitive seedlings the ratio of GSH to GSSG remained lower. Lipid peroxidation and H_2O_2 content increased more in sensitive seedlings than in tolerant ones. Higher cellular redox state in tolerant seedlings seems to counteract dehydration-induced protein oxidation. The most common protein oxidation is protein carbonylation, posttranslational modification of proteins.
The carbonyl group content drastically rise directly after application of dehydration being almost two-fold higher in sensitive than in tolerant seedlings. There were no significant differences in number of proteins synthesized *de novo* upon dehydration and further carbonylated. Among proteins upregulated during water deficiency in tolerant seedlings enzymes related to the Calvin cycle (large subunit of Rubisco) and glycolysis (triosephosphate-isomerase, chloroplastic and chloroplastic-like fructose-bisphosphate aldolase) as well as quinone oxidoreductase-like protein, and chitinase 2 were carbonylated. In sensitive seedlings large subunit of Rubisco, vip1 protein responsible for regulation of osmosensory signaling, cytosolic L-ascorbate peroxidase 1, proteasome subunit beta type-1 and eIF5A1 were carbonylated. The observed specific changes in protein carbonylation pattern seem to be required for counteracting the negative effects of water deficiency.

P9.13

Alternative splicing events in two maize lines under herbicide stress conditions

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Plants, as sessile organisms, must adapt their growth and metabolic style to a changing environment. Splicing is one of the mechanisms which play an important role in plant adaptation and is an additional element of fitness benefit adjusted to the limited capacity of genome size.
Studies of splicing and its role in diverse aspect of cell biology, pathology and stress response, has remained undescribed for many plant species, including maize. Through the mechanism of alternative splicing, exons from primary transcripts (pre-mRNA) with multiple introns may undergo ligation in many different ways generating multiple proteins from single gene. This process can affect mRNA stability and translation efficiency as well as activity, cellular localization, regulation and stability of coding protein.
For better characterization of alternative splicing role in plant herbicide stress response, we sequenced transcriptomes of two maize breed lines – sensitive and tolerant to herbicide RoundUp. We used Illumina next-generation sequencer Genome Analyzer IIx and we conducted pair-end sequencing. As a result we obtained 35 to 76 mln 50nt reads per sample.
Using bioinformatics tools such as BowTie, TopHat, Cufflinks, Cuffdiff and CummRbund we managed to identify between sensitive and tolerant maize line. We also managed to identify different types of splicing events with java script.

Acknowledgements

Ministry of Science and Higher Education 3098/B/P01/2010/39;National Science Center DEC-2011/01/N/NZ9/02900.

P9.14

The Arabidopsis SWI/SNF ATP-dependent chromatin remodelling complex responds to environmental changes in temperature-dependent manner

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The SWI/SNF chromatin remodeling complexes (CRCs) have been shown to play important roles in regulation of gene expression throughout eukaryotes.
The Arabidopsis genome encodes four SWI2/SNF2 ATPases, four SWI3, a single SNF5 and two SWP73 subunits. Most of the genes encoding these core components of Arabidopsis SWI/SNF CRCs have critical but not fully overlapping roles during plant growth, including embryo- and sporophyte development.
During our study we found that genes encoding the SWI/SNF CRC subunits are ubiquitously expressed and that their expression levels depend on the temperature regime of growth. Furthermore, Arabidopsis mutants impaired in several of these genes growing at lower temperatures show partial alleviation of their phenotypic defects, including reduced fertility, root development, and others.
In summary, our data provide novel insight into potential regulatory role of SWI/SNF CRCs activity during plant growth at different temperature ranges.

Acknowledgements

This work was supported by: the Diamond grant No DI 2011 0269 41 Ministry of Science and Higher Education Republic of Poland for D.M.G and National Science Centre (NCN) grants: 2011/01/N/NZ1/01525 for A.T.R., 2011/01/N/NZ1/01530 for E.B., N302 142739 for T.J.S., Marie Curie Reintegration Grant FP7-PEOPLE-2010-RG 268313, 2061 7PR/2011/2 (MNiSW) for T.J.S. and N301158735 (MNiSW) for A.J.

P9.15

Adenylylsulfate:ammonia adenylyltransferase activity of yellow lupin (*Lupinus luteus*) Fhit protein

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Recently, our interest on the metabolism of uncommon nucleotides has been focused on adenosine 5'-phosphoramidate, NH_2-pA, [1-5]. The compound was identified 30 years ago among cellular nucleotides purified from the green alga *Chlorella pyranoidosa* [6] and proved to be a product of the following reaction catalyzed by an enzyme classified as adenylylsulfate:ammonia adenylyltransferase (EC 2.7.7.51): SO_4-pA + NH^+_4 → NH_2-pA + SO_4^{2-} + $2H^+$ [7]. We found this activity in the extract of yellow lupin seeds and purified to homogeneity a protein catalyzing that reaction. Electrophoresis in denaturating conditions (SDS-PAGE) and gel filtration showed that the enzyme functions as a dimer of 38 kDa, and MALDI-ToF mass spectrometry revealed its similarity to plant diadenosine triphosphate hydrolase. The latter activity was demonstrated to be an inherent property of Fhit protein [8]. We also showed that both human and plant (*Arabidopsis*) Fhit proteins have the ability to produce NH_2-pA from adenylylsulfate (SO_4-pA) and ammonia. In conclusion, these results provide evidence for a novel function of Fhit proteins.

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Acknowledgements

This work was supported by the National Science Center (grant 2012/05/B/NZ1/00025).

P9.16

Introduction of mutated version of *AtDEG2* gene into destination vector for studies on functional significance of AtDeg2 chloroplast protease/chaperone

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Deg/HtrA proteases occur in almost every organism representing all domains of life from Bacteria through Arechea to Eukarya. Deg/HtrA proteases are members of the ATP-independent endopeptidases belonging to the serine-type chymotrypsin family S1C type according to the MEROPS nomenclature. These proteases have been identified and described for the first time in *E. coli.* (DegP, DegQ and DegS). Chloroplast AtDeg2 is encoded by in *A. thaliana* by a nuclear gene, orthologous to DegP, DegQ and DegS. Linear structure of AtDeg2 comprises the protease domain and two PDZ domains (PDZ1 and PDZ2). The protease domain contains catalytic triad composed of His, Asp and Ser, whereas PDZ domains play essential role in oligomerization of AtDeg2. In spite of a considerable progress which has been made in recent years, extremely little is known with respect to functional importance of AtDeg2 for *A. thaliana* growth and development under non-stressing conditions at different levels of plant body plan, including discrimination between AtDeg2 function as a protease and as a chaperone. The fist step toward understanding AtDeg2 functional significance is to construct *A. thaliana* mutant with mutated version of AtDeg2 which was deprived of its proteolytic activity by S268G point mutation. This communication provides with the data demonstrating a successful introduction of mutated version of *AtDEG2* into a destination vector, which, in its turn was introduced into *Agrobacterium thumefaciens* cells.

Acknowledgements

The project was supported by grant from Polish National Science Center based on decision DEC-2013/09/B/NZ3/00449.

P9.17

Drought stress responses and nitrogen fixation efficiency in *Medicago truncatula*: unravelling the role of ABA transporters

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Abscisic acid (ABA) is one of the foremost signaling intermediate controlling plant's growth and development as well as response to environmental stresses. In response to water deficit, the level of this phytohormone in leaves fluctuates dramatically, leading to stomatal closure and consequently to minimization of water loss. Moreover, the hydraulic conductivity and elongation of the root are also being increased by ABA, enabling plant's recover after water stress (Kudoyarova *et al*., 2011). Interestingly, effects of ABA on root morphogenesis, mainly on the lateral root formation in Arabidopsis and legume plant *Medicago truncatula* are opposite, indicating differences in specifics of ABA action between plant species. As far as legumes are concerned, ABA promotes the growth of lateral roots and simultaneously affects the nodulation process, acting as negative regulator in its early stages and suppression of symbiotic nitrogen fixation is one of the first responses during drought stress (Ding & Oldroyd, 2009). In the regulation of water stress responses significant role play ABA transporters. Hitherto, two members of the ABC transporter family (ABCG40, ABCG25) and one member from a nitrate transporter family (AIT1) have been reported as ABA transporters in Arabidopsis. The efflux transporter ABCG25 is expressed mainly in vascular tissues, while the ABCG40 and AIT1 importers, are in guard cells and vascular tissues, respectively (Boursiac *et al*., 2013). Here we address a question about role of ABC transporters in attenuation of drought stress and nodulation efficiency in a model legume plant *M. truncatula*. Based on the expression analyses several genes encoding ABCG proteins from *M. truncatula* have been selected as a potential ABA transporter.

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P9.18

Metabolic response of *Brachypodium distachyon* to the fungal pathogen *Staganospora nodorum*

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Brachypodium distachyon, as the first Pooideae whose genome has been sequenced, became a model grass worth to explore given the fact that the acquired knowledge could be transferred to the species of agronomical interest. However, little is known about the defense mechanism induced by the pathogenic microorganisms in this species.
During this study we investigated effects of the infection with the necrotrophic fungal pathogen *Stagonospora nodorum* on the content of secondary metabolites in leaves of *B. distachyon*. The responsive metabolites were recognized by comparing HPLC-UV profiles of metabolites between challenged and unchallenged plants. Several compounds, which concentrations were modified by the pathogen attack, were identified by HPLC-MS^n analysis. Among the identified metabolites we found serotonin, a tryptophan derivative already reported to be induced upon fungal infection in closely related species, like *Triticum* spp. [1] and *Oryza sativa* [2].
In addition, we monitored infection-triggered changes in the expression of selected genes encoding key enzymes involved in the biosynthesis of the affected metabolites (phenylpropanoid and tryptophan metabolism). This allowed us to correlate changes on the transcriptome and metabolome levels, and to recognize the several isoforms of biosynthetic enzymes, which are directly involved in the defense responses.

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P9.19

Effect of auxin (IAA) on slowly activating vacuolar (SV) channels in red beet (*Beta vulgaris* L.) taproots

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In the present report, by using a patch clamp technique, the effect of indoleacetic acid (IAA) on SV channel activity in red beet taproot vacuoles was studied. Patch-clamp experiments were performed on the whole-vacuole and excised cytosolic side-out patch configurations using EPC-7 Plus amplifier. It was found that in the control bath the macroscopic currents showed the typical slow activation and a strong outward rectification of the steady-state currents. An addition of 1 μM IAA to the bath solution blocked SV currents in red beet vacuoles. Removal of IAA from the both solution did not restore the macroscopic currents. When single channel properties were analyzed, only little channel activity could be recorded in the presence of 1 μM IAA. Auxin decreased significantly the open probability of single channels. The recordings of single channel activity measured in the presence and absence of auxin showed that 1 μM IAA only slightly decreased the unitary conductance of single channels.

P9.20

Changes in the chromatin state and gene transcription in response to salinity stress in T87 *Arabidopsis thaliana* cells

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Chromatin is a nucleoprotein complex encompassing DNA and core histones in the nucleus of an eukaryotic cell. Stability of chromatin structure is crucial for the proper regulation of all nuclear DNA-templated processes — transcription, replication, repair and recombination. Emerging studies show that various cell signaling pathways trigger changes in the chromatin state and thus incoming signals can regulate gene expression through chromatin reorganization. Plants as sessile organisms developed diverse mechanisms allowing for quick response and adaptation to abiotic stress conditions. One of the first levels of the plant cell response to stress is induction of transcription of different gene classes. Defining how changing environmental conditions influence the chromatin state is essential for understanding regulation of plant gene transcription critical for improvement the resistance of cultivated plants to environmental stress. We studied in parallel the quick changes at both levels: transcription and chromatin structure in response to salt stress. As a model we chose T87 *Arabidopsis thaliana* cell line grown in suspension. Analysis of transcriptional changes was performed on Affymetrix GeneChip ATH1 microarrays. We carried out microarray experiments of treated with 250 mM NaCl T87 cells in 6 time points (0', 20', 40', 60', 80' and 100'). A number of known (cor15a, dreb2A) and unknown genes were identified to have changed transcription levels in response to salinity, which was confirmed by qPCR analysis. Our transcriptomic analysis identified new genes potentially crucial for plant adaptation to salinity stress. Chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) with the use of antibodies against characteristic for transcriptional regulation core histones modifications revealed global changes in chromatin state under salt stress conditions, which correlated with changed expression of early responsive (ER) genes. Our results show the relations between cell signaling, chromatin state and gene regulation in response to environmental stress in plants.

P9.21

Pea and bean plants adaptation to low light conditions is achieved by different molecular strategies

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Pea (*Pisum sativum* L.) and bean (*Phaseolus coccineus* L.), plants belonging to *Fabaceae* family, have different chlorophyll-protein (CP) complexes organization within thylakoid membranes, which determines distinct thylakoid spatial structure. Pea chloroplasts contain large distinctly separated appressed domains while in bean chloroplasts less distinguished appressed regions are present. Since light intensity and quantity are the most important factors which determine the efficiency of plant photosynthesis, we examined how growth at low light (LL) intensity influences thylakoid membrane structure and organization of CP complexes of pea and bean plants. Using electron and confocal laser scanning microscopy we found that under LL conditions in both species the grana diameter increases while the grana height decreases – especially in bean chloroplasts. Despite similar alterations in pea and bean thylakoid spatial structure under LL conditions, changes in CP complexes organization proceed in different directions. In pea thylakoids, among other processes, partial migration of LHCII antenna to PSI was observed. On the contrary, in bean thylakoids this migration is hampered by changes in membrane lipid composition and protein aggregation level, which are factors affecting the flexibility of thylakoid membranes. Simultaneous analysis of PSII and PSI activity showed that photosynthetic apparatus of both examined species works at optimal efficiency, however pea plants (grown under LL conditions) show better capability of short-term adaptation to high light intensity.

P9.22

Inhibitory effect of heterocyclic phosphonic and phosphinic acids on diphenolase activity of tyrosinase from *Agaricus bisporus*

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Tyrosinase (EC 1.14.18.1) belongs to the class of enzymes oxidoreductases. The enzyme possess two different activities: monophenolase which is connected with hydroxylation of monophenols to *o*-difenols and diphenolase activity realted to oxidation of *o*-diphenols to corresponding *o*-quinones [1-3]. Tyrosinase is widespread in the environment. It is found in bacteria, fungi, plants and animals. Among the many functions of the enzyme we can include the involvement of tyrosinase in the molting process of insects. In plants, the occurrence of browning in some fruits and vegetables is related to enzymatic oxidation of phenolic substrates into quinones catalyzed by tyrosinase. This phenomenon is undesired because of decreasing the value of food [3, 4]. The most important function of tyrosinase activity is involvement in the first two limiting steps of melanogenesis pathway, during the melanins are produced. They are agents responsible for skin, eye and hair color in humans. Abnormal activity of the tyrosinase is associated with occurrence of numerous disorders in skin pigmentation such as hyperpigmentation, age spots or melasma, malignant melanoma, Parkinson and Huntington diseases [5]. There are known the numerous inhibitors of tyrosinase. These enzyme inhibitors are very important because they can be used in various fields, including cosmetic, medicinal and food industries. Many tyrosinase inhibitors such as hydroquinone, ascorbic acid derivatives and kojic acid are commonly used in cosmetic preparations as skin whitening agents. However, their clinical potential have been questioned because of a numerous side effects during its application such as contact dermatitis, leucoderma, hypochromia and ochronosis [6]. Because of the role, which abnormal activity of tyrosinase plays in numerous pathological states, it seems reasonable to search new and effective inhibitors of this enzyme, which do not cause any cytotoxic side effects.Here, we present the obtained results of biological research of presented heterocyclic phosphonic and phosphinic acids as a novel possible potent inhibitors of tyrosinase, which could be base to further research.

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P9.23

The differences in gene expression and catalytic activity of galactinol synthase isoforms of higher plants

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Galactinol synthase (GolS) is the key enzyme in the biosynthetic pathway of raffinose family oligosaccharides (RFOs). GolS synthesizes galactinol from UDP-galactose and myo-inositol. This reaction constitutes the first committed step in the biosynthesis of RFOs. Accumulation of RFOs in maturing seeds is correlated with seed desiccation tolerance. RFOs also accumulate in vegetative tissues in response to drought and low temperature and may enhance plant's resistance to these abiotic stresses. Plants often contain several isoforms of GolS which may be expressed in different tissues or under different conditions and therefore perform distinct biological functions.The aim of this study was to analyze structural differences between two types of GolS proteins from Vicia hirsuta and possible effects of these differences on catalytic activity.
The protein sequences have been downloaded from GenBank database: GolS1 (AGW51291.1) and GolS2 (AGW51290.1). The sequences have been used to generate structural models of both proteins using homology modeling. The ligand (UDP-α-D-galactose) docking in the active center and the whole complex energy minimization has been conducted for both isoforms. The molecular dynamics (using CHARMM36 forcefield) has been utilized to verify the complex stability and the forces of ligand binding.
The first in silico results of molecular modeling show that there are no significant differences in complex stability and ligand binding strenght between GolS1 and GolS2. It is therefore most likely that both enzymes have very similar properties.
Our preliminary analyses show that both GolS genes have different expression patterns, which is consistent with publicly available microarray data for Medicago truncatula, where the two GolS homologs are also expressed differently. Above results indicate that the differences in biological functions of both enzymes stem only from differences in their expression patterns.

Acknowledgements

This research was supported by NN310088137 funds.

P9.24

EPR analysis of the temperature effect on molecular dynamics of thylakoid membranes isolated from diatoms, green algae and higher plants

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Although diatoms are the dominant organisms in cold habitants and provide 40% of the marine and 20% of the global primary production, there are only a few data on acclimatization to low temperatures of these algae. It is known that the low temperature leads to membrane rigidification in model lipid bilayers. Organisms possess several mechanisms which prevent the disadvantageous changes in the molecular dynamics of the membranes. The changes of the fatty acids composition of the membrane phospholipids and glycolipids are one of the most important s mechanisms observed in plants and algae to maintain the optimal fluidity of the membrane at low temperature. Membrane lipids of diatoms contain fatty acids with longer chains and higher degree of unsaturation than higher plants or green algae (20:5 or 22:4). The purpose of the study was a comparative analysis of the influence of temperature on fluidity of thylakoid membranes isolated from representatives of three different groups of phototrophs: diatoms (*Thalassiosira pseudonana*, *Phaeodactylum tricornutum*), green algae (*Chlamydomonas reinhardtii*) and higher plants (*Spinacia oleracea*). The experiments included both effect of growth temperature of the organism studied and the changes of measuring temperature in the range from 0 to 40°C on fluidity of thylakoid membranes. The growth temperatures tested were 12 and 20°C. Thylakoid membranes were isolated according to standard methods, adequately to the species. Chorophyll a concentrations were analyzed by Jeffrey and Humphrey (1975) and Lichtenthaler (1987) methods for diatoms and higher plants respectively. The membrane molecular dynamic of fresh thylakoid membranes were monitored by EPR spectroscopy using two spin labels 5-SASL and 16-SASL and values of order parameter (S) values were calculated. Thylakoid membrane fluidity analyzed with SASL-5 shown that differences between all tested plants were not statistically significant. The S-parameter values decreased in the range from 0.8 to 0.5 independently on growth temperatures. On the other side, the use of 16-SASL revealed that molecular dynamics of hydrophobic region of the membranes was higher for two tested diatoms species than that observed for *Chlamydomonas* and spinach thylakoids. Whereas *Chlamydomonas* and spinach thylakoid membranes shown the same values of S parameter, among tested diatoms the highest molecular dynamic of membrane were recorded for *Th. pseudonana*. Mentioned above observations were the same for the two growth temperatures studied. The higher fluidity of the diatom membranes can be explain by different fatty acids composition. Main thylakoid lipids of diatoms have higher unsaturated degree of the fatty acids than respective lipids of green algae and higher plants. Apparently, the level of unsaturated fatty acids of *Th. pseudonana* thylakoids is higher than that detected for *P. tricornutum*. Obtained results also showed that lipids and their fatty acids composition seem to be independent on growth temperature.

Acknowledgements

This work was supported by project No. 2011/01/M/NZ1/01170.

P9.25

The conjugated auxin, indole-3-acetyl-aspartate, induces the changes in pea responses to some abiotic factors

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Auxin (indole-3-acetic acid, IAA) acts as a pivotal plant hormone that controlls variety physiological processes. Moreover, IAA is also involved in plant response to both biotic and abiotic stress conditions. The concentration of active auxin in plant tissues must be tightly regulated by processes including: IAA synthesis and degradation, transport to or from cells, and reversible or irreversible conjugation with amino acids, peptides, sugars or proteins. The conjugation reactions involve IAA conversion to IAA-amide (IAA-amino acid, IAA-peptide, and IAA-protein) or IAA-esters (IAA-sugar, IAA-*myo*-inositol) compounds. IAA-aspartate (IAA-Asp) is the most commonly amide linkage of auxin in pea tissues. Previously, we identified and described the specific IAA-Asp synthetase in pea seeds. Despite, IAA-Asp acts as an intermediate in auxin catabolism, the more recently investigations suggest a direct biological function of this conjugate during grape berry ripening and in promotion of disease development in *Arabidopsis thaliana*. To date, the involvement of IAA-Asp in mentioned above processes has not been clearly described. In this study we investigate the effect of IAA-Asp on expression and activity of ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT) during cultivation of pea seedlings on NaCl (salt stress) and CdCl2 (heavy metal stress). Moreover, we assayed the protein carbonylation and H_2O_2 concentration. Our results indicate that IAA-Asp can modulate the effect of NaCl and $CdCl_2$ on abiotic stress effectors. Pea seedlings which were cultivated on 100 μM IAA-Asp for 24 hours exhibited about 3-fold higher level of H_2O_2 than control plants. GPX activity decreased under these conditions. On the other hand, IAA-aspartate strongly induced the effect of NaCl and $CdCl_2$ on APX and CAT activity after 24 h. Moreover, we observed additional protein bands displaying catalase activity after native PAGE/zymography assay. All tested samples revealed higher level of carbonylated proteins in comparison with control. Results of this study suggest that IAA-Asp can affect the plant responses to abiotic stress.

P9.26

Using the yeast two-hybrid system to identify proteins interacting with calcium dependent protein kinase (CDPK)

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CDPK kinases are specific regulatory enzymes present exclusively in plants and some protists. CDPK kinases which are single polypeptides contain variable N-terminal domain, highly conserved Ser-Thr catalytic domain and CAD domain which comprises of an autoinhibitor domain and calcium-binding EF-hands resembling those present in calmodulin. The analyses of various CDPK transcripts and protein levels proved the essential roles of these kinases during plant growth and development as well as in biotic and abiotic stress responses. However, the remaining mystery is what the specific substrate and partner proteins of particular CDPKs are. To help understand, we used the Matchmaker Gold yeast two-hybrid system (Clontech) to find proteins interacting with CDPK during specified plant physiological response. cDNA library was constructed and introduced into Y187 yeast strain cells with "Mate & Plate" system (Clontech). To increase the chance of true positive interactions, 5 different plant CDPK isoforms were prepared by conducting directional cloning with highly effective In-Fusion system (Clontech) and directed mutagenesis (Agilent Technologies). The wild type full length kinase and 4 different combinations of CDPK mutants (full length kinase catalytically impaired, constitutively active kinase without CAD domain, kinase without CAD domain catalytically inactive and constitutively active F343A isoform) were used as baits. All the prepared CDPK isoforms were cloned in pGBKT7 bait vector and introduced into Y2H-Gold yeast strain cells through the PEG-lithium acetate method. The tests for bait protein toxicity and autoactivation were performed. During the screening, four different reporter genes were analyzed: *AUR1-C*, *HIS3*, *ADE2*, *MEL1*. Obtained results showed that the wild type CDPK bait detected three prey proteins corresponding to ascorbate peroxidase protein, CBF nucleic acid binding protein and a ribosomal protein. Moreover, we present how the use of constitutively active CDPK baits improved the efficiency of recovering positive interactors relative to the wild type kinase and the catalytically impaired versions.

Acknowledgements

This work was supported by National Science Centre (Poland) grant No. UMO-2011/03/B/NZ9/00752.

P9.27

Identification of novel functions for Arabidopsis RCF (ERECTA) proteins in gibberellin signalling pathway

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Gibberellic acid (GA) is one of the five classical plant hormones. GA regulates growth and influences a number of developmental processes, including seed germination, stem and root elongation, flower induction, development, dormancy, and leaf and fruit senescence. Here we show that the RCF1-3 (Receptor for GA Signaled Flowering 1,2,3/ ERECTA) family of membrane-localized LRR receptor-like kinases act as components of GA signaling. We found that triple *rcf1-3* mutant exhibited strong dwarf phenotype, resembling features of triple *gid1* mutant. None of these phenontypic features could be reverted by gibberellin treatment, indicating that *rcf1* mutant is GA-insensitive. In addition, the phenotype of the *ga1-3* mutation was strongly enhanced in *rcf1/ga1-3* double mutant line. Furthermore, the Relative Growth Rate (RGR) analysis showed that *rcf1* mutant responded with similar kinetics as wild type plant. In contrast, *rcf2* and *rcf3* mutant plants displayed an accelerated first phase (indicative of function in the quick GA response). On the other hand, without GA treatment there was a dramatic reduction of growth potential in *rcf1* mutant and relatively higher RGR in *rcf2* and *rcf3* mutants. Based on these observations, wehypothesize that in the context of a single mutant, RCF1 acts as a positive while RCF2 and RCF3 act as negative components of GA signaling.
During our previous study we discovered functional interdependence between SWI/SNF ATP- dependent chromatin remodeling complexes and DELLA proteins, the growth repressors involved in GA signaling pathway. Here, we report a direct link between regulation of SWI/SNF complexes' stability and the function of DELLA protein in GA mediated growth. We show reduction in the *ga1-3* mutant of the protein level of of one of the core components of SWI/SNF complex, the SWI3B subunit. Importantly, the SWI3B protein level was restored in *ga1-3* mutant after GA treatment leading to degradation of DELLA proteins. To test the relationships between GA signaling and SWI/SNF complex in *rcf1-3* triple mutant, we analyzed in this line the abundance of SWI3B protein. Interestingly, we found the level of SWI3B protein reduced in the *rcf1-3* line and discovered that in contrast to *ga1-3* mutant, GA treatment did not lead to increase of the SWI3B level. This suggests regulation of SWI/SNF stability by *RCF1-3* and explains the reduced GA response in the triple mutant.

Acknwledgements

Financial support: 2011/01/B/NZ1/00053 National Science Centre (NCN).

P9.28

Functional characterization of SWP73A and SWP73B — subunits of Arabidopsis SWI/SNF chromatin remodeling complexes

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The SWI/SNF-type chromatin remodeling complexes (CRCs) are evolutionary conserved amongeukaryotes. SWI/SNF CRCs are involved in regulation of transcription, DNA replication and repair, and cell cycle. So far, there is no data about the function of SWP73A in *Arabidopsis*. Plants with RNAi silenced *SWP73B* gene show dwarf phenotype and *SWP73B* is involved in flowering time regulation by directly control of *FLC* and leaf growth through interaction with AN3.
Our current work focuses on genetic and biochemical characterization of *Arabidopsis* SWI/SNF complexes carrying SWP73A or SWP73B subunits and their role in vegetative and generative development. We found that *swp73a* mutant does not show significant morphological defects under normal growth conditions, while *SWP73B* modulates most of the developmental processes including root growth, maintenance of leaf symmetry and fruit development, mutation of *SWP73B* causes characteristic alterations in the development of vegetative and reproductive organs. We also confirmed using BiFC and YTH assays that SWP73A and SWP73B proteins interact directly with core subunits of SWI/SNF complex and regulator of gibberellins (GA) signaling pathway.
Our further study using qRT-PCR, ChIP, Mnase digestion, Nomarsky optics, electron and confocal microscopy showed that SWI/SNF complexes containing SWP73B are important platform integrating various regulatory processes of plant growth such as embryogenesis, cell cycle, leaf and flower development as well as hormone signal transduction. Our findings provide novel insight into the biological importance of SWI/SNF complexes containing SWP73A and SWP73B.

Acknwledgements

Financial Support: Grant No. N N301 645040 by National Science Centre.

P9.29

Chloroplast biogenesis of different *Arabidopsis* ecotypes

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Chloroplast biogenesis is a multistage process leading to fully differentiated and functionally mature plastids. Differentiation of proplastids to etioplasts with paracrystalline prolamellar bodies (PLBs) and prothylakoids (PT) occurs during the natural scotomorphogenesis, when the initial seedling growth takes place under the soil surface, during germination of the seed in the dark [1].
Thus this process, called etiolation, is a natural stage during the initial plant ontogenesis Photomorphogenesis of young etiolated seedlings takes place upon illumination which causes etioplasts to differentiate into chloroplasts through the PLB transformation and the thylakoid network development together with acquisition of the photosynthetic capacity.
Arabidopsis thaliana is a model organism for plant biology because of its size, availability, rapid growth, and low soil requirements. Arabidopsisis also well suited for studies of the ecotype natural variations. Arabidopsis ecotypes growing in various places around the world exhibit striking differences e.g. light and hormone sensitivity, seed size, the light-dependent hypocotyls and seedlings growth in various environments [2].
In our studies we compare chloroplast biogenesis in three Arabidopsis ecotypes: Col-0, Col-1, Col-2, analyzing the chloroplast ultrastructure with the help of transmission electron microscopy and the photosynthetic complex formation using low-temperature (77K) fluorescence measurements. In this way we correlate, at the same time intervals, the structure of etioplasts transforming into chloroplasts with the function of photosynthetic apparatus. For this presentation we choose four stages of plastid internal membrane arrangement: directly after 8 days of etiolation, after two hours of light exposition, after eight hours of illumination and after 8 hours of re-etiolation during the second day of the experiment. The differences and similarities between various Arabidopsis ecotypes can provide the base for further investigations on the chloroplast biogenesis in Arabidopsis lipid mutants.

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P9.30

Cloning, purification and activity determination of HpCDPK1(Calcium-Dependent Protein Kinase from *Hippeastrum hybr.*) recombinant protein

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CDPKs are ideal candidates for perceiving intracellular changes in Ca^{2+} concentration and translating them into specific phosphorylation events to initiate further downstream signaling processes. The resolution of kinase-specific phosphorylation patterns within a target protein provides evidence suggesting that CDPKs act as signaling hubs in plant stress signaling and development.
In our research we are endeavoring to find the elements of the calcium signaling pathway taking part in defence responses after mechanical wounding and pathogen attack on *Hippeastrum* bulbs. The one of the tasks is identification and characterization of calcium-dependent protein kinases (CDPKs).
Here, we present cloning technique, purification procedure and activity determination of a recombinant kinase Hp-CDPK1 and prove that this enzyme belongs to a CDPKs family.
To obtain purified recombinant protein the pGEX-HpCDPK1 plasmid construct contained the glutathione-S-transferase (GST) sequence joined to 5' coding region of the *HpCDPK1* cDNA was made. *HpCDPK1* ORF was amplified by PCR and subcloned into the pGEX-6P-2 expression vector at *SalI* and *NotI* restriction sits. The pGEX 5' and 3' sequencing primers were used to verify the proper orientation and correct in-frame junctions of cloned insert. The *E. coli* BL21 strain was used to produce the GST-tagged protein and the expression was induced by the addition of isopropyl β-D-thiogalactoside (IPTG).
GST-HpCDPK1 fusion protein was purified by affinity chromatography using glutathione immobilized to a Sepharose matrix. HpCDPK1 was released from the fusion protein by proteolytic cleavage with PreScission protease. For a control expression pGEX-6P-2 vector was used or GST alone was purified.
The homogeneity and purity of eluted protein fractions were analyzed by SDS–PAGE. For Western blotting analysis polyclonal anti-GST antibodies or anti-CDPK antibodies were used. The blots were visualized by a chemiluminescence. Activity of enzymes was determined *in vitro* by measuring the incorpotation of ^{32}P from [γ-^{32}P] into a H-III as a substrate.

Acknowledgements

This work was supported by the National Science Centre (Poland) grant No. UMO-2011/03/B/NZ9/00752.

P9.31

Physiological and antioxidant responses of two *Arabidopsis thaliana* accessions in different light and temperature conditions

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Light and temperature are factors that affect growth of plants to the highest extent. In this study, we investigated physiological and antioxidant responses of two *Arabidopisis* accessions, Shahdara (Sha) from Tadjikistan and Lovvik-5 (Lov-5) from northern Sweden. We showed that both accessions improved their growth at high-light and lower temperature reflecting adaptation to their growth habitats. Moreover, non-photochemical quenching was higher when both Sha and Lov-5 were grown at lower temperature but the Sha accession, originating from the Shokhdara valley (Pamir-Alay Mountain, Taijkistan, Central Asia), showed higher values of non-photochemical quenching. Under higher light intensities and especially at the lower temperature, both Sha and Lov-5 showed no increase in the antioxidant prenyllipids level in contrast to the numerous literature data regarding Columbia-0 (Col-0), an accession of temperate climate. Both the examined accessions showed also higher oxidation level of PQ-pool than that reported for Col-0 under low-light conditions. Mechanisms responsible for keeping PQ-pool in a more oxidized state are most likely the main factors responsible for the adaptation of the investigated accessions to the climate conditions of their origin.

P9.32

Qualitative and quantitative analysis of non-polar lipids isolated from plants with different chilling sensitivity

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Thylakoid membrane represents the most common type of lipid bilayer in the world. It consists of polar lipids, specific proteins of photosynthetic complexes and related molecules, such as non-polar lipids, in particular photosynthetic pigments [1]. Non-polar lipids are recognized to play several important physiological roles, including: antenna function and photoprotection of photosynthetic apparatus, scavenging active oxygen species and regulating the physical properties of biomembranes. Orientation of the non-polar lipids in the membrane, possible change in the membrane fluidity and its thermodynamic and mechanic features depend on the structure of the pigment. Pigments lacking polar groups are randomly spread across the membrane e.g carotene, while polar pigments, which stabilize and protect the membrane lipid phase span the membrane decreasing its fluidity e.g lutein, zeaxanthin [2].

Our previous studies of the chilling sensitive bean (*Phaseouls vulgaris* L.) and chilling tolerant pea (*Pisum sativum* L), demonstrated that the mature chloroplasts differ in the thylakoid organization [3]. The aim of this study was a qualitative and quantitative analysis of non-polar lipids isolated from plants with different chilling sensitivity. Analyses were carried out using μUPLC method. Leaves of studied plants were collected and the isolation procedures were used to receive intact thylakoids. The extraction method was optimized using hexane as the main extraction solvent executed in the temperature of 4°C and perfused with argon. Separation of samples was monitored by a photodiode array at 200–750 nm range for better identification. A single chromatogram at 436 nm (characteristic for carotenoids) was extracted and exported for further analysis.

Significant differences between studied plants both in quality and quantity of non-polar compounds were observed. These changes appear also during the biogenesis process when the formation of stacked thylakoid membranes from prolamellar body was observed. Our results indicate adaptation of chloroplast membrane to low temperature.

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P9.33

A first approach of comparative EDS and ultrastructural analysis of stalks of the diatom *Didymosphenia geminata*

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Diatoms are unicellular, eukaryotic and photosynthetic organisms living as single cells or forming colonies via mucilaginous material. *Didymosphenia geminata* is the freshwater diatom that grows attached to various substrates (e.g. rock surfaces) via mucilaginous stalks. The massive production of extracellular stalks is responsible for the negative ecological impacts of *D. geminata* due to formation of mats covering up to 100% of substrates with thicknesses exceeding 20 cm. Extracellular polymeric substances that comprise the stalk are predominantly composed of sulfated polysaccharides and proteins with some uronic acid content, which form complex structures. From morphological point of view, stalks are fibrous, possess cross-linked structure and nanocrystalline fibers, which most likely determine their mechanical properties.

We studied ultrastructure and chemical composition of *D. geminata* stalks by SEM, TEM and X-ray microanalysis (EDS). Sample preparation was based on Moffat (1994) and Aboal *et al.* (2012). Our studies showed the complex internal structure of *D. geminata*: the cross-section of stalks revealed two or — depending on preparation procedure — three layers. The most inner layer forms "core" with reticular structure whereas the middle one is composed of densely disposed crystalline fibers. The outermost layer is smooth and acts like a mantle. EDS analyses of different areas of stalk cross-section revealed elements: C, O, Ca, Si, S and Fe in various concentration. The highest concentration of Ca and S was noted in the inner layer.

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Acknowledgments

Authors would like to express their gratitude to Prof. Elżbieta Wyroba for support and help during investigations and to my supervisor Prof. Krzysztof J. Kurzydlowski.

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The influence of blue light dependent plastid and nuclear movements on UV caused DNA damages

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Being photoautotrophic organisms, plants depend on light. In their evolution they developed several mechanisms that enable them to optimize the solar light absorption. One of them is a well-known chloroplast accumulation reaction in weak blue light, which helps to increase photosystem saturation in light deficience conditions. The opposite chloroplast avoidance reaction takes place during strong blue light irradiation and it is believed to serve as the photosynthetic apparatus protection against excessive irradiation which may lead to its damage. Blue light receptors responsible for chloroplast movements are phototropins. In Arabidopsis thaliana, phototropin 1 (phot1) and phototropin 2 (phot2) were discovered. Phot1 answers solely for accumulation reactions of plastids, while phot2 is responsible for both movements. The nuclear movements were also observed in strong blue light. This may suggest that the movement may serve as a mechanism protecting genetic material from damages caused by UV, which always accompany the visible light in the environment. The nuclear avoidance reaction is observed only in a presence of functional phot2, no phot1 influence has been observed. This reaction appears not only in photosynthetically active mesophyll, but also in epidermal cells that contain no or lack of small plastids. The aim of the study was to determine the influence of chloroplast and nuclear movements on the level of damages in nuclear and chloroplast DNA and its predicted protective role.

Columbia wild type (Col-0) plants and photoreceptor mutants (phot1, phot2, phot1phot2) were examined. After dark positioning of chloroplasts, leaves or whole plants were treated with weak or strong blue light to achieve the face or the profile position, respectively. This was followed by irradiation with different UV doses. The nuclear and chloroplast DNA was isolated separately and the thymidine dimers level was measured by ELISA test. The obtained results clearly confirm the decrease of damages of both nuclear and chloroplast genome in the avoidance reaction.

Acknowledgments

The study was supported by Polish National Science Centre, a grant no. UMO-2011/03/D/NZ3/00210.