Plenary lecture

PL5.1

Subcellular antioxidative defense of plants during abiotic stress

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Ascorbate and glutathione are the most important antioxidants in plants. They are involved in the detoxification of reactive oxygen species, redox signaling, the modulation of gene expression and are important for the regulation of enzymatic activities. Inter- and intracellular ascorbate and glutathione contents and their ratio between certain cell compartments are important measurements of the plants ability to sense and fight oxidative stress and can give key information about the physiological condition of the plant. This presentation will give an overview about the compartment specific importance of ascorbate and glutathione in *Arabidopsis thaliana* Col-0 plants during abiotic stress conditions such as high light, drought and the exposure to cadmium. By comparing the situation between wildtype plants and mutants with altered glutathione metabolism (*cat2* and *pad2-1*) it was possible to gain thorough knowledge about the subcellular distribution of ascorbate and glutathione in plants and on the importance of these antioxidants in certain cell compartments in the protection against abiotic stress. The author would like to thank the Austrian Science Fund (FWF P22988) for financial support.

PL5.2

Generation of reactive oxygen species in plants during high light stress and their role in signaling

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Electron transfer reactions in the presence of O_2 can lead either directly or indirectly to a significant production of reactive oxygen species (ROS) which can damage the cell. Singlet oxygen ($^1O_2$) and the three intermediate redox states, superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$), are much more reactive than the relatively stable molecular oxygen ($^3O_2$). ROS are produced during normal cell metabolism. This production is drastically enhanced when plants are exposed to biotic or abiotic stresses like high light intensities, extreme temperatures, drought or heavy metals. The main site of ROS generation upon light stress is the photosynthetic electron transport chain. Singlet oxygen. $^1O_2$ is generated by charge recombination reactions in the reaction centre of photosystem II (PSII), while superoxide is mainly generated at the acceptor side of photosystem I (PSI). We have shown that the midpoint potential of primary quinone acceptor plays an important role in controlling the yield of $^1O_2$ generation. In PSII with an up-shifted midpoint potential of Q_A, charge recombination is more likely to occur via a direct recombination pathway that does not lead to the formation of ^{3}Chl and $^1O_2$. This control mechanism will be discussed. Furthermore, we have investigated the role of $^1O_2$ in retrograde signalling by following changes in the expression of reporter gene constructs which are responsive to either $^1O_2$ or H_2O_2 and of *gpxh*, a homologue of the glutathione peroxidase in *Chlamydomonas reinhardtii* H_2O_2 signalling. Recently, we have investigated H_2O_2 signalling and observed that H_2O_2 accumulated transiently upon exposure of *C. reinhardtii* cultures to high light. The accumulation of H_2O_2 was correlated with a reversible inactivation of catalase activity. We propose that under high light intensity the redox state of the photosynthetic electron transport chain is sensed and transmitted to the cytosol to regulate the catalase activity. This allows a transient accumulation of H_2O_2 in the chloroplast inducing a signaling event that is transmitted to the nucleus to modulate the expression of chloroplast-directed protection enzymes. $O_2^{\bullet -}$ generation. At present we investigate the generation of $O_2^{\bullet -}$ at the acceptor side of PSI. Leaves of tobacco or Arabidopsis plants grown in short days (8 h light) generate more ROS in the light than leaves of plants grown in long days (16 h light).

A two fold higher level of $O_2^{\bullet -}$ production was observed even in isolated thylakoids from short day plants. By using specific inhibitors of photosystem II or the cytochrome b_6f complex, the site of O_2 reduction could be assigned to photosystem I. Preliminary data indicate that the reduction of O_2 at the acceptor side of PSI is redox-controlled.

PL5.3

The role of PsbS and chloroplast retrograde signaling in regulation of various Darwinian fitness traits in *Arabidopsis*

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Various environmental stresses cause absorption of light energy in excess of that required for photosynthesis. Foliar heat dynamics measurements combined with time-resolved fluorescence demonstrates that higher plants evolved genetic and physiological global regulatory system, which optimizes photosystem II quantum-molecular functions and the fate of photons absorbed in excess. Our results indicate the role of the PsbS and photosystem II antenna organization in efficient and discrete global regulation of the rates between photochemistry, fluorescence, and heat (*3*). This is associated with generation of reactive oxygen species (ROS), changes in chlorophyll *a* fluorescence parameters and ROS-hormonal homeostasis that induce retrograde signaling and promotes plant acclimation. Obtained results suggest that LESION SIMULATING DISEASE 1 (LSD1), enhanced disease susceptibility 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4) constitute at least tree component molecular machinery regulating retrograde signaling and plant Darwinian fitness traits. Our field and laboratory experiments suggests that changes in chlorophyll fluorescence parameters, water use efficiency, foliar hormonal and reactive oxygen species homeostasis, and seed yield of *Arabidopsis* are conditionally regulated and integrated by these regulators. Mathematical modeling suggests that changes in chlorophyll fluorescence parameters, water use efficiency, hormonal and reactive oxygen species cellular homeostasis, and seed yield of *Arabidopsis* could be defined by the exponential function and simple equation with natural logarithm ($y = y_0 * e^{-Kx}$), that depends on these regulators. We concluded that *Arabidopsis* plants during growth perform discrete global regulation of the rates between photochemistry and retrograde signaling thus perform biological processing aimed at optimizing and integrating photosynthesis, water use efficiency, ROS and hormonal cellular homeostasis. This processing allows reaching the best possible seed yield and Darwinian fitness in multivariable natural environment.

Oral presentation

O5.1

High temperature-regulated expression of microRNAs in barley

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Micro RNAs (miRNAs), a class of small RNAs, usually of 21 nucleotides long, are acting as key regulators of eucaryotic gene expression at the posttranscriptional level. Despite the crucial role of miRNA molecules in plant development, organ formation and responses to various stresses, the structure of miRNA genes, transcription and processing of miRNA precursors in crop plants remains largely unknown. Previously we examined the biogenesis of barley miRNAs and the developmental regulation of their pri-miRNA processing (1). The aim of this study was to

investigate the role of selected miRNAs in barley response to high temperature. We started by determining the gene structure and the processing of their transcripts of eight novel barley micro RNA genes - miR160a, 166a, 167e, 1120b, 1318-5p, 530-5p, 5180b, and 5175a. Apart from miR166a and 167e, the other genes contain from 1 to 10 introns. Most frequently micro RNA and micro RNA* are found within the first exon of intron-containing genes. However, three examples have been found with the miRNA/miRNA* located within introns – miR160a, 1120b and 5175a. We investigated also the effect of high temperature on the level of mature micro RNAs in barley spring cultivar Rolap using northern hybridization. In the case of three micro RNAs – miR160a, 166a, and 167e – we observed an increase in the level of mature miRNAs up to 2.3 times compared with the control conditions. We determined also their pri-miRNA levels by quantitative PCR. For miR167e precursor but mainly for pri-miR166a we detected increased level of the transcripts in heat stress conditions. In addition, RT-PCR analysis revealed changes in the level of different splicing isoforms of pri-miR160a and 5175a precursors. We conclude, that during heat stress the level of barley miRNAs is regulated not only by transcription, but can also be posttranscriptionally regulated at the step of pri-miRNA processing. As the miRNAs function as the key regulators of the gene expression we are currently investigating the potential target transcripts of the high temperature-responsive micro RNAs and build an metabolic network involving these miRNAs. This work was sponsored by POLAPGEN-BD UDA.POIG.01.03.01-00-101/08 "Biotechnological tools for breeding cereals with increased resistance to drought", subject 20: "The role of micro RNA in regulation of mechanisms leading to drought adaptation in plants", executed within Innovative Economy Programme 2007-2013, subject "Biological progress in agriculture and environment protection".

O5.2

Protein carbonylation and hydrolysis in wheat seedlings under drought

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One of the inevitable consequences of drought is enhanced ROS production in the different cellular compartments, namely in the chloroplasts, the peroxisomes and the mitochondria. In plants, low concentration of ROS activates defense responses, whereas higher level seems to be responsible for oxidative damage of proteins. The consequence of oxidative stress is protein carbonylation, which involves the modification of the side chains of certain amino acids e.g. Pro, His, Arg, Lys, and Thr to produce ketone or aldehyde derivatives that are reactive with 2,4-dinitrophenylhydrazine. Such oxidative protein modification might lead to alteration in protein activity, its breakdown or, in the opposite, aggregate formation. In the comparative examination of metabolic response to water deficiency at the dehydration tolerant (four-day-old) and dehydration sensitive (six-day-old) phase of wheat (*Triticum aestivum* L.) growth it has been shown that the carbonyl group concentration in whole tissue extracts from both types of seedlings is maintained at the same low level. However, the response of seedlings to water deficiency was different. The highest increase in concentration of carbonylated proteins was observed at the beginning of drought (15-25% WSD) being almost two-fold higher in sensitive (six-day-old) than in tolerant (four-day-old) seedlings. Such a rapid increase in ROS-modified proteins may be associated with their function in the cell signaling under drought. In a sensitive phase of seedlings growth (six-day-old) the azocaseinolytic activity of wheat seedlings was higher than in the resistant phase of seedling growth. Similarly, the share of cysteine proteinases in total proteolytic activity was higher in drought sensitive seedlings. In response to water deficit, the proteolysis was reduced 2-fold in sensitive seedlings at 15% WSD, whereas in the resistant six-day-old seedlings proteolysis increased slightly but significantly. This result may suggest that increased concentration of carbonylated proteins and decreased intensity of protein hydrolysis in drought sensitive seedlings may be related to the accumulation of abnormal proteins and resulted in the loss of tolerance to drought. Although carbonylated proteins seem to be preferentially degraded by non-vacuolar proteasome-dependent pathway with the 20S proteasome and not 26S the involvement of vacuolar pathway in degrading oxidized proteins cannot be excluded.

O5.3

Metal transporter AtNRAMP1 is essential for optimal photosynthesis and growth under manganese deficiency and low temperature

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Manganese (Mn) is an essential micronutrient required for plant growth and development. It is of particular importance in the process of photosynthesis where Mn has an essential role in both the structure and functions. Photosynthetic performance is directly related to crop yields and is influenced by various abiotic stresses like water stress, salinity, high or low temperatures and nutrient deficiencies. In present work, an automated screening system detecting alterations in the effective quantum yield of photosystem II (PSII) was used to quantify photosynthetic performance of 108 *Arabidopsis thaliana* accessions grown at 28 °C and 16 °C. Chlorophyll fluorescence is a widely used tool to estimate PSII activity, which is an important target of abiotic stresses. Under high temperature, no significant differences in photosynthetic yield between accessions were observed, whereas under lower temperature only one accession (Hog) showed pale green leaves and a reduction in photosynthetic yield. Moreover, Hog accession showed induction of a severe leaf chlorosis after transfer to 4 °C. Interestingly, the presence of chlorosis was dependent on the soil mixes used. The observed genetic x environment (GxE) interaction was associated with impaired plant growth and a large reduction in photosynthetic performance. This led to lethality when Hog seedlings were directly grown at low temperature. In order to map the genomic region(s) involved in the observed GxE interaction, a backcross population was developed between Hog accession and L*er* as a recurrent non-chlorotic parent. Consequently, a region of 0.06 Mb at the bottom of chromosome 1 was identified to be responsible for the above described traits. Based on predicted functions and ontologies for genes present in the mapped region, twenty candidate genes were selected, for which T-DNA insertion mutants were analyzed. Results from the allelism tests clearly identified *NRAMP1* as the causal gene. Moreover, Hog *NRAMP1* sequence showed histidine to tyrosine substitution only present in Hog plants from the laboratory in the MPIPZ (Germany), underlying the presence of spontaneous mutation at this position. We revealed that Arabidopsis plants carrying mutated alleles of *NRAMP1* show severe chlorosis and impaired growth after transfer to low temperatures. Importantly, the chlorotic phenotype could be reversed by watering plants with chelated manganese fertilizer. These results together with chemical analysis of soil mixes indicated that Mn deficiency is the major cause of the observed chlorosis. The identification of mutants with altered Mn phenotypes can increase our understanding of mechanism of Mn toxicity and tolerance in plants. Therefore, further functional analyses of AtNRAMP1 underlying responses to low temperatures are being performed. This research was supported by grants from the Max Planck Society and the University of Gdansk (Gdansk University Grant 538-M031-B145-13).

O5.4

Changes in content of polar and non-polar lipids in plants with different chilling sensitivity

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Chilling stress i.e. the ability of plant to tolerate low temperature in range of 0-15 °C is one of the most important factors affecting plant growth. It limits the geographical range of many economically important crops. Low temperature causes visible disorders in cellular processes in chilling sensitive plants e.g. presence of necrotic spots on lea-

ves, partial necrosis of leaves and decline in content of chlorophyll and polar lipids. Chloroplasts are plant cell organelles most exposed to low temperatures. Furthermore, the first damages associated with chilling stress appear in thylakoid membranes. Chloroplast membranes mainly consist of lipids (polar and non- polar) and proteins. Lipids represent about 20% of the dry weight of chloroplast, in which 90% constitutes of polar lipids. We already shown that formation of stacked thylakoid membranes from prolamellar body is correlated with changes in photosynthetic proteins' levels and the function of photosynthetic apparatus in two plant species: bean (*Phaseouls vulgaris* L.) and pea (*Pisum sativum* L.). We selected these plant species since we demonstrated previously that the mature chloroplasts differ in the thylakoid organization. The aim of this study was to separate and characterized the changes in polar and non-polar lipids' content in chloroplast membranes using non-isocratic HPLC-ESI/MS and UPLC methods. Plants were exposed to low temperature (5°C) at night while the day- time temperature at the climate room was optimal (22°C). Temperature changes weren't immediate but ramped for three hours. We show differences between studied plants both in quality and quantity of polar and non-polar compounds. We analyze both plants during periodic chilling stress. Demonstrated differences in lipid content in leaves of chilling sensitive bean and chilling tolerant pea are observed. Our results indicate adaptation of chloroplast membranes to low temperature. This research was supported by Polish Ministry of Science and Higher Education funds (N303 530438).

Posters

P5.1

Transcriptomic responses of cauliflower (*Brassica oleracea* var. *botrytis*) mitochondria under thermal stress

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Plant mitochondria belong to the cell compartments dynamically involved in stress response and stress modulation. Transcriptomic changes (related with mRNA turnover and abundance) of messengers encoding for mitochondrial proteins are not always accompanied by variations on the protein level. This implies the dynamic regulation of the mRNA pool that is accessible for synthesis of mitochondrial proteins. Analysis of coordination of those responses within plant mitochondria under some abiotic stress conditions including thermal treatments is also particularly interesting for species important in agriculture. The aim of our study was to perform semi-quantitative and quantitative analyses of response of cauliflower transcriptome encoding for mitochondrial proteins in relation to cold and heat stress and also after post-stress plant recovery. Plants were grown either in control conditions or they were submitted to short heat stress (42°C, 2 h) or 10-day-long cold treatment. Some plants were also cold- or heat- recovered. Total RNA was isolated from cauliflower 3-month-old curds as well as from young (1-month-old) leaves using Trizol reagent. cDNA was synthesized using M-MLV reverse transcriptase and random hexamers, and amplified by multiplex RT-PCR or q-RT-PCR (on Applied Biosystems 7900 HT system). We analyzed the level of mitochondrial transcripts (*nad*3, *nad*6, *nad*9, *cox*II, *atp*1) as well as nuclear ones for some mitochondrial proteins that appeared stress- responsive in our previous studies (*AOX1a*, *CPN10*, *HSP81, PDH-E1, ProDH, P5CDH, MPP*b, *SCL*b, *3-PGDH, ATP2*). The level of mitochondrial *nad* messengers was differently regulated in various stress conditions and tissues; more evident their co-expression was expected. In some cases, the level of investigated nuclear- encoded mitochondrial transcripts (for instance, *AOX1a*, *ProDH*, *MPP*b, *SCL*b, *3-PGDH, ATP2*) evidently did not correlated between themselves in stress conditions and depended on tissue. The accumulation of some messengers (*AOX1a*, *CPN10*, *Hsp17.6-CII*, *HSP81*) was diversely induced by cold and heat. The level of cauliflower *AOX1a* mRNA (accession no. KC631778) in stress validated by q-RT-PCR followed results from semi- quantitative assays. *AOX1a* mRNA level was increased in all stress conditions (3.2-5 time-fold in heat especially), however in cold stress and cold and heat recovery a significant imbalance between the accumulation of various AOX isoforms on transcriptomic and

proteomic levels, indicating for adaptative over-accumulation of *AOX1a* messengers. Overall, the respective transcriptomic and proteomic responses for expression pattern of the most of investigated genes were different. This work was supported partially from grant no. N N303 338835 (2008-2010) of Polish Ministry of Science and Higher Education as well as from OPUS grant no. 2011/03/B/NZ9/05237 (2012-2015) of the National Science Centre, Poland.

P5.2

Energy flows in PSII as an alternative indicator of winter damages of wheat plants: a mass-scale approach

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Chlorophyll fluorescence measurements are used as the sensitive and non-destructive indicator of stress reaction. On the other hand the mass-scale use of these methods, as an alternative for the commonly used selection methods are not sufficiently supported by the experimental evidences and thus not practiced. In the three-years research program we tested the chlorophyll fluorescence measurements as an alternative for the winterhardiness and freezing tolerance tests used in wheat breeding. Winter hardiness and freezing tolerance of 75 winter wheat accessions were investigated in the field (7 locations) and in multiple field-laboratory tests. Chlorophyll fluorescence measurements were performed twice during each winter after freezing of detached leaves collected from the plants field-growing in boxes using HandyPEA fluorimeter (Hansatech). The results showed that the new method is the powerful alternative to traditional testing. The correlations between the results of fluorescence tests and either the field winter survival or freezing tolerance of plants measured as % survival were higher than those observed between field survival and % plants survived freezing tests. The best results were achieved if the measurements were made in February or early March and in contrary to traditional methods a single assessment seems to be sufficient for the proper estimation of winter hardiness and freezing tolerance of the plants. The greatest accuracy, however, seem to have measurements taken in the winter with the optimum cold acclimation conditions (2012/13). The degree of freezing tolerance was correlated with chlorophyll fluorescence parameters describing the energy amount trapped in PSII reaction centers and energy flow downstream of PSII reaction centers calculated for the leaf area unit (TRo/CS and ETo/CS, respectively) or characterized the amount of active PSII reaction centers per leaf area (RC/CSo, RC/CSm). The parameters describing the energy flows in the individual, active reaction centers (ABS/RC, TRo/RC, ETo/RC and DIo/RC), however, are entirely useless in predicting of plants freezing tolerance. This observation confirms that level of freezing tolerance is related to the stability of cellular membranes after freezing affecting photosynthetic activity at the level of leaf sections (reflected in the changes of ETo/CS, TRo/CS, or RC/CS), than with damages of individual PSII reaction centers. In the case of plants badly damaged in the field before collecting of the leaves, parameters describing the overall PSII efficiency (PI_{CSm}, PI_{CSo}) and Fv/Fm (the efficiency of energy transfer between PSII antennas and the reaction centers) showed the highest correlation with freezing tolerance of the plants, which reflects the effect of the post-freezing photoinhibition of photosynthesis occurring in the field on the final result of the measurements. Funding: the study was supported by the Ministry of Agriculture and Rural Development (HOR hn 078-801-7/13).

P5.3

Vitamin C reduces the symptoms of oxidative stress in *Chlorella vulgaris* cells under Pi deficit

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The photoreduction of oxygen in chloroplasts of photosynthetic organisms, including algae *Chlorella*, leads to the production of reactive oxygen species (ROS), under normal conditions. The abiotic stresses, such as mineral nutrient deficiency, can suddenly increase ROS level, which results in oxidative damage of cell structure and function. To overcome the oxidative stress, plants have developed several antioxidant defence mechanisms. Hydrogen peroxide is one of reactive oxygen species involved in plant stress response and the L-ascorbate (vitamin C) is known as an important component for H_2O_2 detoxification in photosynthetic organisms. The aim of this study was determine the changes in antioxidant capacity of *Chlorella vulgaris* under phosphate (Pi) deficiency and ascorbate participation in defense against oxidative stress. Unicellular algae, *Chlorella vulgaris* Beijer., were cultured two weeks in buffered, sterile media with different content of phosphorus: complete (control, +P), with lowered phosphate content (1/4P) and without phosphate (–P), under controlled conditions in phytotron. It has been shown that Pi deficiency significantly decreases the growth of algae and phosphate concentration in cells but increases photosynthetic pigments content and rate of photosynthesis. Visible symptoms of oxidative stress were observed in *Chlorella* cells, under Pi deficit increased lipid peroxidation and hydrogen peroxide level. However, the total ascorbate content and antioxidant capacity of cells significantly increased in Pi-deficient algae. In addition, the *provision* of *exogenous* ascorbate to the growth medium caused reduction in lipid peroxidation products and increased capacity of hydrogen peroxide removing by *Chlorella* cells. The obtained results indicated that ascorbate has a significant role in reducing the negative effects of oxidative stress and improving antioxidant capacity of *Chlorella* cells grown under low Pi supply.

P5.4

Oxidative stress in paraquat-treated lupine seedlings

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Paraquat (1,10-dimethyl-4,40-bypiridilium, PQ) is one of the most widely used herbicides in the world because of its great efficiency and low cost. This herbicide is adsorbed very quickly by plant leaves and blocks photosynthesis by accepting electrons from photosystem I (PSI) in plants. This action interferes with photosynthetic electron transport systems and prevents the formation of NADPH. The electrons are transferred directly to molecular oxygen producing superoxide radical and subsequently hydrogen peroxide and hydroxyl radical. These reactive oxygen species (ROS) may interact with unsaturated lipids, proteins and nucleic acids resulting in the destruction of cellular organelles and can lead to necrotic and apoptotic death. To neutralize ROS and counteract their deleterious effects, cells may activate antioxidant enzymes. The purpose of our study was to investigate the influence of light on PQ-induced oxidative stress in lupine seedlings (*Lupinus luteus* L. cv. Juno). The seedlings were grown in 1/4-strenght Hoagland medium for 9 days at 23C with 18/6 photoperiod and irradiance of 150 mM m^{-1} s^{-1}. On the 9^{th} day of culture seedlings were randomly allocated into six groups. Since PQ is related to photosynthesis and acts only under irradiance, three groups (control) were kept in dark for 3 days before PQ treatment. Then they were sprayed with 0, 0,1 mM and 1 mM PQ respectively and kept in the dark for one more day. The other three groups (test) were grown with 18/6 photoperiod for 3 days. Subsequently they were sprayed with 0, 0,1 mM and 1 mM PQ and kept at irradiance for 1 day to enable PQ to induce oxidative stress. In paraquat-treated leaves exposed to light the correlation between herbicide concentration and depletion of leaf fresh weight was noticed. Moreover visible wilting was followed by the appearance of brown, desiccated or chlorotic tissue. The accumulation rate of some peptides

decreased, however the most significant effect was observed in the region about 55 kDa corresponding to a large subunit of ribulose-1,5 bisphosphate carboxylase (RuBisCo, EC 4.1.1.39). Paraquat treatment in the light caused increase in the activity of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POX EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) in lupine leaves when compared with control groups which were cultured in the dark. These results indicated that both low and high concentrations of paraquat induce the response of antioxidant enzymes in plants under light condition. However it seems that the formation of reactive oxygen species is beyond the capacity of antioxidant system. ROS molecules may contribute to the damage of the cell membranes allowing water to leak from the leaf cells which in turn leads to the rapid desiccation of the foliage. The water content reductions as well as leaf necrosis were also provoked by paraquat in lupine seedlings exposed to the light.

P5.5

Changes in melatonin content in conditioned corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) seeds

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Exogenous melatonin (MEL) applied into seeds could be a good biostimulator improving seed vigour, germination and seedling growth. Since MEL is safe for animals and humans as well as inexpensive, a conditioning technique supplemented with this indoleamine could be a good, feasible and cost-effective tool for positive seed quality modification and may be economically benefitial for organic farming. There is still lack of information clearly explaining the role of MEL in plant physiology. In the presented work the efficiency of MEL application methods into seeds was verified. The following methods were tested: osmopriming (PEG – 2 MPa at 25 °C for 4 days) in the case of dicot cucumber and hydropriming (Water/MEL solution at 25 °C for 3 hours) in the case of monocot corn. Conditioning techniques were chosen as optimal for seeds of particular species. In both species four variants of seeds were compared: control – non treated ones, conditoned with water and conditioned witch MEL water solution of 50 and 500 μM (cucumber seeds: C, O, OMel50, OMel500; corn seeds: C, H, HMel50, HMel500). The changes in the content of MEL and the appearance of its potential metabolites were monitored by HPLC-MS quantitative and qualitative analyses of seed extracts for one year starting from the conditioning and idoleamine application moment. In both species the control seeds and these conditioned with water, contained a small amount of MEL: 7-40 ng/g_{FW} in cucumber and 4-2 ng/g_{FW} in corn. Both conditioning techniques supplemented with MEL proved to be a highly effective tool for this idoleamine forcing into the seeds. In both species its levels increased proportionally to the concentration of MEL applied. Interestingly, it was noticed that MEL was metabolized differently in each of the tested species. Moreover, it is worth mentioning that in the seeds not treated with exogenous MEL (variants: C, O, H) seasonal changes in this indoleamine concentration were observed – a significant increase in the winter months. Research work financed in the years 2011/2014 by the NCN N N310 111940.

P5.6

Identification of new *Arabidopsis thaliana* pri-miRNAs and mature miRNAs responsive to drought conditions

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Micro RNAs are key regulators of eukaryotic gene expression. However, the expression of *MIR* genes themselves is a subject of careful control. A high throughput real-time PCR platform (mirEX) has been developed to discriminate all individual known primary miRNA precursors (pri-miRNAs) and to analyze reliably their individual expression patterns (Bielewicz et al., 2012, http://comgen.pl/mirex). In progressive drought experiment, the stress was applied to Arabidopsis plants at 1.10 growth stage (Boyes et al. 2001) by water withholding, and continued until the soil moisture level reached 30% field capacity (FC) (2 days before wilting) and 20% FC (wilting stage), both for different plant batches. The experiment was monitored by leaf relative water content (LRWC) measurements. The polyA+ RNA isolated from plants subjected to 30% and 20% FC drought conditions was analyzed by mirEX platform. A dramatic up-regulation of Arabidopsis miRNA2111b precursor occurs under both drought stress treatments. This is for the first time that *A. thaliana* miR2111b is shown to be drought-responsive. In 20% FC drought a down-regulation of DCL-1 regulating miRNA162a and 162b genes expression is observed. In the case of multigene miRNA families, like miRNA156 and 395, only 2 defined family member pri-miRNAs undergo significant drought-induced expression. Micro RNA family members may be differently expressed during drought time course. Four out of six miRNA399 gene family members are induced under both drought conditions. 8 out of 14 miR169 family members are down-regulated under 30% FC drought but remain unchanged under 20% FC drought where only two *MIR169* genes are subjects of expression down- and up-regulation. In summary, 22% and 31% pri-miRNAs changed expression levels under 30% and 20% FC drought, respectively. 40% and 60% *MIR* genes were up- and down-regulated under 30% FC drought, respectively. In 20% FC drought, 55% and 45% *MIRs* were up- and down-regulated, respectively. The changes of the mature miRNA levels correlated with the changes of their cognate precursors.

P5.7

Silencing expression of the *CBP80* gene for engineering drought-tolerant *Solanum tuberosum* plants

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According to climate changes, and progressive desertification of the soil in Poland, it is very important to develop new strategies for crop plants to improve their response to drought, which is crucial for their innovative breeding. The down-regulation of nuclear cap-binding proteins in *Arabidopsis thaliana* renders plants resistant to drought. Based on these findings the *CBP80* gene in the *Solanum tuberosum* cultivar Desiree was silenced using artificial microRNAs. Obtained transgenic plants displayed a higher tolerance to drought, ABA-hypersensitive stomatal closing, an increase in leaf stomata and trichome density, and compact cuticle structures with a lower number of microchannels. These features were correlated with a higher tolerance to water stress. The level of miR159 was

decreased, and the levels of its target mRNAs *MYB33* and *MYB101* increased in the transgenic plants subjected to drought. Similar trends were observed in an *Arabidopsis cbp80* mutant in water shortage conditions. The evolutionary conservation of *CBP80*, a gene that plays a role in plant response to drought, suggests that it is a good candidate for genetic manipulations that aim to obtain improved water-deficit tolerance of other crop plants.

P5.8

Biogenesis of cucumber cotyledon chloroplasts under chilling stress

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Abiotic stress in plants can be induced by various environmental factors such as light, low or high temperature or high salinity. Chloroplasts are one of the first stress sensors in plants. Low temperature can cause functional and structural changes of thylakoid membranes. Plants tolerance to the chilling stress oscillates between 0-15 °C and plays an important role in plant development in a temperate climate. In spring or autumn, crops are exposed to the chilling stress during early stages of their growth. Therefore a study on the influence of the chilling stress on plant development is important both for basic research and application in agriculture. For our experiment we selected cucumber (*Cucumis sativus*), a chilling sensitive plant (CS) cultivated in a temperate climate. We focused on chloroplast biogenesis in cucumber seedlings. It is a complex process leading to fully developed and functionally mature chloroplasts. At the ultrastructural level it can be observed as a transformation of prothylakoids (flat porous membranes, PT) and prolamellar bodies (paracrystalic tubular membrane structures, PLB) into grana and stroma thylakoids during the plant photomorphogenesis. To perform our studies we selected main stages of the plastid internal membranes arrangement in optimal (22 °C) and low temperature conditions (4 °C): paracrystalline PLB (before the light was switched on), transformed PLB (two hours of light exposition), first stacked membranes (eight hours of illumination), fully formed grana (third day of the experiment). We followed chloroplast biogenesis on the ultrastructural and functional level using: modulated fluorescence of chlorophyll a, low temperature fluorescence of photosystems and chloroplast ultrastructure visualized by the transmission electron microscopy. We found that low temperature gradually modulates development of the cotyledon chloroplasts. At first the chilling stress accelerates the chloroplast differentiation and after longer exposure it leads to a characteristic membrane damages and functional changes.

P5.9

Pb entrance and accumulation regions in *Populus tremula x P. tremuloides* and *Arabidopsis thaliana* roots

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Here we describe the regions of Pb uptake and plant tolerance strategy where Pb is deposited mainly in the CW regions characterized with high level of low-methylesterified pectins. Root tips of *Populus tremula x P. tremuloides* and *Arabidopsis thaliana* treated with $PbCl_2$ (1000 μMPb 4h, control 4h H_2O) were the object of the study. Low methylesterified pectins were identified by immunogold method (JIM5 antibody); Pb – by rodizonic acid, X-ray microanalysis connected with transmission electron microscopy (TEM) and as electron-dense deposits in TEM. Detection

of Pb by rodizonic acid showed that root tip is one of the root regions which indicate markedly high Pb accumulation. Pb deposits were present in most cells building root tip tissues. However, there was a special zone in the root tip which indicates especially high Pb accumulation. It included meristematic cells and adjacent to them apical cap cells and cortex cells. The high Pb accumulation zone extended above the very tip root tissues and include the epidermal cells and adjacent to them cortex cells. At the ultrastructural level (in TEM) the highest Pb concentration was usually observed in the sites where the highest level of JIM5 pectin epitope was detected in control. There were cell walls (CWs) connections of a few cells, CWs adjacent to intercellular spaces (IS) and inside the IS. In the CWs connections the middle part of this region, indicating the highest level of JIM5 pectin epitope was simultaneously the region of the highest Pb accumulation. Similarly, in the CWs adjacent to the IS Pb was accumulated especially in the inner part of CWs and ISs angles where the JIM5 pectin epitope occurred in the highest level. Hence, the highest level of JIM5 pectin epitope – the highest Pb accumulation. Furthermore immunogold detection of JIM5 pectin epitope in Pb treated material showed undoubtedly the co occurrence of Pb deposits and gold particles indicating JIM5 pectin epitope in these regions. Gold particles were usually placed adjacent to Pb deposits. To sum up, the root tip is one of the main regions of Pb uptake by the roots of poplar and *A. thaliana*. The distribution of accumulated Pb in root tissues indicated close relationship to the distribution of low-methylesterified pectins. Therefore, we can conclude that low-methylesterified pectins participate in the tolerance strategy to Pb in both plants. This work was supported by MNiSW grant no. NN 303 801 940.

P5.10

Antioxidants and allergenic proteins as compounds produced by spice plants under stress

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Plants are endangered during their development by the influence of various stressful factors, both abiotic and biotic. Transitory action of stressor is leading to mobilization of the organism in order to defend against its consequences. One of common reactions to stressor action is the increase in the concentration of reactive oxygen species (ROS). Plant's tissues possess the deftly functioning defence system against ROS relying on action of enzymes neutralizing ROS (superoxide dismutase, catalase, peroxidase) and on the interaction with antioxidants. To the last belong: ascorbic acid, compounds from the group of terpenoids (carotenoids, tokoferols), but above all, polyphenols. Antioxidants with small molecular mass react directly with reactive forms of oxygen or indirect metabolites of oxidoreduction, not preventing the forming of ROS. High content of antioxidants in plants decides on their role in the prevention of civilization diseases. The above mentioned compounds are not synthesized in the human organism, so their delivery in the diet has significant importance in reactions against free radicals, considered the factors responsible for cardiological and oncological diseases. Plant cells in response to abiotic stress are able to synthesise specific proteins moderating the stress through elimination or neutralization of its effects. However some of them protect basic cellular structures and metabolic processes. Some of these proteins have documented allergenic properties and they are able to elicit serious allergic reactions, including life threatening anaphylactic shock. Among plant allergens listed in the official base of allergens, approximately 25% belongs to the group of pathogenesis related proteins (PR). Therefore, impact of the stress on higher synthesis of proteins in the plant is tantamount to higher contents of proteins triggering allergies and food intolerances and which is following by threat to allergic consumers. Spice plants are the unknown source of allergenic proteins frequently apllied in thP cuisine. We examined protein content and allergenicity of specific spices applied in food industry. By immunoblotting we were trying to identify allergenic proteins. The concentrations of polyphenolic compounds and antioxidant activity in the fresh and dried spice plants were determined. Different allergizing and antioxidant properties were found among popular spices. Summing up, diet rich in plant products influences beneficially the human organism on the one hand thanks to the content of antioxidants from the other poses the risk of the allergic reaction.

P5.11

Cr(VI)-influence on aquatic carnivorous plant *Utricularia gibba*

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The scope of the present study was to investigate morphology and physiology of shoots and traps of *Utricularia gibba* subjected to elevated Cr(VI) concentration. This globally-distributed water carnivorous plant occurs in waters with varying levels of contaminations. Cr(VI) is a highly mobile Cr speciation, classified by Environmental Protection Agency (USA) as a priority-toxic pollutant. The plant material was obtained from the collections of the Institute of Botany, Jagiellonian University as well as from the private ones. The experiment was carried out in three independent series and a few independent replicates. Plants were incubated for 7 days in 50 μM Cr(VI) (K_2CrO_4) standard, twice-diluted MS culture medium, pH 5.7, at 24 °C, under controlled light regime: 16 h light/8 h darkness, light intensity 35 $\mu Mm^{-2}s^{-1}$. The analyses cover microscopic observations of traps and shoots morphology. A special *paramecium* test was developed to detect traps activity. Photosynthetic pigment contents were measured in the means of UV-Vis spectrophotometry whereas inductively coupled plasma optical emission spectrometry (ICP-OES) was applied to quantify Cr concentration in plant biomass. The obtained results showed changes in activity as well as morphology of traps subjected to Cr(VI). A number of *paramecium* absorbed by Cr(VI)-treated traps was significantly (t-Student test; $\alpha = 0.05$) lower than in case of control samples. This result was correlated with morphological alterations of traps. In comparison to control, traps of plants subjected to Cr(VI) had relatively low number of gland cells responsible for secretion of digestive enzymes. On the other hand no significant differences were observed between control and Cr(VI)-treated plants in relation to other measured physiological parameters of shoots even though they revealed high Cr accumulation content, 0.78 mgg^{-1} d.w. Our results show that traps of carnivorous plants are far more sensitive to Cr(VI) than shoots. The work was financially supported by the grant DEC-2011/03/B/NZ9/00952 from the National Science Centre, Poland.

P5.12

Antioxidant defence system in the leaves and roots of *A. thaliana* under long-term S deficiency

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Plants often face the challenge to grow under unfavorable environmental conditions. Mineral deficiency stresses exert adverse effects on plants growth and development by inducing many metabolic changes. Sulphur (S) deficiency is nowadays one of the important factors that limit agricultural production in Europe due to strong reduction of SO_2 emission to the atmosphere (Vestreng et al., 2007). Here we present the analysis of enzymatic and nonenzymatic antioxidant systems in the leaves and roots of *Arabidopsis thaliana* grown in S-deficient Knop nutrient medium for 9 weeks. The level of S-containing glutathione severely decreased in both tissues and the ratio of its reduced (GSH) to oxidized form (GSSG) was higher in the leaves but lower in the roots as compared to control. Ascorbate level diminished but the ratio of reduced (AsA) to oxidized (DHA) form of ascorbate became higher in the leaves and roots of sulphur deficient plants. Photosynthetic pigments analysis revealed similar levels of carotenoids. Anthocyanins were elevated and accumulated mostly in leaf stalks and veins along the leaf lamina. Higher superoxide dismutase, catalase and ascorbate peroxidase activities were detected. Our data indicate that elevation of enzymatic antioxidant systems in *Arabidopsis thaliana* acclimated to long-term sulphur deficiency allows them to survive stress. The project

was supported by the Grant N N303 800240 from the NCN (Poland) given to I.M.J. and the intramural grant DSM501/86-104952 from MSHE through the Faculty of Biology, University of Warsaw, given to M.O.

P5.13

The effect of salicylic acid and excess of copper on selected stress parameters induced in *Phaseolus coccineus* leaves

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Salicylic acid (SA) is a broadly distributed phenolic compound in plants. It takes part in defence reactions to abiotic stress, including heavy metal stress. Anthocyanins and proline are known as non-enzymatic antioxidants and their accumulation is an important factor in ameliorating the heavy metal excess. The aim of the study was to determine the effect of SA on selected stress parameters, namely the anthocyanin and proline content, in the runner bean plants treated with copper. The runner bean plants, *Phaseolus coccineus* L. cv. Piękny Jaś, were cultivated on Hoagland nutrient solution in the growth chamber under controlled conditions of temperature, photoperiod, and light intensity. The following treatments were administered: control and 100 μM SA supplemented for 1 h or 24 h; next, the plants were cultivated in the control nutrient solution as SA1h, SA24h (without the presence of Cu), respectively, or as SA1h/Cu, SA24h/Cu (in the presence of 50 μM Cu), respectively and 50 μM Cu alone (Cu). After 5 days of the copper treatment, the leaves were spectrophotometrically analysed for the anthocyanin and proline content. Moreover, the Cu concentration was determined by atomic absorption spectrophotometry. The research has revealed that not only the content of anthocyanins but also proline was higher in SA1h/Cu than in all the treatments without Cu supplementation. There were no significant differences in anthocyanin and proline accumulation among all three treatments without Cu. The proline content in the SA24h/Cu treatment was higher than in the control and SA24h treatments. SA did not affect the Cu content in the Cu-treated and non-Cu-treated plants. The experiments showed the synergistic effect of the anthocyanins and proline content in the SA/Cu treatment. This implies that anthocyanins and proline may be stress indicators in the conditions generated by copper excess. Both compounds may play a protective role by trapping free radicals in plants under copper stress.

P5.14

Influence of short-term ammonium supply on ROS metabolism in *Arabidopsis*

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When comparing reductants and energy demand under nitrate nutrition with ammonium nutrition, it may be expected that the favorable N form for plants is ammonium. Surprisingly, when ammonium is supplied as an exclusive N source, it causes symptoms of toxicity called "ammonium syndrome". Recently, we have shown that long-term ammonium nutrition causes an overreduction of the extrachloroplast fraction of Arabidopsis leaf cells and leads to oxidative stress (Podgórska et al., 2013). However, under long-term stress conditions adaptive mechanisms are induced and new redox homeostasis is achieved, therefore some of the changes cannot be recognized. The purpose of our study was to characterize the modifications of ROS metabolism under short-term (30 min – 3 h) ammonium supply. We identified the changes in the level of metabolites involved in ROS metabolism and transcript levels of antioxidant enzymes localized in different cellular compartments. The concentration and reduction level of low-mass

antioxidants were estimated in whole tissue extracts and individual compartments. The obtained results are discussed in comparison to well characterized effects of long-term ammonium supply. This work was supported by grant N NZ3 02953 given to A. P.

P5.15

The influence of phenylpropanoid pathway metabolites on *Lupinus luteus* seedlings exposed to Pb stress

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As a consequence of human, agricultural and industrial activities, the environment is increasingly polluted with heavy metals. Among the heavy metals that affect plants, lead (Pb) is one of the most toxic and frequently encountered. One of its adverse effects on plants is reactive oxygen species (ROS) generation, leading to oxidative stress and in consequence – to cell death. In recent years, there has been increasing interest in the role of phenolic compounds in plants response to heavy metal stress, especially in the possible relation between flavonoids accumulation and heavy metal tolerance. Moreover, the latest reports have highlighted the potential role of these secondary metabolites as effective antioxidants.Therefore, the aim of this study was a better understanding the role of flavonoids in tolerance promotion of lupine roots exposed to Pb stress. It was found that treatment of yellow lupine (*Lupinus luteus* L.) with $Pb(NO_3)_2$ containing 150 mg/l of Pb^{2+} (which corresponds to 724 μM $Pb(NO_3)_2$) caused the statistically significant increase of total flavonoids content in all investigated parts of the plant – cotyledons (by ca. 67%) and roots (by ca. 54%). Furthermore, our experiment revealed that the lupine plants pre-incubated (for 2h) with flavonoid extracts, derived from Pb-treated lupine cotyledons, display enhanced tolerance to the heavy metal. Flavonoid pre-incubated roots of yellow lupine, growing for 48h in the presence of $Pb(NO_3)_2$, showed decreased symptoms of lead toxicity, which was manifested by increase in the root length and its biomass. Additionally, in seedlings pretreated with the natural flavonoid preparations showed a significant decrease of lipid peroxidation, measured as TBARS (thiobarbituric acid reactive substance) content and the number of dying cells. As we found, the protective role of phenolic compounds against Pb was associated with an increase in the antioxidant activity, and correlated with the reduced accumulation of both H_2O_2 and O_2^-. Thus, accumulation of flavonoids could be an effective addition to the plant's spectrum of defense responses to heavy metal stress, and the protective role of flavonoids against heavy metals might be associated with their ability to scavenge overproduced reactive oxygen species.

P5.16

The influence of lead and selenium interactions on shoot growth and antioxidant enzyme activity in *Vicia faba* L. *minor* plants

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Lead (Pb) belongs to the most common and dangerous heavy metals. Pb accumulation in plant tissues results in numerous disturbances of physiological processes. Researchers' attention is focused on identifying factors that could contribute to reduction of Pb absorption or toxicity in plants. Selenium (Se) is one of the potential antagonists of Pb. The effect of selenium (Se) on *Vicia faba* L. *minor* shoots subjected to lead (Pb) stress was studied by investigating shoot growth, viability, and antioxidant enzyme activity. The experiments were carried out on plants grown for 2 weeks on Hoagland's medium supplied with 50 μM Pb in the form of $Pb(NO_3)_2$ and/or Se concentrations of 1.5 μM and 6 μM in the form of Na_2SeO_3. Detection of Pb was performed using the rhodizonic method. Assessment of leaf viability was performed by trypan blue staining. To detect hydrogen peroxide (H2O2), staining with 0.1% DAB

solution was performed. Detection of superoxide anion ($O_2^{\bullet -}$) generation was carried out by staining with 0.05% NBT. Antioxidant enzyme activities: CAT, GPOX, and GSH-Px were determined after the enzymes were extracted in 50 mM potassium phosphate buffer, pH = 7.0 with 1 mM EDTA and 1% PVPP. It was shown that the exposition of the shoots to Pb led to serious damage in the leaves, which was accompanied by metal accumulation in the tissues. Pb caused significant changes in the biochemical parameters: the activity of GSH-Px, GPOX, and CAT increased. Moreover, Pb intensified $O_2^{\bullet -}$ and H_2O_2 production in the shoots. Selenium, especially at the 1.5 μM concentration, alleviated Pb toxicity, which was accompanied by decreased H_2O_2 and $O_2^{\bullet -}$ production and decreased activity of GSH-Px, GPOX, and CAT in the shoots.

P5.17

The effect of osmopriming on proline metabolism in germinating rape (*Brassica napus* L.) seeds under salinity stress

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Seed priming is a presowing treatment that improves the ability of seeds to germinate. Priming involves a period of controlled hydration of the seeds to a point close to the emergence of the radicle after which the seeds are dried back to their initial moisture content before sowing. Priming treatment plays beneficial effects on the vigor and viability of seeds which is manifested by improved germination and seedling growth. It is also stated that seed priming increases the tolerance of germinating seeds and seedlings to biotic and abiotic stresses. The aim of this study was to determine the effect of osmopriming on the acquisition of salinity tolerance by germinating rape (*Brassica napus* L.) seeds. Germination tests performed in the presence of 100 mM NaCl showed that priming increases the rate and uniformity of germination under salinity conditions. In plants, one of the mechanisms determining tolerance to salinity is the accumulation of osmoprotectans such as proline. In the present study proline level and gene expression of the key enzymes of proline turnover were studied. Primed rape seeds imbibing under control conditions accumulated about 2.8-fold more of proline than the unprimed seeds. Under salinity conditions, the primed seeds contained about four times more proline than the unprimed seeds. Moreover, in primed seeds *P5CS* gene encoding pyrroline -5-carboxylate synthethase was up-regulated compared to unprimed seeds and the expression level of *PDH* encoding proline dehydrogenase was down-regulated under both control and salt stress conditions. These results suggest that the one of the advantages of osmopriming which contributes to enhanced salt stress tolerance of germinating rape seeds is modulation of proline metabolism. Enhancement of proline anabolism with depletion of its catabolism in osmoprimed seeds speaks in favor of this hypothesis. This work was supported by grant no. 2011/03/B/NZ9/00068 from the National Science Centre.

P5.18

Transcriptome and proteome changes accompanying increased vigor of osmoprimed rape (*Brassica napus* L.) seeds

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Osmopriming is a pre-sowing treatment that exposes seeds to a low external water potential that allows partial hydration but prevents germination. Osmopriming improves seed germination performance as well as stress tolerance of germinating seeds and seedlings. In this work, rape (*Brassica napus* L.) seeds were osmoprimed with

– 1.2 MPa polyethylene glycol (PEG 6000) for 7 days. A combined transcriptomic, proteomic and physiological study has been performed to understand the molecular and physiological basis of seeds osmopriming and osmopriming-induced stress tolerance. Transcriptomic analysis of dry osmoprimed and control untreated seeds was performed to identify osmopriming-specific genes. We have also conducted comparative analysis of transcriptome of germinating osmoprimed seeds having completed the germination with that of unprimed seeds germinating for the same period. Our results from microarrays experiment showed that the transcript level of 1598 genes was altered 3 fold or more in response to osmopriming and post-priming germination. Among these 1598 genes, 1083 were induced and 515 were repressed. These genes were classified into 15 functional categories. Genes encoding proteins with binding function or cofactor requirements, proteins involved in metabolism, interactions with environment, cell rescue and defense were highly represented. The majority of the genes on the microarray were up-regulated during osmopriming and post-priming germination, confirming the hypothesis that during osmopriming germination-related processes are initiated. To characterize proteins involved in rape seed germination and vigor, a comparative proteomic analysis was carried out between dry untreated and osmoprimed seeds and also with corresponding imbibed seeds collected at the end of germination. Among about 500 total seed proteins resolved in 2D gels, changes in abundance (up-and down-regulation) of 149 proteins were observed during osmopriming and post-priming germination. Among these proteins 89 were up- regulated, including proteins involved in metabolism, transcription and translation, signal transduction, response to stresses and oxidation-reduction processes. Some of them had previously been shown to play role during germination and/or priming. This approach revealed also new proteins associated with seed germination and priming. To understand association of priming with post-priming stress tolerance, the response of antioxidant system and proline metabolism during osmoprimg and post-priming germination under salinity stress was also studied. Results of this work show that the germination performance and increased salinity tolerance of osmoprimed seeds are the consequence of multifaceted changes at various levels of the plant organization. This work was supported by grant no. 2011/03/B/NZ9/00068 from the National Science Centre.

P5.19

High-throughput phenotyping and genotyping in comparative QTL analysis of early short-time drought tolerance in Polish fodder and malting spring barleys

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Mapping populations of Polish fodder and malting spring barleys were used for QTL analysis of the traits describing short-time drought response at the seedlings stage. High throughput genotyping (DArT markers) and phenotyping techniques, including measurements of drought-induced changes in gas exchange, chlorophyll *a* fluorescence, water relations and membrane integrity were performed. The results showed a high genetic diversity of studied populations enabling the creation of high density linkage maps, as well as a high diversity in the physiological response of studied breeding materials. The analysis revealed 18 QTLs for 9 physiological traits on all of the chromosomes except for 1H in malt-type barley. In the fodder-barley population 15 QTLs for 5 physiological traits were found on chromosomes: 2H, 4H, 5H and 6H. Chromosomes 4H and 5H contained the regions explaining most of the observed phenotypic variation in the parameters analyzed in both populations. There was one major QTL for net photosynthetic rate in the malting barley mapping population located on chromosome 5H. Also two major QTL, which seem to correspond to two main genes, were found for PI. One major QTL for q_p was located on chromosome 4H in the fodder barley population. Three QTL regions were common for both mapping populations but explained drought-induced changes in different traits which are in accordance with the previous studies in which different traits were shown as being responsible for the drought tolerance variation within fodder and malting barleys. It seems that the best approach for QTL analysis, effective for further evolution of marker systems, is to create mapping populations for local-adapted gene pools, even separate for different breeding directions (e.g. malting and fodder barleys)

and to phenotype the well-recognized physiological characteristics responsible for the variation in tolerance among the studied group of genotypes and under local conditions.

P5.20

The effect of temperature on growth of *Thalassiosira pseudonana in vitro* system

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The effect of different temperatures on the kinetics of diatom (*Thalassiosira pseudonana*) growth in batch cultures was examined. The experiments were performed using *Th. pseudonana* as a model diatom with known genome sequence. The elucidation of the significance of temperature as an important factor for growing of diatoms plays important role in planning all experiments with these algae in *in vitro* systems. The studies compared the effect of temperature on several parameters important for cultivation of the diatoms in culture medium. These parameters are the concentration of photosynthetic pigments, proteins and Fv/Fm as well as the density of the culture. The diatoms were grown at optimal temperatures: 15 °C and 20 °C and under stress conditions: 12 °C and 23 °C under photoperiod 10/14 h with light intensity of 40 $\mu Em^{-1}s^{-1}$. The observations were made for 14 days, individually for each culture at different temperatures. The analysis of the obtained results demonstrated that temperature had significant effects on the growth kinetics of *Th. pseudonana* growth the higher the temperature, the faster the growth of culture. In cultures growing at 20 °C and 23 °C the highest chlorophylls and proteins concentration as well as OD were observed. The analysis of the relation between investigated parameters shows that in first 5 days after inoculation there are significant positive correlation between OD increase, chlorophyll a, chlorophyll c and protein concentration, and F_v/F_m at different temperature. The pattern and strength of correlations varied with temperatures. Several days after inoculation (from 6^{th} till 14^{th} day) chlorophylls and proteins concentration as well as OD were increasing continually at all growth temperature. On the other hand the F_v/F_m parameter at 12, 15 and 20 °C decreased to the level registered in the first day of observation whereas at the highest temperature (23 °C) the level of this parameter was comparable and low during whole experiments.

P5.21

Accumulation and translocation heavy metals in hyperaccumulating plants (*Brassica juncea v. Malopolska*) exposed to Pb, Cu, Cd and Zn

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Heavy metals are mainly attributed to anthropogenic sources, including various human activities such as mining, smelting and various industrial activities. With the development urbanization and industrialization, soils have become increasingly polluted by heavy metals with threaten ecosystems, surface and ground water, food safety and human health. Phytoremediation is relatively approach to removing contaminants from environment. In phytoremediation used hiperaccumulator plants to remove, destroy and sequester hazardous substances from polluted environment. Hyperaccumulator plants describes a number of plants that belong to distantly related families, but share the ability to grown on metalliferous soils to accumulate high amounts of heavy metals in the above-ground organs, especially leaves, at concentration 100-1000-fold higher then those found in non-hyperaccumulating species. They show no symptoms of phyotoxicity. According to a criterion hyperaccumulators are plants that, when growing on native soils,

concentrate >10 mg g^{-1} (1%) Mn or Zn, > 1mg g^{-1} (0,1%) As, Co, Cr, Cu, Ni, Pb, Sb, Se or Tl, and >0,1 mg g^{-1} (0,01%) Cd in the above-ground organs, without suffering phytotoxic damage. The hyperacculumator plants, in comparison to non-hyperaccumulators, strongly enhanced rate of heavy metal uptake, a faster root-to-shoot translocation and greater ability to detoxify and sequester heavy metals in leaves. The heavy metals (Cd, Pb, Cu, Zn) absorbed by the roots Indiana mustard plants cultivated hydroponically for 96 h on a Hoagland medium with the addition heavy metals solution in combination: CuPb, CuCd, CuZn, PbCd, ZnPb, Zn Cd at the concentration of 25 M: $CdCl_2$, $Pb(NO_3)_2$, $CuSO_4$, $ZnSO_4$ was applied. In experiments was observed inhibition of root elongation growth, decreasing of biomass, root colour changing and root sliming. We shown using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) the highest translocation root-to-leaves for Zn (52%), Cu (37%) and Cd (17 %). We noticed competition among used metals for example: Pb limited collection Cd and Cu but Zn increases collection Cu.

P5.22

Peroxidase activity and soluble carbohydrates content in pea roots in response to soil contamination with oxytetracycline

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Antibiotics applied in medicine and veterinary medicine represent an important group of bioactive compounds which can potentially have a detrimental effect on the environment. Oxytetracycline is a natural antibiotic which belongs to the group of tetracyclines and is produced by *Streptomyces rimosus* bacteria. It is characterized by a wide range of bacteriostatic activity, and antioxidant, immunosuppressive and anti-inflammatory properties. The research was aimed at determining the effect of various concentrations of oxytetracycline on peroxidase activity in pea roots, as well as on germination and elongation of the plants. The content of soluble carbohydrates in roots constituted an additional germination indicator. Peroxidase is an antioxidant enzyme whose main enzymatic function is to reduce hydrogen peroxide and other organic peroxides in the presence of reduced glutathione as a result of increased oxidative stress. The analyzed concentrations of oxytetracycline (from 0.01 to 100 mM) caused an increase in peroxidase activity in peas. In the roots growing in the soil supplemented with 0.01 to 10 mM of oxytetracycline hydrochloride, the enzymatic peroxidase activity increased, with the highest value at 10 mM. In higher concentrations of the antibiotic, peroxidase activity decreased. Yet, in the highest of the analyzed oxytetracycline concentrations (100 mM), the enzyme was still active. Moreover, the research showed that the content of soluble carbohydrates is a good growth indicator, yet it cannot be applied as a contamination indicator. Thus, it should not be used to assess the contamination of the environment with veterinary pharmaceuticals.

P5.23

Determination of epoxides in *Escherichia coli* expressing recombinant zeaxanthin epoxidase

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Zeaxanthin epoxidase (ZEP) is one of two enzymes engaged in violaxanthin cycle, an important photoprotective mechanisms occurring in higher plants, mosses, algae and lichens. ZEP catalyzes conversion of zeaxanthin (an epoxy-free xanthophyll) into violaxanthin (di-epoxide xanthophyll) via intermediate product antheraxanthin. Our studies

on overexpression of ZEP recombinant protein in *Escherichia coli* showed its toxic influence on bacterial cells. It has been assumed that the lack of ZEP substrate, zeaxanthin, may cause nonspecific reactions leading to production of toxic epoxides. We noticed a correlation between ZEP expression in *E. coli* and increase in amount of epoxides. A spectrofluorometric method has been used for the determination of common epoxides according to Sano and Takitani (1985). As a result of standardizing the method the best conditions to carry out the experiment using bacteria cells were worked out. Two steps reaction was performed; the first with sodium sulfide and the second with taurine and o-phthalaldehyde reagents. The studies were conducted on two strains of *E. coli*: *BL21* (*DE3*) and *Origami B* (*DE3*) transformed with *Arabidopsis thaliana* ZEP open reading frame cloned into pDEST17 vector. Differences between bacteria strains, transformed and non-transformed cells, and protein overexpression induction times were observed. Non-transformed *Origami B* strain contains more epoxides than *BL21*. In transformed cells of both strains, an increase in amount of epoxides was detected and this correlated with lower growth rate of bacteria. Higher epoxides content was noticed during the time of ZEP overexpression induction. These results show that the ZEP overexpressed in *E. coli* is responsible for the higher epoxide content which may be the reason of difficulties with production of recombinant protein. A method of epoxides determination in bacteria cells may be useful for studies on proteins engaged in epoxide formation.

P5.24

Drought responsive changes in barley leaf proteome

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Barley belongs to the genus *Hordeum*, in the tribe *Triticeae*, the grass family *Poaceae.* This species was one of the first domesticated cereals. Barley is also one of four most important cereals in worldwide production. Currently, it is considered as a model experimental system due to short life cycle and good morphological, physiological and genetic characterization. Drought is one of the major abiotic stresses that strongly influence plant growth, development and it has a great impact on agricultural production. In response to water deficit, plants developed various biochemical and physiological mechanisms. Adverse environmental factors, such as water deficit, cause significant changes in gene expression profile. Products of stress induced genes are classified as directly involved in tissue protection against dehydration and as proteins related to gene expression control and signal transduction. Specific composition of proteins present in cells in defined environmental conditions reflects the true biochemical outcome of genetic information and indicates the biochemical pathways that may be involved. It is now accepted that explaining proteome changes is critical in developing full understanding of how cells work and adapt towards various stimuli. The aim of conducted research was the analysis of changes in barley proteome under drought stress. Comparative analysis was studied by 2D electrophoresis and MALDI-TOF mass spectrometry. Plants were grown in greenhouse for three weeks under controlled environmental conditions. After this time, plants were subjected to drought stress. After 10 days, leaves were harvested and subjected for proteomic analysis. Leaf proteins were isolated by phenol extraction. The extracts were dissolved in IEF buffer and submitted for separation by 2D gel electrophoresis. Separated proteins were then stained using Coomassie Brilliant Blue in colloidal version. Obtained gels were analyzed in Image Master 2D Platinum 7.0 software. Protein spots, which showed changes in expression profile, were excised from gel, digested with trypsin and analyzed by MALDI-TOF or MALDI-TOF/TOF mass spectrometer. The registered mass spectra (Peptide Mass Fingerprint) were compared with these from databases (MSDB, SwissProt, NCBI), using the dedicated MASCOT server. The list and the expression profile of drought related proteins are presented on the poster. This project is supported with grant from EU funds (grant no. WND-POIG.01.03.01-00-101/08: POLAPGEN-BD).

P5.25

Molecular cloning, characterization and expression of a guanylyl cyclase (*HpGC1*) involved in the stress signalling in *Hippeastrum* x *hybr.*

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Research of the past few decades has shown that cyclic GMP acts as second messenger in a wide range of physiological processes including hormone signal transduction, light signal transduction, abiotic and biotic stress-response signaling in plants. Despite the widely recognized importance of cGMP little is known about the mechanism of its synthesis and structure of enzymes involved in this process. Guanylyl cyclases (GCs) catalyze the formation of the second messenger – guanosine 3′:5′-cyclic monophosphate (cGMP) from guanosine 5′-triphosphate (GTP). Only a few studies on plant GCs have been conducted. Both soluble and transmembrane GCs were discovered in higher plants, but the knowledge about mechanisms by which activity of plant guanylyl cyclases can be regulated is still insufficient. In the light of our interest in studying the role of cyclic GMP and the intracellular signaling processes leading to stress tolerance in *Hippeastrum*, we have cloned cDNA that represents a putative member of the guanylyl cyclase gene family in plants. Using the RACE-PCR method we isolated and characterized *HpGC1* (accession no. HM481265.1), which is 1157 bp long and codes a 256 amino acid peptide (accession no. ADJ94125.1) with a theoretical molecular mass of 28.8 kDa. The predicted amino acid sequence alignment indicates that the identified cDNA shares high similarity with other plant guanylyl cyclase. Analysis of the deducted amino acid sequence shows that HpGC1 contains all important residues, responsible for substrate specificity for GTP, transition state stabilization and binding the essential metal ions. The changes in transcriptional activity of *HpGC1* gene were tested in response to abiotic and biotic stress conditions. The investigations were conducted on bulbs of *Hippeastrum* x *hybr.* after mechanical wounding and fungal pathogen *Phoma narcissi* attack. The significant changes in transcript level were observed in response to both stresses, thus suggesting that guanylyl cyclase is involved in both stress factors. Obtained results seem to be quite interesting especially in comparison with the expression of adenylyl cyclase gene (*HpAC1*) studied in the same conditions. We also obtained a recombinant HpGC1 protein using *E. coli* BL21 strain and pGEX-6P-2 expression vector system. The molecular mass of protein was 28 kDa and corresponded to the *in silico* prediction. The analysis of recombinant HpGC1 protein activity revealed the formation of cGMP from GTP *in vitro* in the presence of Mg 2+ and Mn 2+ ions. Based on the obtained gene sequence and enzyme activity it was shown that *HpGC1* has features and properties typical of plant guanylyl cyclase.

P5.26

Molecular cloning, characterization and expression of a calcium dependent protein kinase (CDPK) involved in the stress signalling in *Hippeastrum* x *hybr.*

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Ca^{2+} ions are an important second messenger in plant cells. They have been linked to the perception of and response to biotic and abiotic stimuli, as well as regulation of many aspects of growth and development. One of the best known calcium downstream effectors is calcium dependent protein kinases (CDPKs). Although CDPKs have not been found in animals, they appear to be widespread in flowering plants, algae and some protists. In the light of our interest in studying the role of calcium and CDPKs in the response of *Hippeastrum* to abiotic and biotic stress, we have cloned cDNA, designed as *HpCDPK1*, which belongs to putative CDPK gene family in plants. The *HpCDPK1* open reading frame is 1596 bp-long which encodes 531 amino acid peptide with a predicted molecular mass of

59,5 kDa and an isoelectric point of 6,85. Predicted amino acid sequence alignment indicates that the identified cDNA shared high similarity with other plant CDPKs. The highest amino acid identity is observed with CDPK1 from *Dendrobium officinale* (79% identity) and CDPK2 from *Oryza sativa* (76% identity). Analysis of the deducted amino acid sequence shows that HpCDPK1 contains all important domains characteristic for CDPK family. It contains a highly variable N-terminal region, a conserved kinase catalytic domain typical for the serine/threonine protein kinases and an autoinhibitory domain joined to the C-terminal CaM-like domain with four conserved Ca^{2+}-binding EF hands. Analysis of gene transcriptional activity using Real-Time PCR technique gives an insight into its biological functions in biotic and abiotic stress conditions. The studies were carried out on bulbs of *Hippeastrum* infected by fungus *Phoma narcissi* and mechanically wounded. Analysis of *HpCDPK1* gene transcriptional activity showed changes in its expression level as a result of both mechanical damage and pathogen attack.

P5.27

Modulation of antioxidative metabolism in response to osmopriming in *Brassica napus* seeds improves germination under salt stress

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Salinity causes a variety of physiological, biochemical and metabolic dysfunctions in plants. Salt stress may inhibit or delay seed germination by lowering osmotic potential of soil solution, the toxic effect of salt ions or a combination of these factors. Under salt stress elevated level of reactive oxygen species and activation of antioxidant systems are observed. Seed priming is one of the presowing techniques adapted to achieve tolerance to stresses. Priming treatment plays beneficial effects on the vigor and viability of seeds which is manifested by improved germination performance and seedling growth, especially under adverse environmental conditions. To understand osmopriming physiology and its association with post-priming stress tolerance, we investigated the response of antioxidant system during osmopriming and post-priming germination of rape (*Brasscia napus* L.) seeds under salinity stress. Seed priming activated antioxidant metabolism by increasing activities of antioxidant enzymes such as ascorbate peroxidase, catalase and superoxide dismutase. Enhanced activities of APX, CAT and SOD in primed seeds were correlated with increased expression rate of genes encoding these enzymes. Despite higher activities of antioxidant enzymes, we observed higher level of H2O2 accumulation in primed seeds during germination in water and 100 mM NaCl. However, the level of lipid peroxidation, measured as an accumulation of tiobarbituric reactive substances, was lower in primed seeds germinating both in water and NaCl. We conclude that there is a correlation between the activation of antioxidant metabolism in primed seeds and increased tolerance to salt stress during germination of rape seeds and that antioxidative capability acquired by priming seeds may be one of the mechanisms promoting better tolerance of salt stress during germination. This work was supported by NCN no. DEC-2011/03/B/NZ9/00068.

P5.28

Markers of physiological stress in common pea (*Pisum sativum*) in response to soil contamination with tetracycline

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Pharmaceuticals, applied in medicine and veterinary medicine constitute a potential source of environmental contamination. Tetracycline is a natural bacteriostatic antibiotic produced by some strains of *Streptomyces*. A wide range of tetracycline activity includes Gram-positive and Gram-negative bacteria, *Chlamydia spp.*, *Rikettsia spp.*,

Mycoplasma and some *Protozoa*. Tetracycline is used, among other applications, in GIT infections, chronic pneumonia and bronchitis, pyoderma, and in prolonged treatment of acne. In prophylaxis, the antibiotic is administered prior to and after surgical interventions. The present study aims at determining effects of various concentrations of tetracycline on peroxidase activity in pea roots. The effect of tetracycline on germination and root elongation was also checked. The soluble carbohydrates content in pea roots was an additional indicator of germination. Peroxidase (POX) activity, germination and roots elongation were determined to be seedlings' sensitive endpoints in the evaluation of toxic effects of tetracycline. The enzyme's activity is a good marker of physiological stress in the plants. The analyzed concentrations of tetracycline (from 0 to 100 mM) caused a decrease in POX activity in pea roots. An increase in peroxidase activity appeared in seedlings which grew in the soil supplemented with 0.05 to 1 mM of tetracycline. In line with growing concentrations of the antibiotic (from 5 to 100 mM), POX activity decreased, yet it still appeared in the highest analyzed concentration. The dynamics of seedling growth proved to be the best indicator of soil contamination with tetracycline. Soluble carbohydrates content was also a good indicator of seedling growth. Yet, it is not a contamination indicator and thus cannot be used to assess soil contamination with antibiotics.

P5.29

Involvement of salicylic acid in cucumber response to a combination of salt stress and *Pseudomonas syringae* pv. *lachrymans* infection

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Leaves, stems and roots constitute an integrated physiological unit that receives stress signals and establishes acclimation responses. Communication within this system, occurring via signaling molecules, metabolites and phytohormones, is important for integrating the whole-plant response to stresses. Salicylic acid (SA) has received particular attention as an important signaling molecule mediating plant responses to abiotic and biotic stresses. It has also been shown to mitigate the deleterious effects of some abiotic stresses. We found that salt stress impaired the defense response of cucumber (*Cucumis sativus*) to *Pseudomonas syringae* pv. *lachrymans* (*Psl*) infection as shown by more severe angular leaf spot disease symptoms and enhanced electrolyte leakage when bacterial infection was combined with salinity. To analyse how salinity interacts with biotic stress we determined the effects of sequentially applied NaCl and *Psl* on SA content in cucumber leaves. Moreover, the impact of exogenously applied SA on infection development and endogenous SA concentration in plants grown under salt stress or not affected by salinity was studied. Cucumber plants were exposed for 7 days to salt stress (50 mM NaCl) and thereafter infected with *Psl*. Alternatively plants were sprayed with 0.25 mM SA and after 3 weeks treated with 50 mM NaCl and infected. Infection development, leaf cell damage recognized by Evans blue staining, electrolyte leakage and SA content were analyzed 7 days after inoculation. For quantification of free and conjugated SA (SAG) HPLC method was used. In cucumber leaves SA occurred mostly as SAG. Bacterial infection induced a significant increase in SA and SAG concentrations, however the intensity and dynamics of SA and SAG accumulation differed in leaves of NaCl-treated and non-treated plants. Foliar application of 0.25 mM SA caused an increase in the endogenous content of SA and SAG and alleviated disease development, especially when *Psl* infection was combined with salt stress. It is proposed that the significantly increased free SA found in 0.25 mM SA pre-treated plants grown under salt stress and infected could help cucumber plants to relieve the adverse effects of salinity and could have a positive impact on plant defence against *Psl*. The results have been discussed in relation to the role of SA in plant response to a combination of abiotic and biotic stresses.

P5.30

The relations between the effect of Cd and Pb on the growth of maize seedlings, and their IAA and H_2O_2 content

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Experiments were carried out with etiolated 4-day-old maize seedlings cv. Kosmo 230, which grew for the last 24 h in hydroponic systems filled with solution containing 1,0 mM KCl, 0,1 mM NaCl, 0,1 mN $CaCl_2$ (control medium) or in the control medium with $PbCl_2$ or $CdCl_2$ at the concentration of 10^{-5}, 10^{-4} or 10^{-3} M. After 24 h of incubation in the presence of the metals, the growth of seedlings organs (roots, mesocotyls, and coleoptiles) was measured and the content of IAA and H_2O_2 in the organs was determined spectrophotometrically. It was found that toxic effect of Cd on the elongation growth of seedling organs increased with increasing concentration of this metal in the hydroponic solution. Lead diminished growth of organs in comparison with control but there was no statistically significant difference in growth of mesocotyls and coleoptiles in the presence of 10^{-5}, 10^{-4} and 10^{-3} M $PbCl_2$. Both metals induced oxidative stress in seedlings. The content of hydrogen peroxide (H_2O_2) in the organs increased with the increasing concentration of Pb in the hydroponic solution. Similar effect was observed in seedlings treated with Cd. The content of IAA depended on the metal concentration and the type of organ. In mesocotyls and coleoptiles, the content of IAA increased with increasing concentration of Pb or Cd in the hydroponic solution. In roots treated with Cd or Pb in the concentration of 10^{-5} and 10^{-4} M the content of IAA did not differ when compared with the control, whereas in roots treated with Pb 10^{-3} M the content of IAA was significantly higher, which was correlated with short but thick roots. By contrast, Cd at the concentration of 10^{-3} M diminished considerably the content of the phytohormone in comparison with control roots. The results presented above suggest that the toxic effect of Cd on roots growth could be related with oxidative stress, whereas it is not dependent on the content of IAA in this organ. It seems that the high content of IAA in roots treated with Pb 10^{-3} M caused considerable diameter-increment growth, whereas inhibition of the elongation growth of the organ was due to high content of H_2O_2. Since the content of IAA and H_2O_2 in mesocotyls and coleoptiles was not diminished by both metals, it is postulated that growth decrease of both organs was mainly the effect of oxidative stress.

P5.31

Aeroponics as a tool in reintroduction of endangered *Orchideceace* and *Droseracae* species

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The presented study was conducted in order to develop a reliable method for acclimatisation and reintroduction of endangered native orchid species and carnivorous plants. In Poland, all orchid species are endangered (Dz. U. Nr 27, poz.134). Two orchid species: *Cypripedium calceolus* L. and *Liparis loeselii* L. C. Rich. are most threatened in Europe and require the development of recovery plans. Both have been propagated by means of *in vitro* culture in the Department of Plant Protection and Biotechnology at the Intercollegiate Faculty of Biotechnology, University

of Gdańsk and Medical University of Gdansk. Our current efforts focus on establishing successful transfer conditions to *ex vitro.* Micropropagation has been extensively used for the rapid multiplication of many plants. However, its more widespread use is restricted by a high percentage of plants frequently being lost or damaged when transferred to *ex vitro* conditions. Plantlets in *in vitro* culture grow under special conditions in relatively air-tight cultivation vessels, e.g. air humidity is higher and irradiance is lower than in a conventional culture. The use of closed vessels decreases air turbulence, limits the inflow of CO2 and outflow of gaseous plant products from the vessels. What is more, cultivation media are supplemented by saccharides, which considerably decrease the water potential of the medium. Furthermore, during *in vitro* culture the plants' metabolism can be mainly or partly heterotrophic whereas plants during the acclimatisation period have to increase their photosynthetic activity. All the above-mentioned factors may lead to a high mortality rate during acclimatization to *ex vitro* conditions. Similar factors apply to plants which produce secondary metabolites. It is well known that the metabolomic profile varies between *in vitro* and *ex vitro* conditions, carnivorous plants of the Droseraceae family being a prime example. Obtaining a tool of plant cultivation in the laboratory which would eliminate the use of heterotrophic conditions can provide useful new data especially regarding endangered species. Aeroponic cultures are well known and their applicability is established especially in low annual rainfall regions and where water is a highly valued commodity. There are few reports concerning the laboratory-scale use of this technique. Aeroponics may provide favourable conditions for the growth of *ex vitro* plants and their acclimatisation to phototropism, enabling an unperturbed plant reintroduction while also providing a valuable tool for plant cultivation for metabolomic analysis. We present our approach developed for peat or bog grown endangered species and data concerning our plant cultivation technique. Our findings regarding the stress induced when transferring plants from *in vitro* to aeroponics are based on the measurements of the maximum quantum efficiency of photosystem II, Performance Index and observed morphological changes.

P5.32

The effectiveness of DArT markers conversion to PCR markers useful for the discrimination of Polish barley genotypes with higher spring drought tolerance

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The specific molecular markers based on PCR may be effectively used in genotypes selection programs. The two DArT (*Diversity Array Technology*) markers based genetic maps of Polish fodder and malting barley populations were used to find markers associated with physiological parameters characterizing plant response under limited watering conditions: electrolyte leakage, the relation between quantum efficiency of PSII and the efficiency of carboxylation, and leaf water content. Tests were carried out within parental specimens which were used before to create mapping population of spring barley. For malting barley population, 27 markers were selected on chromosomes 1H, 2H, 3H, 6H, 7H. The 8 out of 19 clone sequences were converted to SSR (*Simple Sequence Repeated*) markers and 3 out of them had a polymorphic character. The 11 STS (*Sequence Tagged Site*) out of 17 additional markers obtained from GrainGenes database were polymorphic. For fodder barley population 26 markers locating on chromosomes 2H, 3H, 5H, 6H were chosen. The analysis of 16 clone sequences carried out by MISA software (Thiel et al., 2003) showed the presence of SSR motifs within 10 sequences. After the polymorphisms analysis, 9 sequences proper to further mapping populations' tests were obtained. Within fodder barley population, 21additional sequences obtained from GrainGenes database were tested resulting in 13 polymorphic STS markers selection. The effectiveness of obtaining PCR markers within DArT sequences for both analyzed barley populations was 60.24%, whereas the effectiveness of DArT markers to SSR markers conversion was 66.67%. The fodder barley population analyzes showed the higher efficiency level to obtain PCR markers (64.44%) than malt-type barley (55.26%). The study confirms that DArT markers may be an efficient startpoint for generating PCR-based markers which can be used in plant breeding programs.

P5.33

Measurements of physiological parameters to evaluate *in vitro* selected *Alyssum* lines

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Application of biotechnology techniques enables rapid progress in the knowledge concerning mechanisms of plant cell metabolism shifted in order to alleviate heavy metal stress. The studies on adaptations of plant species to colonize substrates enriched with compounds containing heavy metals has been conducted for many years, but the utilization of aseptic methods to pursue such goal was relatively limited. This is why the aim of conducted experiments was the elaboration of optimal method of *in vitro* selection using *Ayssum montanum* – metallophyte species from *Brassicace* family. Plant material to start *in vitro* cultures were seeds sampled from specimens representing population from Olkusz Ore-bearing Region Lines were selected to obtain plant material which will tolerate gradually increased level of Pb^{2+}, Cd^{2+} and Zn^{2+} ions in the substrate. At the same time, the protocol allowing to effective micropropagation of studied material was optimized. The morphogenetic reaction and physiological activity of respective cultured line have been studied. Obtained results will be presented and discussed during poster session.

P5.34

Selected biochemical parameters of macrophytes applied in the Model Biofiltering System (MBS)

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Macrophytes constitute inherent element of wetlands. Due to their environmental properties they can be used in sewage treatment plants where on the one hand they can directly participate in biochemical mechanisms of soil and water purification and on the other they can create favorable conditions for the maintenance and intensification of these mechanisms. Some of common plant species are so resistant to contamination and have such a great biological potential for assimilation and accumulation of nutrients and heavy metals that they could be used in constructed wetlands for water and wastewater treatment. The research was carried out in the Model Biofiltering System (MBS) located at the outflow of wastewater from sewage treatment plant in Rozprza town in the Pilica River catchment (central Poland). Constructed wetlands constituted the fourth part of MBS, and were overgrown by four species of plants: *Glycera maxima*, *Acorus calamus*, *Typha latifolia* and *Phragmites australis*. MBS was developed based on the concept of ecohydrology and phytotechnologies. Activities of glutathione transferase (GST) and guaiacol peroxidase (POx) were measured in leaves and roots of the above mentioned plant species. Additionally, chlorophyll concentration in plant leaves was determined. Almost in all examined plants GST demonstrated opposite dynamics of activity compared with POx. Moreover, differences in dynamics of enzyme activities were determined between organs of examined plants. The highest chlorophyll concentration was detected in *Glycera maxima* leaves. Of all the tested species growing in the constructed MBS *Glycera maxima* and *Acorus calamus* were least effected which indicates that they are best suited to deal with the difficult conditions of flowing wastewater. The research was supported by the Polish Ministry of Science and Higher Education, Project: N N305 365738 “Analysis of point sources pollution of nutrients, dioxins and dioxin-like compounds in the Pilica River catchment and draw up of reclamation methods”.

P5.35

Agrochemicals applicable in phytoextraction combined with plants biomass production

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The clean-up of metal-polluted soils is important for the protection of human and environmental health but also becoming interesting from the economical point of view. Effects of heavy metals on plants result in growth inhibition, structure damage, a decline of physiological and biochemical activities as well as of the function of plants. Phytoextraction is a large scale, *in situ* method which uses plants and their associated microorganisms to remove pollutants from the environment. For the removal of heavy metals from large areas with relatively low concentrations of pollutants plants with added economic value are proposed as more cost efficient then hyperacummulator plants. Application of energetic plants in phytoextraction is a relatively new concept that shows good potential. The aim of this research was the evaluation of influence of commercially available agrochemicals on growth, development and uptake of heavy metals by energetic plant *Salix viminalis.* Two commercially available agrochemicals: Asahi SL and Biojodis were tested in this research for the potential application in cultivation of willow *Salix viminalis* for biomass combined with phytoextraction. Plant model: willow *Salix viminalis* is a fast growing, high biomass plant widely used for biomass production. Plants were cultivated in pots on soil contaminated with cadmium and fertilized with recommended doses of N:P:K. Cultivation took place from April to October. Agrochemicals were applied according to manufacturers' recommendations. Growth of plants, chlorophyll content and photosynthesis intensity was measured during the whole season. Cadmium content, fresh and dry biomass as well as energetic properties of biomass was measured and analyzed at the end of experiment. All data was statistically analyzed. Applied agrochemicals increased the yield of biomass and partially mitigated the toxic effect of cadmium on photosynthesis. Phytoextraction efficiency of cadmium characterized by bioaccumulation factor (BAF) was increased significantly. Applied agrochemicals increased the uptake of cadmium as well as biomass of plants. Biojodis was more effective than Asahi SL. The translocation factor (TF) and transfer factor (TFr) values were calculated to complete information about heavy metal ion transport from soil to plant. Applied agrochemicals reduced the stress in plants and increased the biomass yield and cadmium accumulation. Application of Biojodis to enhance phytoextraction and biomass accumulation might be a viable method of increase the efficiency, and the cost-effective of the process. Research where sponsored by Ministry of Science and Higher Education in Poland, Grant No N N304 385338.

P5.36

Growth parameters and ionic versus chelated zinc uptake in *Pisum sativum* L.

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EDTA and recently also EDDS (as more easily biodegradable and environmentally friendly) are postulated for use in chelant-assisted phytoextraction. The aim of this work was to compare the effect of Zn^{2+}, Zn-EDTA and Zn-EDDS on growth parameters of *Pisum sativum* seedlings as well as metal uptake and its translocation after 7-day treatment. Zn was added in the form of $ZnSO_4$ up to Zn^{2+} concentrations 0.8 and 1.6 mM and chelats were equimolar mixtures of Zn^{2+} and EDTA or EDDS. Zn concentration in roots and shoots was determined by FAAS. Zn^{2+} at the lower concentration stimulated *P. sativum* root growth, but the higher level of this metal limited root elongation by 25%. However, the fresh weight (FW) of roots was not affected by ionic zinc at either dose. Zinc chelated by EDTA had

markedly worse effect on root growth then its ionic form. The root elongation was 18- and 31-fold lower then that at 0.8 and 1.6 mM Zn^{2+}, respectively. The roots FW decrease was less dramatic (by 21 and 38%). Zn-EDDS had less negative effect on pea roots than Zn-EDTA. Admittedly, its lower concentration reduced root growth by 45% but the higher dose did not change this parameter as compared to Zn^{2+}. Zn-EDDS at either examined concentration did not significantly affect root FW. Pea shoots were more sensitive to zinc ions than roots. Their elongation declined by 19 and 50% after treatment with 0.8 and 1.6 mM Zn^{2+}, respectively, but is FW remained at the control level. Zn-EDTA reduced shoot growth by 43 and 50% and its FW by 14 and 35%, at the lower and higher examined concentration, respectively. 0.8 mM Zn-EDDS did not change shoot growth and their FW and its higher dose even stimulated shoot elongation as compared to ionic zinc. Under normal zinc supply pea seedlings accumulated 0.2 mg Zn/g dray weight (DW). Plants treated for one week with 0.8 or 1.6 mM Zn2+ contained 5.6 and 9.9 mg Zn/g DW, respectively. About 60% of the taken up metal was located in roots. The chelated forms of zinc were less available to pea seedlings. Zinc concentration in the plants treated with the chelates was always lower than in the material exposed to the ionic form of metal. Comparing the two examined chelators, at their lower concentration a little more Zn was taken up by the plants from Zn-EDDS solution while at higher – from Zn-EDTA. The translocation factor of Zn in the plants incubated in 1.6 mM Zn-EDTA was at least 14-fold higher than in other experiment variants. The obtained results indicate that Zn^{2+} at the higher tested concentration (1.6 mM) had negative effect on pea root and shoot elongation. Zn-EDTA, but not Zn-EDDS, had significantly worse effect on seedling growth then its ionic form. The examined chelators reduced Zn uptake by pea seedlings. EDTA markedly stimulated translocation of the metal from roots to shoots.

P5.37

Cadmium accumulation and localization in the leaves of two ecotypes of *Dianthus carthusianorum* differing in metal tolerance

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Dianthus carthusianorum is one of the dominant species spontaneously colonizing calamine waste deposits left over Zn-Pb ore mining and processing in Bolesław, South Poland. Our previous studies have shown that the significant morphological and genetic differences between plants inhabiting metallicolous and non-metallicolous sites were accompanied by higher metal tolerance of the metallicolous ecotype. However, accumulation of intracellular ligands – thiol peptides and organic acids was not responsible for enhanced Cd tolerance of the metallicolous ecotype. Therefore, the present study was conducted to investigate the accumulation and spatial distribution pattern of Cd in the leaves of the two ecotypes of *D. carthusianorum*. Plants were cultivated hydroponically under controlled photoperiod, light intensity, and temperature conditions in the presence of 50 M Cd for 14 days. Metal accumulation in the roots and shoots (as determined by atomic absorption spectrometry, AAS) was similar in both ecotypes; however, significant differences were found in the distribution of the metal within leaf tissues. Histochemical analysis (using dithizone staining) revealed the presence of Cd mainly in the vascular bundles and trichomes in the metallicolous ecotype, whereas in the non-metallicolous ecotype the majority of Cd was found in the epidermis cells, and especially in the cells of stomata. More precise semi-quantitative analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) confirmed the presence of high amounts of Cd in the vascular bundles and trichomes followed by stomata and mesophyll cells in the metallicolous ecotype. Surprisingly, trichomes appeared the main site of Cd accumulation, followed by vascular tissues, and stomata in the non-metallicolous ecotype as revealed by the LA-ICP-MS method. The results showed that multiple-approach studies are indispensable to determine precisely the metal distribution pattern in plant leaves. In both ecotypes, Cd seemed to be excluded from the mesophyll cells and deposited in leaf trichomes. Further investigations of the metal localization and speciation at the ultracellular level are necessary to explain the mechanism of the elevated Cd tolerance of the metallicolous ecotype of *D. carthusianorum*.

P5.38

Gamma-tubulin distribution in root cells of soybean (*Glycine max* L.) seedlings under cadmium stress

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The microtubule (MT) cytoskeleton in combination with associated proteins, provide support for the most important processes in plant cells such as assembly of mitotic spindle, maintenance of internal order or construction of the cell wall. It is well established that MT arrays are sensitive to different stress factors, particularly to heavy metals such as cadmium, but mechanisms of that sensitivity are still obscure. Gamma-tubulin (g-tubulin) functions as a MT nucleating factor that is localized in different size complexes over the plant cell. The complexes are essential for acentrosomal MT nucleation and crucial for proper functioning of MT cytoskeleton in plants. In the present study, the distribution of g-tubulin in root tip cells of soybean at 85 mM and 170mM Cd^{2+} concentrations was investigated by means of different immunological approaches. After exposure to cadmium, the disrupted MT arrays were associated with decreased level of g-tubulin immunofluorescence signal. At the ultrastructural level, the cadmium treatment resulted in an increased yield of immunogold labeling of root cells sections, but the clusters of gold particles were smaller as compared to control ones. Biochemical results obtained by immunoblot techniques after 1D and 2D electrophoresis revealed the gradual disappearance of high molecular bands and simultaneously appearance additional low molecular bands after cadmium treatment. Moreover, the increased number of g-tubulin spots in cadmium treated cells was observed, especially at 170mM Cd^{2+}. The obtained results seemed to indicate that after cadmium treatment the gradual disintegration of g-tubulin complexes might occur, which in turn influences the nucleation of MT and contribute to functional impairment of MT cytoskeleton under cadmium stress. The research was supported by Ministry of Science and Higher Education (grant no. N N303 537938).

P5.39

Influence of lead (Pb^{2+}) on chloroplast distribution patterns in *Lemna trisulca* L. mesophyll cells in darkness

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Lead (Pb^{2+}) is one of the most important heavy metals polluting the natural environment due to human activity. Uptake of this metal may cause many destructive changes in plants, among others in the photosynthetic apparatus. However, so far it has been unclear whether the mechanisms of its toxic effect on chloroplast include disturbances in the distribution of these organelles in the mesophyll cell. In this study we investigated the effects of lead on chloroplast distribution patterns in mesophyll cells of *Lemna trisulca* in darkness. An analysis of confocal microscopy images of *Lemna* fronds treated by lead revealed differences in distribution patterns of chloroplasts in comparison to untreated plants. In dark-adapted control fronds, chloroplasts were uniformly distributed along all cell walls (dark position). In the fronds treated with lead ions nearly all chloroplasts accumulated at the anticlinal walls; only a few remained near periclinal walls. A similar arrangement of chloroplasts is observed also in control plants in response to strong light (avoidance response). Is the reaction of *Lemna* chloroplast to lead also an avoidance response? To address this question, we examined the colocalization of chloroplast and lead deposits. TEM X-ray microanalysis showed that intracellular chloroplast arrangement was independent of the localization of Pb deposits, suggesting that in the presence of lead the redistribution of chloroplasts, which looks like a light-induced avoidance response, is not a real avoidance response to the metal. Furthermore, we observed a similar redistribution of chloroplasts in *Lemna*

control plants in darkness after exogenously applied another stress factor - hydrogen peroxide. These results suggest a non-specific nature of the reaction of *Lemna* chloroplast to lead. A water angiosperm *Lemna trisulca* L. was treated with lead nitrate (15 μM Pb^{2+}) in the Wang medium (diluted 1:50) for 24 h in darkness. Some of the dark-adapted control plants were additionally incubated with 10^{-3}M hydrogen peroxide (H_2O_2) for 3 h in the dark or exposed to strong white light (350 μmol $m^{-2}s^{-1}$) for 45 min. Chloroplast distribution was analyzed under a confocal laser scanning microscope LSM 510 (ZEISS). Transmission Electron Microscope JEM 1400 (JEOL Co.) with energy-dispersive X-ray microanalysis system (Oxford Instruments) and high resolution digital camera (CCD MORADA, SiS-Olympus) installed in Nencki Institute of Experimental Biology (EU Structural Funds, SOP ICE) were used to co-localize lead and chloroplasts. This work was supported by the National Science Centre Grant No. NN303 806640.

P5.40

CRK5 as a convergence node between senescence and abiotic stress responses

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In plants, receptor-like protein kinases (RLKs) play essential roles in signal transduction by recognizing extracellular stimuli and activating the downstream signaling pathways. Cysteine-rich receptor-like kinases (CRKs) constitute a sub-family of RLKs and are distinguished by the novel C-X8-C-X2-C motif (DUF26) in the extracellular domains. In Arabidopsis thaliana, 44 members of CRKs family have been identified. One of them, CRK5, raises particular interest as a putative negative regulator of senescence and stress acclimation response. Functional characterization of crk5 shows its impaired adaptation to abiotic stress – UVC radiation, as well as decreased chlorophyll content, lower biomass production and visible symptoms of premature leaf aging. Accelerated senescence of crk5 is even more induced by external stimuli such as continuous dark and low CO2 concentration. CRK5 gene has many W-Box cis-elements in its promoter region specifically recognized by WRKY transcription factors (TFs). Many WRKY, e.g. WRKY53 and 70 are well known regulators of both plant senescence and defence pathways. RT-PCR analysis suggests that CRK5 may overlap WRKY-dependent regulatory pathways.

P5.41

Cucumber HMA5A and HMA5B are two tonoplast-localized homologs of *Arabidopsis thaliana* heavy metal ATPase HMA5 involved in cellular heavy metal homeostasis

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Arabidopsis thaliana has eight P_{1B}-ATPases (AtHMA1-AtHMA8), which are implicated in transition metal transport and homeostasis. Among them, the AtHMA5 protein is probably important for detoxification of plant cells from Cu excess. However, the subcellular localization of AtHMA5 has not been confirmed yet. Due to the lack of genomic resources, the studies on proteins homologous to HMAs in other plants have been limited. Since the genome of cucumber have been already sequenced and released to public, we used eight *A. thaliana* genes *AtHMA1-8* as the query sequences to identify genes encoding P_{1B}-ATPases in cucumber DNA. Similar to *A. thaliana*, cucumber has eight genes encoding putative P_{1B}-ATPases, however, there are two homologs of *AtHMA5* (*CsHMA5A* and *CsHMA5B*) and no homolog of *AtHMA3* in the genome of cucumber. The genes encoding two HMA5 homologs in cucumber roots are differentially expressed under heavy metal stress. *HMA5A* expression is up-regulated by Pb and down-regulated by Cd and Cu deficiency, whereas the transcript of *HMA5B* is markedly elevated upon Pb, Cu, Zn and Cd. Immunolocalization of

CsHMA5A and CsHMA5B with the specific antibodies revealed that both pumps of approximately 100 kDa reside at tonoplast membranes. The level of both proteins is elevated under heavy metal excess but only CsHMA5A is markedly reduced under Cu deficiency. In addition, *CsHMA5B* give rise to two transcripts of different size: *CsHMA5B-1* and *CsHMA5B-2*, depending on the Zn or Cd excess in nutrition media. The heterologous expression of *CsHMA5B-1* and *CsHMA5B-2* in *S. cerevisiae* restored the growth of yeast mutants on media enriched in Cu or Cd. Altogether the data suggest a different function of cucumber HMA5-like pumps in the homeostasis of cellular heavy metals.

P5.42

Changes in DNA methylation of embryonic axes of seeds of oak and beech during *in vitro* culture and cryopreservation

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DNA methylation plays a key role in the regulation of plant growth and differentiation of their organs. The present study determined the total level of DNA methylation in plants growing from embryonic axes before undergoing cryopreservation. The study focused on two species of trees: pedunculate oak (*Quercus robur* L.) with *recalcitrant* seeds, sensitive to dehydration and European beech (*Fagus sylvatica* L.), the seeds represent the category *suborthodox*, i.e. tolerant to dehydration. DNA methylation was determined by a method using an antibody directed to the methylated cytosine in CpG dinucleotide. Analysis focused on tissues treated with: i) dehydration and treatment with a vitrification solution (PVS3) and ii) the vitrification step, and then cryopreservation in liquid nitrogen (LN_2), (−196 °C). DNA methylation was induced mostly in tolerant genotype *F. sylvatica* after 30 days of culture of embryonic axis, where its total level in cryopreserved embryo axes was 30% higher compared with the control, while for *Q. robur* no changes in methylation were observed. After 120 days of plant growth demethylation changes appeared which were also more pronounced in species *F. sylvatica* than in *Q. robur*. This response was more pronounced in plants after cryopreservation stage in LN_2, than in control plants or subjected to vitrification solution. This research was supported by the Polish National Science Centre in Kraków(NCN), grant No. N N309 101836 (to PMP).

P5.43

Vigour of naked and husked oat cultivars under drought stress conditions

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Reproduction of the sowing material characterized by high-vigour seeds creates possibilities for full utilization of the potential of specific cultivars under suitable growth and developmental conditions. One of the problems in seed production is high sensitivity of traditional oat cultivars to water deficits to which this crop is exposed especially in early spring, during seed germination, when low temperature causes the phenomenon of the so-called soil drought which limits the possibilities of using in full the winter water resources. The initiation of the imbibition process, necessary for activation of the enzymes responsible for germination, is determined by the amount of available soil water and by the range of temperatures which are most favourable for the germination process. Water stress occurring in early spring may delay field emergence or completely stop that process. Considering the above, the knowledge of the reaction of naked oat cultivars to drought conditions is still insufficient. The objective of the research was to determine the effect of the drought stress on vigour of naked and husked oat cultivars. The studies covered two husked oat cultivars: Cwał and Stoper, and eight naked oat cultivars: Abel, Avenuda, Bullion, Cacko, Izak, Pikant,

Polar and Saul, characterized by high germinability (above 95%). Naked oat seeds showed susceptibility to varied thermal conditions during drought simulated with the use of PEG at a concentration of −1.5 MPa. A rise in temperature from 10°C to 20°C affected a 36% increase in the number of normally germinating seeds and a 51% increase in germination rate, as well as a 25% decrease in the average germination time. An increase in the osmotic potential from −1 to −2 MPa during initial induction of the drought stress resulted, in husked cultivars, in a 17% decrease in vigour determined on the basis of the percentage of normally developed seedlings. The oat seed vigour, evaluated on the basis of electrical conductivity of exudates, was modified by genotypic variability. Lower, by 60% on average, values were noted for naked cultivars. The coefficients of correlation between electrical conductivity of exudates and germinability $r = -0.784^{**}$) or frequency of normally developed seedlings $r = -0.919^{**}$) confirm the highly significant interrelationship between the methods used for assessing oat seed vigour under drought conditions.

P5.44

The effect of galactinol synthase activity on RFOs content in developing pea seeds (*Pisum* sativum L.) subjected to drying and cold

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Raffinose family of oligosaccharides (RFO) is soluble sugars widely distributed in legume seeds. The most common RFO are raffinose, stachyose and verbascose. The key compound necessary for their synthesis is galactinol [*O*-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol] formed in a reaction catalyzed by galactinol synthase (GolS) (UDP-α-D-galactose:1L-*myo*-inositol-1-α-D-galactopyranosyl transferase, EC 2.4.1.123). The aim of the study was to investigate the effect of drying and cold on galactinol synthase activity and soluble oligosaccharides content in developing pea seeds. Pea seeds at degree of maturity of 12 and 22 DAF (DAF, day after flowering) were exposed to various stressors. Seeds were dehydrated at 12% RH (RH – relative humidity) (fast drying) and dehydrated at 75% (slow drying). The seeds were also exposed to low temperature +4°C (cold). After 4, 8, 12, 24, 192 and 300 h of stress fresh and dry weight of seeds, GolS activity and soluble carbohydrates content were determined. The enzyme activity was measured by determining the amount of galactinol formed, and expressed as nmole galactinol mg protein^{-1} min^{-1}. The formation of galactinol and carbohydrate content was monitored by High Resolution Gas Chromatography (HRGC). It was shown that soluble carbohydrates content and GolS activity depended on the degree of development of the seeds. 12 and 22 DAF seeds contained mainly sucrose and monosaccharides. The amount of RFOs was about 2 fold higher in 22 DAF seeds than in 12 DAF seeds. The enzyme activity in 12 DAF seeds was 4 times lower than in 22 DAF seeds. Both fast and slow drying increased approximately 8-fold the activity of the enzyme in 12 DAF seeds. Untill 192 h of dehydration GolS activity did not change in 22 DAF seeds subjected to drying, and then the activity significantly decreased, as a result of drying seeds at both developmental stages accumulated large amounts of RFOs. Cold did not significantly influence on significant changes in monosaccharides, sucrose, RFOs amount and galactinol synthase activity regardless of the degree of maturity of pea seeds. These results suggest that changes in activity of this enzyme in response to abiotic stresses depended on maturity of pea seeds. In contrast to the dehydration, cold did not induce changes in galactinol synthase activity. In 12 DAF seeds submitted to drying increased GolS activity corresponded to high RFOs accumulation. An increase in GolS activity wasn't reflected into galactinol content. It suggests that galactinol was used as galactose donor during RFO biosynthesis. These results indicate a significant role of galactinol synthase in response of developing seeds to the decreasing water content.
The research was supported by Polish Ministry of Science and Higher Education funds (NN 310088137).

P5.45

Drought-induced changes of actin cytoskeleton organization in barley leaves

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Cytoskeleton rearrangement plays a crucial role in adaptation to dynamically changing conditions of the environment (Kobayashi et al., 1997). Although the physiological and molecular aspects of barley response to drought stress were elderly examined (Rapacz et al., 2010; Gou et al., 2009) nothing particularly is known about the drought induced changes in actin (AKT) expression and remodeling. In the present paper expression profile and configuration of actin filaments (AFs) were analyzed in barley (*Hordeum vulgare* L.) leaves. For that reasons qPCR and whole mount immunodetection techniques were applied. AFs were stained in genotypes varied in susceptibility to drought stress. During the experiment detached leaves were dried under controlled conditions. The drought treatment revealed different expression profiles for *AKT* gene for drought tolerant and susceptible genotypes. After a comparison of the gene expression profiles at five time points (55′, 110′, 165′, 220′ and 275′ under drought treatment) differences were identified between investigating genotypes. Generally, gene expression level decreases in response to drought. To study the distribution of the actin microfilaments in barley leaves, we stained actin with fluorescence-labelled Alexa Fluor 488 phalloidin, and observed actin fluorescence by confocal laser microscopy, xyz-sectioning allowed visualization of AFs very clearly. Significant differences were observed between the organization of the cytoskeleton in the drought treated and control tissues. It can be supposed that AFs rearrangements may be an element of response to water deficit in the cells. This work was supported by the European Regional Development Fund through the Polish Innovative Economy Program 2007-2013, project WND-POIG.01.03.01-00-101/08 POLAPGEN-BD "Biotechnological tools for breeding cereals with increased resistance to drought". This work was supported by The National Science Centre awarded by decision number DEC-2012/07/N/NZ9/02412.

P5.46

Biosynthesis of defensive secondary metabolites of spice plants in answer to heavy metal stress condition

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During normal vegetation plants are exposed to many environmental pollutants such as presence of heavy metal ions in the soil, chemical pesticides, excessive UV radiation or attack of pathogenic microorganisms. One of the first defense reactions of living cells is an increase in the concentration of reactive oxygen species. To protect living cells plant synthesizes a series of defensive secondary metabolites, among which the polyphenolic compounds and defensive proteins play the most important role. Defensive proteins known as pathogenesis related proteins (PR proteins) exhibit different antimicrobial activity. Some of these proteins have documented allergenic properties and can cause severe allergic reactions, including anaphylactic shock. In our work we examined the concentrations of polyphenolic compounds in the tissues of popular spice plants, their antioxidant properties as well as the concentration and identification of defensive proteins. We compared the content of these secondary metabolites in control plants vs. plants under heavy metal stress condition. During our research we were trying to check dependence between plant breeding conditions and the actual contents of defensive metabolites. We were trying to answer the question if there is any correlation between concentration of antioxidant compounds and the content of pathogenesis related proteins, which are a source of potential allergens.

P5.47

In vitro evolution and characterization of a copper resistant strains and populations of *Chlamydomonas reinhardtii*

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Chlamydomonas reinhardtii P. A. Dang is used as a model green alga in research on photosynthesis and environmental stress (Hanikenne, 2003). As shown by Prasad et al. (1998), CW15 mutant of *Chl. reinhardtii* is more sensitive to copper excess than wild type. Copper is an example of heavy metal which is an essential micronutrient for all organisms. However, in higher concentrations Cu^{2+} can disrupt photosystems and it promotes very efficiently formation of reactive oxygen species (Yruela, 2009). Moreover Cu^{2+} excess causes change in level of mRNAs related to stress response, glycolysis and gluconeogenesis (Jamers et al., 2006; Simon et al., 2008). There are three main ways cells may protect themselves against copper excess: i) extracellular complexation of metal excess outside the cell (mainly by cell wall components); ii) precipitation in the cytoplasm or vacuole and iii) binding by polypeptides or proteins (for review see Mallick and Rai, 2002). It is well known (rev. in Lindberg and Greger, 2002), that protective mechanisms can be constitutive or inducible and that there are genetic differences between Cu-tolerant and Cu-sensitive organisms of the same species. Metal-resistant populations have been already isolated and physiologically characterized in experiments with arsenic (Fujiwara et al., 2000) and cadmium (Collard and Matagne, 1990; Collard and Matagne, 1994; Nagel and Voigt, 1989). From the Cu^{2+}-excess (5 mM) tolerant population of *Chl. reinhardtii* CW15 (cell wall free mutant) we obtained genetically homogenous strains. One of these strains, Cu3, was characterized in presented experiments. We compared growth rate and photosynthesis parameters between the copper-excess-adapted and control (typical) strains of *Chl. reinhardtii* during exposition to high concentrations of Cu^{2+}, Zn^{2+} and Cd^{2+}. We used maximal chlorophyll fluorescence (F_M) for the measurement of growth rate showing that this parameter correlates very strongly with chlorophyll concentration measured spectrophotometrically. Cu3 strain in comparison to the control strain showed significantly higher tolerance towards copper ions, whereas tolerance towards cadmium and zinc ions was similar in both strains. Photosynthetic electron transport in Cu3 was also less sensitive towards acute Cu^{2+} stress (300-1000 mM, 24 h) than in control strain, as was showed by measurements of fast chlorophyll fluorescence induction kinetics. In the control strain we observed more pronounced decrease of quantum yield of photosynthesis (F_V/F_M) and of T_{Fm} values (time at which the maximal fluorescence level occurs). They can be considered as signs of stronger PS2 inhibition. From Cu3 strain we have also obtained populations that exhibit further enhanced tolerance for copper ions. Cells from one of them can complete their life cycle at Cu^{2+} concentrations as high as 200 μM.

P5.48

Molecular and physiological aspects of the role of root hairs during drought stress in barley

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Roots are the first plant organ that senses drought and it is well known that root system architecture changes in the response to water deficit. Root hairs, tubular-shaped outgrowths of root epidermis, are one of the elements of root system, but the knowledge about their role in plant response to drought is very limited. Even if it is generally accepted that they play an important role in water and minerals uptake from the soil, the molecular mechanisms linking root hairs as plant organs with their presumable role, are largely unknown. Here, using a series of physio-

logical tests and the global transcriptome differentiation methods in the wild-type/root hairless mutant system we deducted the role of root hairs in the adaptation to drought conditions in barley. A root hairless barley mutant *rhl1.a* obtained in our department by N-methyl-N-nitroso urea (MNU) treatment and its parent variety "Karat", producing normal root hairs, were used in this study. Severe 10-day-long drought treatment resulted in similar decrease of RWC values in both genotypes to 40-50% of plants grown in control conditions. Similarly, two weeks of rehydration allowed to restore the value of RWC to the level observed in control. However, the lack of root hairs in the *rhl1.a* mutant strongly affected photosynthesis under drought stress. All parameters of the maximal efficiency of PSII photochemistry and electron transport were much more decreased in *rhl1.a* mutant than in its wild-type parent, reflecting a strong inhibition of photosynthetic processes and significant damage of photosynthetic apparatus. Accordingly, comparative analysis of drought-regulated transcriptomes of root-hairless mutant *rhl1.a* and its parent variety "Karat" revealed drastic changes of transcriptomic response in both genotypes. Much more drought-related differentially expressed genes were observed in leaf and root transcriptome of root-hairless mutant *rhl1.a* than in its parent variety "Karat" and transcriptomes of both genotypes differed in differential expression of both, down- and up-regulated drought-related genes. Gene Ontology enrichment analysis, carried out on genotype-dependent groups of differentially drought-regulated genes, resulted in identification of biological processes involved in root hair phenotype-dependent response to drought stress. Moreover, the selected genes distinguishing root transcriptomes of root-hairless mutant *rhl1.a* and its parent variety "Karat" in normal control conditions at 6-d-old seedlings stage showed genotype-specific expression pattern during drought stress.

P5.49

DNA methylation dynamics under drought stress in barley

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DNA methylation is one of the epigenetic phenomena that play a crucial role in regulation of gene expression in plants in response to environmental stimuli. In the presented study, the effect of drought stress on DNA methylation level, pattern and its correlation with gene expression in *Hordeum vulgare* L. were assessed. Drought stress study was carried out with spring barley variety "Karat". Plants were grown in pots with soil of controlled moisture. During the 39-days-long experiment, leaf and root tissues were collected in the four time points: [1] in normal conditions, 12 days after germination, with 12% of soil moisture, [2] after three days of mild drought stress, when the soil moisture gradually decreased to 3%, [3] after 10 days of severe drought with 1.5-3% soil moisture and [4], after 14 days of water re-supply. In order to evaluate changes in the level and pattern of cytosine methylation under drought stress, a methylation sensitive amplification polymorphism (MSAP) technique was used on leaf and root samples. The results indicate that drought stress induced global-wide changes in the DNA methylation pattern in both, leaves and roots. More methylation changes were observed in leaves than in roots of plants grown in water-limited conditions. In leaves methylation events occurring under severe drought dominated, and, interestingly, they went back to their initial status after recovery. In roots, many hipermethylations were observed but, in contrast to leaves, they occurred earlier, under mild stress, and the new methylation pattern remained unchanged after re-watering. Moreover, re-watering caused numerous demethylation changes in loci unaffected by the drought stress. To evaluate the hypothesis that drought-induced changes in barley epigenome could specifically influence differential expression of drought stress-related genes, the analysis of DNA methylation of previously identified candidate genes was performed. The Bisulfite Patch PCR technique allowed the detailed analysis of drought-induced alterations in methylation patterns within proximal promoters and 5′ UTRs of selected drought stress-related genes. A link between induced changes in cytosine methylation and differential expression of drought stress-related genes was indicated.

P5.50

PsbS is required for local and systemic integration of the photosystem II quantum-molecular functions with foliar heat dynamics, acclimatory and defense responses in *Arabidopsis*

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In a simplified model of photosynthesis, light energy absorbed by chlorophylls of photosystem II is distributed between photochemistry, fluorescence, and heat. Regulation of this distribution is important for stress responses, but its role and mechanism are not known. To get a new insight into this mechanism we measured time resolved chlorophyll *a* fluorescence and foliar temperature dynamics in wild type and recessive mutant of non-photochemical quenching (*npq4-1*) in *Arabidopsis thaliana* rosettes partially exposed to excess light. In wild type foliar temperature and fluorescence decay (FD) exhibits specific regulation between leaves directly exposed to excess light and leaves undergoing systemic acquired acclimation. In contrast in directly exposed *npq4-1* leaves we observed higher foliar average temperature, temperature dynamics and much faster FD, which is insensitive to excitation power. However, in systemic *npq4* leaves foliar temperature and FD changes are insignificant. This complex fluorescence dynamics can be described by a set of maximum three FD times. Interestingly the appearance of the longest FD time correlates with higher average foliar temperature and deregulated foliar temperature dynamics. This influence the expression of the robust molecular markers for light acclimation, heat shock and defense responses. We concluded that higher plants have developed global regulatory system of absorbed photon fate and PsbS integrates changes in quantum-molecular functions of the photosystem II with regulation of light acclimatory, heat shock and defense responses.

P5.51

Influence of drought stress on water relations and photosynthetic apparatus in spring barley plants (*Hordeum vulgare* L.)

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Among the environmental stresses, drought stress is one of the most limiting factors of plant growth and productivity. Environmental stresses can cause a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical responses to drought is essential for an overall perception of plant resistance mechanisms to water-limited conditions. A research was conducted on spring barley drought-treated in seedling stage. Water management, stability of cell membranes, activity of photochemical processes in PSII, CO_2 assimilation intensity, non-photochemical excitation energy dissipation and damage to the photosynthetic apparatus were investigated. Drought level of soil used in the study resulted in a clear decline in the value of physiological parameters such as net photosynthesis rate and transpiration. Parameters describing the photochemical activity of individual reaction centers of PSII were slightly sensitive to drought. Drought stress has highlighted the correlation relationship between water management and physiological parameters, especially for RWC (Relative water content) parameter. POLAPGEN-BD project financing and conformity with Operational Programme Innovative Economy 2007-2013.

P5.52

Short-term heat stress effects on a *cyp*11A1 canola plants

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Resistance to abiotic stresses becomes an essential characteristic of plants because of the climate changes. High temperatures of leaves reduce plant growth and limit crop yields. Earlier we have obtained spring canola (*Brassica napus* L., cv Mariia) lines bearing bovine *cyp*11A1 gene in their nuclear genome using *Agrobacterium tumefaciens*-mediated leaf disk transformation. This gene encodes cytochrome P450SCC of adrenal cortex mitochondria and was shown to affect the biosynthesis of steroid compounds in transgenic tobacco (*Nicotiana tabacum* L.). *Bar* gene was used in a transformation cassette as a selective marker, so *cyp*11A1 canola plants were resistant to BASTA herbicide treatment in greenhouse conditions. Some of the transformants accumulated an increased amount of total soluble proteins in leaves and seeds. They have enhanced antioxidant activity in leaf tissue. Some of them flowered 5-7 days earlier than the wild-type plants. Homozygous *cyp*11A1 lines (T21a and T22c) obtained by self pollination of primary transformants under greenhouse conditions were selected as the most tolerant to osmotic stress induced by mannitol in the previous *in vitro* experiments. In the present work, the growth features of these plants were investigated under short-time heat stress (42 °C) in a growth chamber. Electrolyte leakage in *cyp*11A1 canola leaves was 40% lower and relative water content keeps up by 13% higher in comparison with wild-type plants under stress. The fatty acid content and palmitic acid (PA) level were increased (up 25% and 19%, respectively) in transgenic leaves while palmitlinolenic acid (PLA) content were decreased (up by 33%) under 42 °C. Both saturated (PA) fatty acids increase and trienoic (PLA) fatty acid content decrease are characteristically for heat resistant plant. Linoleic acid content was rose and linolenic acid content did not change during heat stress in all plants tested. Chlorophyll *a* and carotenoids content was increased under stress conditions in control as well as *cyp*11A1 canola, but quantity of these photosynthetic pigments became lower in the latter_which is typical for plants with improved thermotolerance. Chlorophyll *b* content retained without changes except for the increase in transgenic T21a line. Superoxide dismutase (SOD) activity in transgenic leaves was up 1.76-fold higher than in control at 22 °C. It remained unchanged under heat in *cyp*11A1 leaves whereas SOD activity increased in control. Manifestation of mammalian *cyp*11A1 expression in transgenic canola is the same as the heterologous *sod* overexpression. SOD activity increase could be caused by cytochrome P450SCC expression which resulted in the superoxide radical formation. We suppose that SOD activity increase plays a defining role in the biochemical alterations in tested transgenic canola metabolism, which allows thermotolerance improvement. These plants might be resistant to other stress conditions both of abiotic and biotic origin.

P5.53

The properties of peroxisomal ascorbate peroxidase from sugar beet (*Beta vulgaris*) and expressed in *E. coli*

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A family of ascorbate peroxidases (APX) plays a key role in antioxidant protection in plant cells. The APX compartmentation and activity levels strongly affect the efficiency of adaptation to adverse environmental conditions. Studies on the role of these enzymes in the stress response in sugar beet are seriously hampered by the absence of data regarding the molecular and biochemical characteristics of APX in this plant. Recently we cloned and cha-

racterised a peroxisomal isoform of APX from sugar beet. The ORF of *Bv*pAPX was amplified by PCR and cloned into the pF1A T7 Flexi expression vector (Promega). Induction of cultures of BL21 cells carrying the pF1AT7/*Bv*pAPX construct resulted in the elicitation of ascorbate peroxidase activity in the soluble fraction of cells. The molecular weight of overexpressed protein was estimated to be ~32 kDa. APX activity in the *E. coli* expressing *Bv*pAPX was dependent on the presence of ascorbate as the electron donor. Replacing ASC by alternative electron donors, such as guiacol, diaminobenzidine or NADPH, resulted in strongly reduced peroxidase activities in protein extracts from induced BL21 cells. The enzyme's kinetics was studied using a series of ASC or H2O2 concentrations in the reaction mixtures. At fixed ASC concentration, the Km and Vmax values for H2O2 were 27.03 μM and 13.5 μM/min, respectively. At fixed H2O2 concentration, the Km and Vmax values were 95.24 μM,and 16.4 μM/min, for ASC. The enzyme was active in pH ranging from 5.5 to 8.0, however, the highest activity was observed at pH 7.0. The ascorbate-dependent peroxidase activity in extracts from cells expressing *Bv*pAPX was inhibited by KCN and NaN3, the heme inhibitors, as well as thiol group modifier; *p*-chloromercuribenzoic acid. The APX activity was also inhibited by hydroxyurea and p-aminophenol, which were reported to act as suicide inhibitors of APX.