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Opening lectures

Evolutionary aesthetics as a meeting point of philosophy and biology

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Metaphysics, or the knowledge of what there is, has been traditionally placed at the pinnacle of philosophical hierarchy. It was followed by theory of knowledge, or epistemology. Practical knowledge of proper modes of conduct, ethics, came third, followed by aesthetics, treated usually in a marginal way as having to do only with the perception of the beautiful. The hierarchy of philosophical disciplines has recently undergone a substantial transformation. As a result, ethics has assumed a central role. The aim of this paper is to suggest that the hierarchy of philosophical disciplines is not yet complete and that one further step needs to be taken. According to the claim advocated here, it is not metaphysics, epistemology or ethics, but aesthetics that is the first and foremost of all philosophical disciplines. This claim is argued for by references to findings of evolutionary aesthetics, especially to Charles Darwin's idea of sexual selection as elaborated in *The Descent of Man*. I also argue that Darwinian approach to morality is, and should be, derivable from an Darwinian aesthetics which lies at the core of his conception of sexual selection.

From molecules to trees: exploring plant development with computational models and simulations

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As early as the 1960s and 1970s, computer models were introduced to investigate the relations between local control of development and the global form of plants. Over the last decade, models rooted in molecular-level data and hypotheses have offered further insights. At the basis of these models lies a postulated feedback between the concentrations and flow of auxin, and the distribution of auxin transporters, in particular PIN1 proteins. Operating in the shoot apical meristems, this feedback can explain the spatial arrangement of buds around their supporting axes (phyllotaxis). Operating along the plant axes, a related feedback mechanism explains which buds will eventually develop into branches (bud activation). Of particular interest are strategies that plants employ to avoid overcrowding if the branching processes repeat and several orders of branches are formed. One strategy, found primarily in herbaceous plants and inflorescences, is to gradually reduce the size of branches, producing fractal patterns. Another strategy, widely found in trees, is to avoid overcrowding through competition between buds and branches for light and space. Recent models show that a wide variety of temperate-climate tree architectures results from different biases in this competition. Although many gaps in our knowledge remain, the link between molecular-level processes and the architecture of herbaceous plants and trees begins to appear.

Purkyně Day – organismal level

Jan Evangelista Purkyně (1787 – 1869) and the establishment of cellular physiology - Wrocław as a Central European cradle for a new science

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Purkyně was a strong character driven by honest rational thinking – after the education by Piarists he studied medicine at the Charles University in Prague. He appreciated religion and philosophy very much, yet he ardently defended laboratory experiment as an ultimate way to the truth in science. He put regularly his whole personality into experimental situations – when he explored subjective visual phenomena or tested effects of drugs on human apprehension. Purkyně was nominated as a professor of physiology in Prussia governed Breslau/Wrocław in 1823. For his crucial scientific achievements he rose into high esteem. His observations made him one of the fathers of the cellular theory; he devised the name «protoplasm». Combination of physical, chemical and microscopic experiments and observations made him father of modern physiology. The Physiology institute he established in Wrocław on November 8, 1839 was the first physiological Institute worldwide. His name is connected to many crucial discoveries – some of them rediscovered later by others. All the years in Wrocław Purkyně was a focal personality of Slavic cultural life in the city. He was as devoted teacher as researcher – he insisted that teaching should be based on practical demonstrations of phenomena allowing students to participate actively in conceptual work. His two sons (Karel is considered one of the founders of modern Czech painting) brought with them regularly their schoolmate Julius Sachs. With him and two sons he succeeded to come back home to Prague in 1850. Without Purkyně support and guidance Julius Sachs would not become a founder of modern plant physiology. Back home he became crucial personality and cornerstone of modern Czech scientific and cultural life. Not only genius in science, Purkyně is also an inspiration how to be truly humanistic European and yet responsible patriot.

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Session 1
Signaling networks regulating environmental stress responses

Plenary lectures

Apoplastic ROS perception and signaling

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Effective responses to both external and internal stimuli will ensure optimal growth and survival in an environment where productivity and product quality are adversely affected by biotic and abiotic stresses. Plants must have effective means of defending themselves against invading pathogens and adapting to changes in their environment. The main features of such defence measures involve early recognition and perception of the developing stress, and subsequent activation of induced adaptive and defensive responses leading to both local and systemic resistance. Reactive oxygen species (ROS) that are formed in plant cells by several stresses may be one of the factors that contribute to, and regulate plant stress sensitivity/ tolerance. Strong evidence has accumulated that ROS play an important role in the signaling resulting in induction of plant defence responses. The recognition of a stress is followed by involvement of a small number of signal transduction pathways. They also seem to control and potentiate each other's activities, indicating that cross talk between these pathways may be very common in defence gene regulation. Several stresses are experienced by plants much in the same way. Therefore, it seems obvious that the signal transduction pathways involved are, at least partially, identical and combinations of signal molecules might direct the activation of certain defence responses. The air pollutant ozone generates reactive oxygen species (ROS) in the leaf apoplast. Consequently, ozone has been used as a tool to unravel *in planta* ROS-induced processes and apoplastic ROS sensing. Two examples of interaction between multiple regulatory cascades discovered with this approach are presented.

Cell death during late senescence depends on Whirly1

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Senescence is the last phase of leaf development preceding death of entire plants or organs such as leaves. It is accompanied by massive changes in metabolism and gene expression. One of the genes with enhanced expression during barley leaf senescence is the *HvS40* gene. Its expression is induced by signalling compounds originating from chloroplasts, abscisic acid, jasmonic acid and salicylic acid, as well as by fungal pathogens (Krupinska et al. 2002).
One of the proteins binding to the promoter of the *HvS40* gene is Whirly1, a plastid/ nucleus-located DNA-binding protein (Grabowski et al. 2008). Transgenic barley plants with a knockdown of Whirly1 show a delayed senescence phenotype. The phenotype was only visible when plants were grown at high light intensity. It is hypothesized that Whirly1 is a downstream component of salicylic acid inducing light dependent cell death during late senescence. It is furthermore proposed that Whirly1 is retranslocated from chloroplasts to the nucleus in response to salicylic acid. Transplastomic tobacco plants synthesizing an HA-tagged version of Whirly1 inside the plastid were used to show translocation of Whirly1 from chloroplasts to the nucleus.

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Oxidative stress signal transduction. Gathering the pieces.

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Different biotic and abiotic stresses adversely affect plant growth and development leading to worldwide yield losses. A common theme within these environmental factors is the perturbation of reactive oxygen species (ROS) homeostasis. Despite the great economic importance, the signal transduction mechanisms of the oxidative stress response in plants are poorly understood. We conducted several (forward and reversed) genetic and chemical screens, together with proteomic approaches in order to obtain a more comprehensive overview on the different components of the network that govern the oxidative stress response. These efforts identified several genes as new members of the oxidative stress gene network in plants, together with small molecules that are able to interfere with specific events during the oxidative stress response in plants. The potential of these genes and molecules for providing abiotic stress tolerance in commercially relevant plants will be assessed in the near future.

Oral presentations

Nitric oxide contributes to distal signal generation from roots to leaves in Cd-challenged seedlings of yellow lupine

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Cadmium (Cd) is one of the most toxic elements occurring in polluted environments. Because of its high solubility in water it is rapidly taken up by plant roots, in which the most evident phytotoxic symptoms including cell death are observed. In the present study, we examined the effect of cadmium stress on the endogenous nitric oxide (NO) generation and salicylic acid (SA) level in 12- day old seedlings of yellow lupine (*Lupinus luteus* L. cv. Juno). On the 12th day of plant culture the roots were treated with cadmium ($CdCl_2$) at concentration 10mg/l and after 24h of stress duration the root tips, hypocotyls and primary leaves were taken for analysis. Furthermore, the determination of metal content and localization of Cd by dithizone complex in root tissues was examined.
Cd ions treatment of lupine seedlings resulted in temporary NO generation starting from the apical zone through the elongation and finally to differentiation zone of the root. At the same time point an enhanced NO synthesis was observed along with the main veins of primary leaves. Additionally, a visible modulation of salicylic acid (SA) level in root and hypocotyls cells was noted. Remarkably, the statistically significant increase (*ca*. 20%) in SA level after Cd treatment was found in lupine primary leaves as well. The observed changes in generation of both signals were correlated in time with programmed cell death (PCD) of root cells, which was evidenced by TUNEL positive fluorescein assay. It is possible that through Cd-challenged PCD plants might generate a mobile distal signal in order to enhance the tolerance responses of other upper plant organs, e.g. leaves. However, this assumption seems to be possible only at low Cd doses, which are able to trigger protective responses.

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Distribution of some reactive oxygen species during rhizogenesis *in vitro* from hypocotyls of *Mesembrynthemum crystallinum* L.

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Reactive oxygen species (ROS) play important role in the regulation of plant development (Gapper and Dolan 2006). Recent discoveries suggest that ROS may control development through regulation of cell growth. ROS production and accumulation during acquisition of competence to rhizogenesis as well as during early stages of root development were analyzed by imaging techniques for superoxide radical ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) visualization. Superoxide radical has been produced in the response to wound stress on the both ends of the explants that has been just cut off from the seedling. This effect has been no more visible just after one day of culture when $\cdot O_2^-$ has been uniformly distributed throughout the hypocotyls. In the following days of culture high level of superoxide radical deposits has been preferentially found in cells involved in *de novo* root formation (that is, in the cells of inner cortex) before any visible organized root primordia has been observed. During early stages of root growth superoxide radical has been localized in meristematic zone of newly formed roots. Hydrogen peroxide has been mainly visualized in veins of hypocotyls and during following days of culture level of H_2O_2 precipitates have grown up uniformly throughout hypocotyl in the cells surrounding main vein. Hydrogen peroxide distribution was also visualized on the cell level under electron microscopy by cytochemistry.
Inclusions of cerium-H_2O_2 has been detected in mitochondria, chloroplasts and in the cell wall but only in hypocotyls cut off from the seedlings. During following days of culture hydrogen peroxide localization has been limited to apoplast of meristematic cells of cultured hypocotyls and developing roots.

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Photo-electrophysiological and quantum-redox retrograde signaling in plants under excess light stress

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Plants have developed a highly responsive and flexible physiology and photochemistry which allows them to function in the naturally variable environment. Plants naturally absorb more light energy than they need to drive photochemistry, therefore light energy absorbed in excess must be dissipated. This in turn induces quantum-redox changes in photosynthetic electron carriers of photosystem II (PSII) and in its proximity (e.g., in singlet oxygen levels, nonphotochemical quenching and redox status of the glutathione, ascorbate and plastoquinone pools). Furthermore, chloroplast originated photo-electrophysiological retrograde signals could be transmitted intra- and extracellularly by the chloroplasts stromules that form network of extended chloroplast envelope membranes connecting different cellular organelles or by the bundle sheath cell layer connecting different plant organs. Obtained results suggested that photo-electrophysiological signaling triggers changes in *APX1* and *APX2* gene expression, reactive oxygen species/hormonal circuits, stomatal conductance, programmed cell death, and in consequence systemic acquired acclimation and systemic acquired resistance (Karpinski et al. 1999, Jabs et al. 1996). Moreover, *Arabidopsis* rosette partially exposed to excess light with different wavelength but similar provided energy is specifically memorized by leaves for at least several days. We concluded, that plant leaves adjust their dynamic and discrete in time and cellular network space optimal light acclimation, photosynthesis, gas exchange and transpiration processes in a way similar to that defined by the cellular automation algorithm and complex biological computation that is depending on absorbed light energy in excess (Peak et al. 2004, Szechyńska-Hebda et al. 2010). Our results suggests that plants can be intelligent organisms capable to utilize the memorized information encrypted in spectral composition of light in order to improve their Darwinian fitness and survival chances.

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NO modifies the expression of genes implicated in the ethylene biosynthesis and signaling pathway during dormancy alleviation of apple embryos

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Short term pre-treatment of embryos isolated from dormant apple (*Malus domestica* Borkh. cv. Antonówka) seeds with nitric oxide (NO) stimulates their transition from dormancy to germination and removes morphological anomalies of developing seedlings, such as asymmetric growth and greening of cotyledons. NO induces emission of ethylene by germinating embryos. As a gaseous phytohormone, it breaks dormancy of seeds of various plant species. Ethylene is produced from *S*adenosyl-methionine in reactions catalyzed by ACC synthase (ACS) and ACC oxidase (ACO). The signal transduction pathway includes four receptors (ERS1, ETR1, ETR2 and ETR5), which appear to interact with CTR1 (a Raf-like kinase). Then the signal passes through the protein EIN2. Downstream components of the ethylene signal transduction pathway include the EIN3/EIL and ERF families of transcription factors. We analyzed the expression of *MdACS1; MdACO3; MdERS1; MdETR1,2,5; MdCTR1*; *MdEIN2, MdERF1* and *MdERF2* genes, using semi-quantitative reverse transcription –polymerase chain reaction. We investigated the expression profiles of genes mentioned above in axes and both cotyledons separately, isolated from dormant or NO pre-treated embryos. The analyzed genes were differently affected during dormancy alleviation and *sensu stricto* germination. In axes isolated from NO pre-treated embryos, the transcription of *MdACS1* and *MdACO3* as well as of *MdERF2* increased markedly, while the expression of *MdETR2* decreased in comparison to axes isolated from dormant embryos. However, in cotyledons isolated from dormant and NO pre-treated embryos no significant alterations were observed in the expression profiles of these genes.

Response of pea to pea aphid *Acyrthosiphon pisum* infestation

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The aim of study was to examine defence responses of *Pisum sativum* L. cv. Cysterski to infestation by pea aphid *Acyrthosiphon pisum* Harris. The first objective determined was activity of important enzymes in the salicylic and jasmonic acid biosynthesis pathway, i.e. phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX), in response to varying numbers of pea aphids. Recognition of aphid feeding by plant receptors and ensuing defence responses are followed by the transmission of signal cascades that involve various signaling molecules, including jasmonic and salicylic acids (Morkunas et al. 2011). The second objective was to examine whether and which degree oxidative stress was induced in pea leaves by aphid infestation. Therefore, concentration of free radicals and the relative release of superoxide anion were assessed.
PAL and LOX activity in leaves of pea seedlings increased from 0 to 96h cultivation. The highest value was recorded at 96h. Activity of these enzyme in leaves infested by varying numbers of aphids was significantly higher than in the control. Electrophoretic data showed the presence of two LOX-isoforms in pea leaves; intensity of one isoform strongly increased from 72 to 96h after infestation. Concentration of semiquinone radicals EPR detected with two g-values of 2.0020 and 2.0035 also increased during infestation time and was higher than in the control. The highest concentration was found in leaves infested by 30 aphids at 96h. Superoxide anion was assayed by using specific indicator, dihydroethidium (DHE). The DHE-derived fluorescence was covered a much larger area in leaves infested by larger number of aphids from 0 to 96h. These results suggest that the change of PAL and LOX activity may be related with the oxidative responses of pea seedlings induced by aphid infestation.

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A functional study of *Arabidopsis thaliana* sirtuins

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Abiotic stresses are the major causes of yield loss in cultivated crops. These losses are the result of multiple mild stresses episodes throughout the growing season as well as periodical severe stresses. Therefore, breeding of crop varieties with improved responses to environmental changes is one of the important goals in modern agriculture. Crop varieties with enhanced tolerance to abiotic stress will broaden the window of optimal growth conditions for cultivated crops, thereby increasing average yield, yield stability and productive acreage. Such traits will provide substantial benefits to farmers and processors; may reduce costs in seed production and may lead to implementation of new strategies in plant breeding. Today the molecular and biochemical mechanisms involved in abiotic stress responses are still poorly understood and the signaling networks remain elusive. Recent data indicate that an efficient energy homeostasis contributes to stress tolerance and that the modulation of cellular NAD(P) homeostasis is an attractive and innovative strategy to improve plant performance in stress conditions. Functional studies in various eukaryotes indicate a potential important role for sirtuins in NAD+ dependent stress tolerance (Lagouge et al. 2006). Sirtuins catalyze protein and histone deacetylation and have been suggested to play a role in the suppression of recombination, chromosome stability, metabolic regulation and ageing (Denu et al. 2003). Through stress related phenotyping of transgenic plants with perturbed sirtuin levels, molecular phenotyping and protein-protein interaction studies, we provide new insights in the molecular function of sirtuins within the plant stress response.

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Poster presentations

1.1. The effect of cadmium stress on peroxynitrite generation in lupine roots

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The reaction of nitric oxide (NO) and superoxide anion ($\cdot O_2^-$) in the biological milieu leads to peroxynitrite ($ONOO^-$) formation. This is a short-lived oxidant and nitrating species, commonly known as a mediator of cellular injury in many biological systems. However, in contrast to animal systems, $ONOO^-$ itself is not so destructive for plant cell metabolism (Delledonne et al. 2001). Although the detection of the protein tyrosine nitration phenomena allows us to speculate that $ONOO^-$ is generated during various plant stress responses associated with pathophysiological mechanisms, there is no information regarding peroxynitrite during cadmium stress.

We showed that short-term (24h) exposure of yellow lupine (*Lupinus luteus* L.) roots to 10 mg/l $CdCl_2$ resulted in a slight increase in the production of $ONOO^-$, what was supported by the fluorometric assay with using folic acid. Simultaneously, the analysis showed a relatively high the steady-state level of $ONOO^-$ in non-stressed roots of lupine seedlings. In turn, bio-imaging with using the fluorochrome aminophenyl fluorescein (APF) revealed that the accumulation of $ONOO^-$ in response to cadmium was strictly localized in the meristematic zone of lupine roots. Interestingly, artificially produced peroxynitrite from donor SIN-1 did not cause decrease in cell viability and was even effective in alleviation of cell dying caused by cadmium stress. It is possible that formation of $ONOO^-$ in root cells exposed to Cd might reset the Cd-induced excess of the NO signal. Furthermore, $ONOO^-$ generation in plant cells could provide an important regulatory loop for NO activity.

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1.2. Physiological status of *Callitriche cophocarpa* under chromium influence

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The aim of the present work was the analysis of the photosynthetic performance of *Callitriche cophocarpa* exposed to high concentration of trivalent or hexavalent Cr ions. This worldwide distributed aquatic macrophyte was recently identified as an outstanding Cr phytoremediator (Augustynowicz et al. 2010, 2011). In the study reported here, shoots of *C. cophocarpa* collected from the natural stands were incubated in 1 mM (52 mg l^{-1}) Cr(VI) or Cr(III) solutions for five days in seminatural conditions. Kautsky induction curves were recorded (Handy PEA, Hansatech Instruments) following calculations of the changes in the chlorophyll fluorescence yield. Moreover, spatial heterogeneity in fluorescence emission of whole plants was visualized (FluorCam, Photon System Instruments). Additionally, chlorophyll a, b and carotenoid contents were evaluated. Differences in some parameters of photosynthesis were shown with respect to the metal oxidation state. As compared with control, plants influenced by Cr(VI) exhibited significant decline in photosynthetic pigment contents as well as pronounced decrease in photosynthesis quantum efficiency. Only some symptoms of Cr(III) stress correlated with the season of plant collection were observed.

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1.3. How melatonin can improve cucumber seeds germination?

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Seeds are the plant material difficult for investigation because their populations are usually heterogenic and they do not germinate uniformly. Seed conditioning is one of the techniques solving this problem. It equalizes the rate of seed germination and kinetics of plant emergence. Plants after conditioning exhibit better vigour under stress conditions. Seed priming may be supplemented with application of growth regulators, protective substances and biostimulators (*e.g.*melatonin). Melatonin (MEL) applied into cucumber (*Cucumis sativus* L.) seeds with osmoconditioning can improve their metabolism during germination under chilling (10°C) stress. They exhibit higher tolerance to secondary oxidative stress. Biological effects of oxidative stress are the following: lipid peroxidation as well as oxidative damage of proteins and DNA. If plants have efficient enzymatic and non-enzymatic antioxidant systems they can effectively neutralize reactive oxygen species (ROS). It has been shown that MEL can increase the activity of antioxidant enzymes, such as: superoxide dismutase (SOD) and glutathione reductase (GSSG-R), it can also influence changes in the oxidized glutathione (GSSG) and reduced glutathione (GSH) pools measured in embryonic axes isolated from germinated cucumber seeds. This positive effect was also confirmed by the biochemical test concerning hydrogen peroxide (H_2O_2) level measured in the same tissues. Although, the function of MEL in plants is poorly understood the results show its positive effect with regard to modifications of the antioxidant defence system under stress conditions.

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1.4. Lead-induced autophagy in root meristem cells of *Lupinus angustifolius* L.

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Lead poisoning constitutes one of most detrimental environmental hazards to all living organisms. Plants grown in Pb contaminated soils are an important avenue for toxic pollutants entering the human food chain.

The effects of $Pb(NO_3)_2$ at concentrations ranging from 0.1 mM to 1000 mM on the growth of *Lupinus angustifolius* seedlings in hydroponic cultures were investigated. Our researches concentrated on root meristematic cells. After 14-day incubation in metal solution 2-fold reduction of shoot and root growth parameters was observed at concentrations above 100 mM and 33 mM, respectively. The effect of lead at the concentration of 100mM on root meristems was investigated. The mitotic index after 6, 12, 24 and 48h of metal treatment was 1.1, 1.0, 0.8 and 0.3 respectively as compared to 4.2% in the control. Dividing cells were sporadically seen after 7-day incubation. These effects were associated with an increase in the number of cells exhibiting disturbances. Moreover, cytophotometric measurements of DNA content revealed cell cycle blockade mainly in G2.

Analysis of semi-thin root meristem sections showed progressive cell vacuolization during lead treatment. In consequence, after 48h of incubation vacuoles occupied greater part of a cell. Additionally chromatin condensation and dramatic changes in nucleolus structure were noticed. The above changes in root meristem cells indicate autophagy induction. Autophagy is a conserved mechanism degrading cellular contents in order to recycle nutrients or eliminate damaged organelles or toxins. This process could be used to remove lead contamination from root meristem cells and/or to reduce nutrient availability, but the mechanisms are still unknown. The presence of tested metal seems to intensify an autophagy process but not cell death.

1.5. Involvement of *Medicago truncatula* full-size ABCG transporter in a basal immune response

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Full-size ABC transporters belonging to ABCG subfamily are found only in plants and fungi (Crouzet et al. 2006). Little is known about these proteins in legumes, which represent the second most important crop. Previously, we have shown that the expression of *MtABCG10* in *Medicago truncatula*, a model legume plant, is strongly up-regulated during infection with pathogenic fungi *Phoma medicaginis* and *Fusarium culmorum* (Jasinski et al. 2009). Here we demonstrate that the *MtABCG10* transcript is present mainly within vascular tissues and corresponding protein is located in the plasma membrane. Different PAMPs (pathogen associated molecular patterns), like polysaccharides from fungal cell wall, as well as flagellin, increase *MtABCG10* accumulation. Interestingly, almost identical expression patterns were observed for two defence-related genes: *phenylalanine ammonia-lyase (PAL*) and *isoflavone synthase* (*ISF*) – key enzymes of isoflavonoid biosynthesis (Zipfel 2008).
We also observed that *MtABCG10* silencing significantly lowered the amount of various isoflavones both in root tissues and in the external medium. The differences between silenced and wild-type plants are even more pronounced upon elicitation with PAMPs, and concern precursors of medicarpin - a phytoalexin of *Medicago* sp. In general, our data suggest that MtABCG10 is an essential component of the plant basal immune system, which defends plants against infection in a non-specific manner.

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1.6. Expression of Zn transporter *AhHMA4p1::AhHMA4* in tomato affects the Fe accumulation

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It is known that a plant exposure to a range of Zn concentrations involves complex interactions between this micronutrient and other elements. Proteins and compounds involved in Zn uptake, short- and long distance transport, chelation as well as sequestration in cellular compartments possesses varying affinity to nutrient elements. For example, AtIRT1 from *Arabidopsis thaliana* (the high-affinity transporter of Fe^{2+}) can also transport Zn^{2+}, and excess Zn induces in this organism significant Fe deficiency. However, the knowledge about the cross-talk between Zn- and Fe homeostasis mechanisms is still very limited. Our study contributes to these relationships. *AhHMA4* from *A.halleri* encodes P1B-ATPase exports Zn from the cytoplasm to the apoplast and is involved in the control of root-to-shoot Zn translocation. Expression of *AhHMA4::AhHMA4* in tomato results in enhanced Zn accumulation upon exposure to 10 µM Zn in ¼ Knop's medium, and in increased Zn sensitivity (lower than in wild-type biomass and leaf chlorosis). To gain an insight into the underlying mechanisms of the generation of Zn-related phenotype, the expression of the transgene and Fe-deficiency responsive tomato endogenes were investigated.
We showed that increase in Zn concentration in the shoots was accompanied by decreased level of Fe. Expression analysis demonstrated involvement of Fe-deficiency responsive genes in the modification of Zn/Fe partitioning due to the expression of *HMA4* in tomato.

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1.7. Analysis of interaction between Arabidopsis PCNA and cell cycle proteins

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Proliferating cell nuclear antigen (PCNA) is one of the most conserved proteins present both in animal and plant organisms. PCNA functions as an auxiliary factor of DNA polymerase delta. Moreover, it also plays an important role in DNA repair and in regulation of the cell cycle. PCNA naturally forms a trimeric ring encircling DNA that serves as a platform for binding various proteins involved in DNA metabolism. Although the role of PCNA in animal and yeast cells has been studied extensively, only limited information concerning the role of PCNA in plant cells, especially in DNA repair, is currently available. Human PCNA was shown to interact with p21 (cyclin dependent kinase inhibitor, CDKI) and cyclin D within the group of cell cycle proteins. In contrast, plant PCNA was suggested to interact only with A-type cyclin dependent kinases (CDK). The detailed data concerning the interactions between plant PCNA and cell cycle proteins is not available. Our aim is to test interactions between Arabidopsis PCNA and cell cycle proteins including cyclins, CDK and CDKI. Applying *in vivo* bimolecular fluorescence complementation, the map of interactions between plant PCNA and cell cycle proteins will be constructed.

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1.8. An early response to cadmium- and anthracene-mediated stress in a chloroplast space of *Chlamydomonas reinhardtii* cells

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The PSII function, carbonic anhydrase (CA) activity and expression of chloroplastic superoxide dismutase Fe-SOD were analyzed in the 3rd, 6th and 12th h of exposition of green alga *C. reinhardtii* to EC50-corresponding concentrations of cadmium chloride and anthracene. The chlorophyll *a* fluorescence (OJIP test), CA activity and protein amount (WB analysis) and expression of Fe-SOD (enzymatic activity, WB analysis and semiquantitative Real Time PCR) were performed.

Both cadmium and anthracene exhibited transient inhibitory effect on photosynthetic processes. Suppression of oxygen evolution, decrease in fraction of active PSII reaction centers and lowering of quantum efficiency of PSII were observed. The most pronounced effects were visible after 6h of exposition and then photosynthetic parameters returned to the level near the control values. After 3 and 6h of exposure on anthracene, the activity of CA was inhibited by about 42%, whereas after 12h by about 18%. The strongest reduction of CA activity occurred in cells exposed to cadmium for 6h. Longer incubation led to weaker cadmium effect on CA activity. Cadmium decreased the content of CAH3 protein, regardless of time exposure. In anthracene-stressed cells the increase of Fe-SOD transcript was not followed by the increase of protein biosynthesized. In cadmium treated cells, higher Fe-SOD protein amount was noted with simultaneous no effect on its mRNA level. Thus, cadmium and anthracene affect the expression of chloroplastic Fe-SOD on the transcriptional and translational level, respectively, while both toxicants inhibit the enzymatic activity of Fe-SOD protein.

1.9. Tissue- and cell-specific localization of selected genes involved in maize response to low temperature by means of qRT PCR and *in situ* hybridization.

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Maize, a species originated in sub-tropics is a short-day, thermophilic plant. In temperate climate regions, modern maize varieties are insensitive to photoperiod, although they still suffer from low temperatures. Transport processes in leaves have been found to play important role in response of maize seedlings to low temperatures (Bilska and Sowiński, 2010). This study was performed to follow the molecular mechanisms of inhibition of the short distance transport in leaf and phloem loading. To this end, among genes showing change in expression at low temperature as found by means of microarrays technique, we have selected the list of genes coding proteins, potentially involved in transport processes (sucrose transporters, aquaporins, proton pump, plasmodesmata-related proteins). Thus, by means of qRT PCR and *in situ* hybridization we have searched for genes expressed specifically in Kranz mesophyll cells, bundle sheaths, vascular parenchyma cells and companion cells. Those genes will be studied further for their role in maize response to low temperature.

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1.10. Hydrotime analysis of the hormone effect on germination of *Taraxacum officinale* seeds

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Taraxacum officinale is a perennial globally distributed weed. Detailed knowledge about the ecophysiology of its seed dormancy could enable to create biological or integrated systems of weed control. The aim of this study was to explain the effect of gibberellins, abscisic acid and inhibitors of their synthesis on germination of *T. officinale* seeds.
Ripe achenes were soaked in solutions of GA_{4+7}, paclobutrazol, GA_{4+7} + paclobutrazol, ABA, fluridone, or ABA + fluridone. Pretreated seeds were incubated at reduced water potentials. Germination time courses were analysed according to the hydrotime model. The release of dormancy by gibberellins was mostly related to a decrease in the mean base water potential accompanied by a simultaneous widening of its distribution and an increase in the value of the hydrotime constant. Similar changes of the model parameters during a short stratification period suggest a crucial role of gibberellins in this process. The inhibitor of gibberellin synthesis almost completely inhibited germination thanks to a considerable shift of mean base water potential distribution towards positive values. It appears that gibberellins were synthesised during imbibition *de novo*. Exogenous ABA did not change the germination percentage of seeds, but shifted towards higher values and narrowed the distribution of the mean base water potential. Changes in the model parameters caused by the inhibitor of ABA synthesis were very similar to changes produced by gibberellins. This confirms the suggestion that seed dormancy and germination may be regulated by a dynamic balance of these hormones in the seeds.

1.11. Expression pattern of newly identified *ClC* cucumber genes under different nitrate provision of plants

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Nitrate is not only the major source of nitrogen for the most plants, but acts also as a signal molecule regulating plant metabolism, growth and development. As its importance, NO_3^- is usually taken up in excess of immediate requirements and is stored in vacuole of plant cells, from where it can be easily remobilized to meet plant demand under nitrogen starvation. Although plasma membrane proteins involved in nitrate uptake from the soil have been well characterized, transporters responsible for NO_3^- distribution within the cell are still under investigation. Studies on *Arabidopsis thaliana* have revealed that AtClCa can act as a tonoplast NO_3^-/H^+ exchanger, suggesting the role of proteins from *C*h*L*oride*C*hanel family (ClC) in nitrate transport through this membrane.
Based on the nucleotide sequences of *Arabidopsis ClCs* we have identified six homologous in cucumber genome. Three of them (*CsClCa, CsClCc* and *CsClCg*) seem to encode the putative tonoplast proteins. The amino acid sequences of CsClCa and CsClCc and presence of glutamate in GKEGP motif suggest their involvement in anion/proton exchange, while occurrence of alanine instead of glutamate in this motif of CsClCg indicates that this protein operates rather as anion channel. Transcription profiles of three *CsClCs* encoding potential tonoplast proteins have been analyzed in organs of cucumbers growing under different nitrate supply. In all organs the level of specific *CsClCg* transcript was much lower compare to *ClCa* and *ClCc.* Also its expression dependency on nitrate availability to plants differs distinctly from expression pattern of *CsClCa* and *CsClCc*. Although obtained data clearly indicate involvement of ClCa, ClCc and ClCg in nitrate distribution in cucumber cells, further functional studies are required to explain they particular role.

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1.12. Antioxidant enzymes induced by soil drought in tubers and leaves of potato

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Water limitation is the most adverse environmental factor having a profound impact on agricultural and ecological systems. Plants respond to all environmental stresses with the enhanced production of reactive oxygen species (ROS) as a consequence of disruption of cellular metabolism (Mittler et al. 2004, Torres 2010, Foyer and Noctor 2011). To control ROS generation and protect plant tissue against oxidative stress, antioxidants and ROS-scavenging enzymes are the most commonly activated as a first line of antioxidant defence system. Among them peroxidase is suggested to be an enzyme responsible for tuberisation of potato plants. It is also in discussion that level of SOD activity could be an important mechanism to explain why some potato, e.g. *Solanum curtilobum* are more resistant to abiotic stresses than others. Therefore, the question arises whether ten day soil drought applying in tuberisation phase of potato development affects activity and pattern of oxidative enzymes like superoxide dismutases, peroxidase, polyphenol oxidase, glutathione reductase and catalase. Activities of above mentioned enzymes in potato tubers of two cultivars (Tajfun and Cekin) which had been grown in pots with optimal water supply and with water shortage have been investigated. It has been shown that Tajfun is cultivar with higher dehydration tolerance than Cekin cultivar. The higher dehydration tolerance of Tajfun cultivar was accompanied by the lower total peroxidase activity in tubers of plants growing thorough experimental period in soil with optimal water supply. However, the electrophoretic pattern of peroxidase isoforms depends both on genotype and on sensitivity to water supply of potato plants. Catalase activity has been found in Cekin and Tajfun tubers only at higher molecular weight. More results will be presented on poster.

1.13. Response to oxidative stress in transgenic tomato plants constitutively expressing a dehydrin gene

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Active oxygen species, generated by stress, trigger the expression of defence genes and consequently the production of stress proteins in plants. Some of these proteins may have different roles in signal transduction processes and induction of a large number of new genes in plants. Transgenic technology is a useful tool for studying the functions and applications of stress induced genes. In the present study, the transgenic tomato (*Lycopersicon esculentum* cv. Heinz 91606) plants expressing a drought induced gene, encodes a dehydrin protein, from a wild tomato species were evaluated for oxidative stress tolerance versus control plants. Defence responses to paraquat-induced oxidative stress were measured in terms of superoxide dismutase (SOD) activity and lipid hydroperoxidation level. Superoxide dismutase activity increased in response to increasing levels of paraquat in all plants. The activity of superoxide dismutase was not significantly different among transgenic plants and non-transgenic control plants. In contrast, a decrease in lipid hydroperoxidation level was observed in two transgenic lines with higher expression levels than those of the control plants and a third transgenic line. This differential response of SOD activity and lipid hydroperoxidation levels to paraquat-induced oxidative stress could correlate with the activities of other antioxidant enzymes in those plants. Further improvement of the whole antioxidant mechanisms of a crop plant may be achieved by the engineering of more than one stress tolerance gene.

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1.14. Molecular analysis of phytocystatins in winter triticale seedlings under drought stress conditions

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Drought is fundamental abiotic stress which causes various morphological and molecular changes in plants limiting their productivity. In the response to this stress factor the expression of specific genes and multiple biochemical pathways are inducted (Ingram and Bartels 2006). Previous studies have shown that under the drought stress the activity of plant proteolytic enzymes including cysteine endopetidases increases (Zagdańska and Wiśniewski 1996). The natural inhibitors called phytocystatins which gene expression could be induced upon various abiotic stresses probably are involved in the regulation of enzymes activity.
The aim of our study was identification and molecular analysis of phytocystatins in triticale seedlings exposed to the drought stress and after returned to optimal conditions. The performed studies show that two genes of winter triticale seedlings phytocystatins (*TrMDC*, *TrHvC*) are induced by drought stress. In leaves and roots of the seedlings, the mRNA level of the inhibitors increased in response to progressive water deficit. The level of both transcripts *TrMDC* and *TrHvC* decreased in seedlings which were rewatered after 48 h of stress factor. Additionally, in vegetative organs of seedlings under stress conditions accumulation of protein TrMDC (Western blot analysis) have been observed. After rewatering seedlings, the level of the protein has been reduced. Understanding the mechanism of protein turnover regulation is very important to obtain plants resistant to stress.

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1.15. Phenolic compounds of fodder grasses – analysis of compounds from leaves of *Phleum pratense* L.

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Phenolic compounds such as chlorogenic acid and its derivatives, and flavonoids are synthesized in leaves of most plant species, including fodder grasses. High Performance Liquid Chromatography integrated with Mass Spectrometry (HPLC/MS) may be successfully used for profiling of these compounds in plant extracts. This approach is particularly useful when small amounts of tissue is available, however in such a case not all compounds present in it may be identified. Preparative flash chromatography on reversed phase column combined with semi-preparative HPLC have been applied for purification of phenolic compounds from *Phleum pretense* cv. Obra. The phenolic compounds have been eluted from the C18 silica gel flash chromatography column with a stepwise methanol gradient in water and the composition of the eluted fractions was established using Ultra-High Performance Liquid Chromatography (UPLC, Waters) and ion trap HPLC/MS. Fractions containing the same compounds have been pooled, evaporated and submitted to re-chromatography at the preparative HPLC. Compounds obtained in result of the above procedure in sufficient amount and purity were submitted to the Nuclear Magnetic Resonance (NMR) analysis.
This approach allowed us to detect much more phenolic compounds than it was possible in previous analyses conducted for *Lolium multiflorum* using only HPLC/MS. We have found more than 100 compounds characterized by a unique combination of the retention times, UV spectra and MSn spectra. Structures of 68 of compounds could be proposed on the basis of spectral data by comparison with those of standards and previously described compounds.

1.16. Protein carbonylation during dormancy removal of apple seeds

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Reactive oxygen species (ROS) play a signaling role in seed dormancy alleviation and germination. Their action may be described by "oxidative window". ROS accumulation in embryos leads to oxidative modification of protein through carbonylation. The specificity of protein carbonylation during seed germination was demonstrated for sunflower and Arabidopsis. Mature apple (*Malus domestica* Borkh. cv. Antonówka) seeds are dormant and do not germinate. Their dormancy may be overcome by 3 month cold stratification or by short term pre-treatment with nitric oxide (NO). NO mediated dormancy breakage involves transient accumulation of ROS in apple embryos (Gniazdowska et al. 2010). The aim of this work was to analyze alterations in ROS production during stratification of apple seeds and carbonylation of soluble proteins in this process.
We observed biphasic changes in H_2O_2 concentration in the embryos during stratification. The first maximum was detected after 14 days of seeds imbibition in 5°C and the second one after next two months. Alterations in ROS accumulation correlated with the level of protein carbonyl groups. The highest protein carbonylation was detected after 21 days of stratification. Our results suggest that protein carbonylation may be one of important factors in seed dormancy removal.

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1.17. Characterization of autophosphorylation in calcium dependent protein kinase 1 from *Cicer arietinum*

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In plant kingdom, various cellular processes like cell cycle progression, cell differentiation and phytohormone signaling are directly or indirectly regulated by calcium ions. Calcium dependent protein kinases (CDPKs) play important roles in decoding calcium signaling. They are unique class of kinases that are able to couple Ca^{2+} sensor to its responder kinase and calmodulin domains, all present in a single polypeptide. In the absence of calcium signal the protein acts as a pseudosubstrate that blocks the active site giving only a low basal activity. In the presence of calcium the protein undergoes conformational changes and autophosphorylation, accompanied by restoration of the kinase activity disengaging autoinhibition.

Not much is known about function of autophosphorylation in CDPKs. We carried out the detail analysis of function of autophosphorylation in CDPK isoform 1 from *Cicer arietinum* (CaCDPK1). Autophosphorylation of CaCDPK1 is calcium-dependent. Consensus on significance of autophosphorylation on the kinase activity eludes in view of reports of multiple effects: stimulatory, inhibitory or without effect. In CaCDPK1 autophosphorylation enhanced its ability to phosphorylate the exogenous substrate. Maximum autophosphorylation was seen in presence of Ca^{2+}. Apart from Ca^{2+}, Mn^{2+} was able to stimulate the autophosphorylation activity but not as strongly as calcium. The autophosphorylation rate clearly depends on the CaCDPK1 concentration which indicates that CaCDPK1 under goes autophosphorylation via intramolecular mechanism. CaCDPK1 prefer ATP as phosphor-donor over GTP with K_m of 2.5 μM for ATP. Autophosphorylated CaCDPK1 protein does not require Ca^{2+} for further activation. Prior autophosphorylation completely abolished lag phase required for enzyme activation. MALDI MS/MS data suggests that autophosphorylation occurs at Thr- 339, Ser-357, and Ser-367 residues.

Phospholipids like phosphotidylcholine and neutral lipid line diacyglycerol (DAG) stimulated its autophosphorylation activity indicating they might be regulated through these lipids.

1.18. Cyclitols in chilling tolerance of germinating pea (*Pisum sativum* L.) seeds

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Cyclitols like *myo*-inositol, d-pinitol, d-*chiro*-inositol are considered as important plant osmo- and cryo-protectants. Beside *myo*-inositol other cyclitols did not occur in pea seeds. To introduce cyclitols to maturing (22DAF) pea seeds (cv. Hubal), stem-leaf-pod explants were fed with d-pinitol and d-*chiro*-inositol, as described previously (Lahuta et al., 2010). This procedure affected accumulation of raffinose family oligosaccharides and induced synthesis of new galactosyl cyclitols in pea embryo. Dry pea seeds with modified composition of α-d-galactosides were imbibed in Petri dishes at 4, 12 and 20°C for 48 h, then transferred into wet germination paper towels and incubated at 20°C for the next 5 days. Chilling treatment delayed seedlings growth, reduced degradation of RFO and galactosyl cyclitols, as expected. After 5 days of germination d-pinitol and d-*chiro*-inositol were still detected in epicotyl, hypocotyl and cotyledon tissues. However, seedlings containing new cyclitols reached length and dry weight content as high as the control. Thus it can be concluded that exogenous cyclitols did not increase chilling tolerance of pea seedlings.

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1.19. The effects of sugars and light on the photosynthetic apparatus of *Arabidopsis thaliana* cultured *in vitro*

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Light and sugars are fundamental elements of plant metabolism and play signaling roles in many processes. They are also critical factors determining the condition of plants cultured *in vitro*. The aim of this work was to investigate the simultaneous influence of irradiance and sugar content in the medium on the growth and photosynthetic apparatus condition of *Arabidopsis thaliana in vitro* (Eckstein et al. 2011). Plants were cultured on media containing 1% or 3% of sucrose or glucose at three irradiances: 25, 100, 250 $\mu mol \cdot m^{-}2 \cdot s^{-1}$ (weak, medium and strong light). Media without sugar were used for control plants. The influence of culture conditions on plant growth was investigated. Measurements of photosynthesis and xanthophyll cycle activity were performed. The expression of genes related to these processes was analyzed.
The presence of sugar in the medium was found to be essential for normal growth of Arabidopsis *in vitro*. Weak light significantly limited growth and the capacity to acclimate to changing light conditions. Strong light during culture was a source of stress in some cases, impairing photosynthetic activity. Contrary to several earlier reports, exogenous sugars showed a positive effect on photosynthesis. At higher concentration they acted as photoprotectants, allowing to overcome the negative influence of strong light on photosynthesis and the xanthophyll cycle. These effects seem to be only partly related to changes in gene expression.

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1.20. The effect of soluble carbohydrates on synthesis of free salicylic and jasmonic acids in yellow lupine infected with *Fusarium oxysporum*

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The aim of the presented study was to verify whether the level of soluble carbohydrates, i.e. sucrose, glucose and fructose, affects the synthesis of free salicylic and jasmonic acids in embryo axes of *Lupinus luteus* L. cv. Juno inoculated with a spore suspension of *Fusarium oxysporum* f. sp. *lupini*.
Analysis of the concentration of free salicylic and jasmonic acids determined by GC-MS method revealed a higher post-infection level of these molecules in embryo axes than that in the control. In infected embryo axes cultured on a medium with glucose and fructose a higher level of salicylic acid was recorded than that in the control axes, as well as higher level in relation to axes cultured on a medium without sugar. At the same time sucrose and glucose alone increase the activity of phenylalanine ammonia lyase (PAL) – an enzyme initiating the phenylpropanoid pathway, in the course of which salicylic acid may be formed. In turn, we need to stress here a very high level of free jasmonic acid (JA) in embryo axes with a deficit of carbohydrates up to 48 hours of culture, both in those infected with *Fusarium oxysporum* and non-infected axes; however, it was considerably higher in infected axes. Analysis of the zymogram for lipoxygenase (LOX) – an enzyme engaged in the synthesis of jasmonic acid, revealed quantitative changes in isoenzymes in the time from 0 to 96 h culture, with their intensity increasing particularly strongly in infected embryo axes cultured on a medium with sucrose.
Results presented in this study indicate that soluble carbohydrates affect the mechanism of synthesis of salicylic and jasmonic acids; however, only the accumulation of free salicylic acid was dependent on the high level of carbohydrates.

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1.21. Role of the galactinol synthase in response of developing pea seeds to drying and cold

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Galactinol synthase (GolS, EC 2.4.1.123) catalyzes the biosynthesis of galactinol (O-α-D-galactopyranosyl-[1→1]-L-*myo*-inositol) from UDP-galactose and *myo*-inositol. Galactinol is the main galactose donor during biosynthesis of raffinose family of oligosaccharides (RFOs), that are represented in pea seeds by raffinose, stachyose and verbascose. The changes in RFOs, galactinol, galactose bound in RFOs levels and GolS activity were measured: 1) during the development of seeds of *Pisum sativum* L. cultivars: Hubal and Kiler differing in the RFOs composition, 2) in response of seeds at two early developmental stages to abiotic stresses: drying (at 12% and 70% RH) and cold (4°C). At different time points, up to 192 h of stress treatments, the GolS activity and soluble carbohydrates level were analyzed. A similar temporal pattern of soluble sugars accumulation was observed in developing seeds of both pea cultivars – the accumulation of sucrose, monosaccharides and galactinol as well as high GolS activity preceded synthesis and accumulation of RFOs. At the late maturation stages of pea seeds development galactinol content and GolS activity declined. All stress treatments resulted in increase of GolS activity in seeds. The activity increased earlier in response to drying than cold. It was also found that increase of GolS activity was followed by accumulation of RFOs. Differences in GolS activity influenced the content of α-D-galactosides, the highest level of RFOs was in slowly dried seeds, and the lowest in cold treated seeds. It was found that the increase in GolS activity was not reflected into the galactinol content in seeds. It suggested that galactinol was used as galactose donor during RFOs biosynthesis. The results confirmed that the activity of GolS increases in response to abiotic stresses and that the activity of this enzyme influences the accumulation of soluble α-D-galactosides indicating the role of GolS in environmental responses.

Supporting Agencies:
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1.22. Involvement of cysteine proteinases in the response of wheat (*Triticum aestivum* L.) seedlings to dehydration

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Cysteine proteinases are widely acknowledged as key regulators of plant responses to unfavourable environmental conditions because they are known to be responsible for removal of abnormal and misfolded proteins induced under drought (Grudkowska and Zagdańska 2004). Previous studies have indicated that activity of cysteine proteinases were preferentially enhanced in dehydration-sensitive wheat cultivar (Grudkowska and Zagdańska 2010). Therefore, the question arose whether changes in the cysteine proteinases activities induced by water deficiency are responsible for acquisition dehydration tolerance or are consequences of drought-induced plant senescence.
Ten-day-old seedlings of two spring wheat (*Triticum aestivum* L.) cultivars, differing in dehydration tolerance, were subjected to drought resulted in 15 and 50% leaf deficit estimated as WSD. All experiments were carried out on dehydrated seedlings and seedlings recovered from a mild water deficit. Enzymes were extracted in acetic acid buffer, pH 5.0 prerequisites to extraction of vacuolar enzymes mainly. Protein separation patterns were assessed after PAGE separation (1D and 2D systems), on Amido Black and silver stained gels while enzyme activities - on gels co-polymerized with 0.1% gelatin.
Electrophoretic analysis showed that in fully hydrated leaves activities of cysteine proteinases were undetectable, in contrast to drought stressed tissues. In leaves of both cultivars, dehydrated up to 15% WSD, two bands appeared. In severe dehydrated (50% WSD) leaves, three bands were detected in extracts from drought resistant seedling leaves, whereas in drought sensitive cultivar, two bands of weak activities were visible. After seedlings rewatering only one activity band revealed both in drought sensitive and drought resistant wheat cultivar. The obtained results suggest that specific vacuolar proteinases are involved in the plant responses to drought.

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1.23. The role of reactive oxygen species and nitric oxide in plant defence responses against mechanical wounding of *Hippeastrum* x hybr. hort bulbs

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Generation of reactive oxygen species (ROS), such as the superoxide radical (O) and hydrogen peroxide (H_2O_2), is a common defence response to wounding. Various reports demonstrated that NO has protective influence on cells against oxidative damage caused by ROS, based on its ability to act as a ROS scavenger.
The aim of our study was to investigate if the increase in H_2O_2 content was coincided with NO accumulation in bulbs after wounding, and to determine whether H_2O_2 and NO could function as the signaling molecules and affect phytoalexin (PA) production. To answer these questions exogenous applications of H_2O_2 and NO were performed and the level of PA was measured. Also endogenous level of H_2O_2, $\cdot O_2^-$, NO were analyzed in *Hippeastrum* bulbs after mechanical wounding.
It was revealed that exogenous application of H_2O_2 on wounded bulbs inhibits PA production. The reaction observed after application of NO donors was different and dependent on used concentration. In extreme concentrations of nitric oxide PA generation was reduced but in 1-2 mM concentrations rapid synthesis of PA was noted. As a next step we assayed the production of H_2O_2 and $\cdot O_2^-$ in response to mechanical damage and noted that NO donors pretreatment reduces ROS level after injury. Above results suggest that concomitant activation of NO and ROS synthesis is required for induction of a defence responses against injury of *Hippeastrum* bulbs, among them synthesis of PA is an important stage.

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1.24. Proteomic analyses of dormant embryonic shoot of Norway spruce (*Picea abies*)

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Temperate trees have developed unique mechanisms which allow them to adapt to the environmental conditions which change during a year. Their developmental activity is highly synchronized with the annual cycle: the periods of activity and dormancy come one after another. Our research concerns the key problems of growth and development coordination and the course of processes of adaptation to the seasonal changes in the environment. Norway spruce embryonic shoot is the model in the research. This embryonic shoot is covered with thick scales and contains all the elements of a mature shoot. The embryonic shoot is only about 1 mm high, when closed in the bud during the winter. Within this small area there are meristematic cells (apical meristem cells, procambial cells) and differentiated cells (pith cells, parenchyma cells of the needles). In the dormant bud, all the anatomical and physiological relationships between the elements of prospective shoot are maintained. They guarantee the integrated development of the embryonic shoot into the mature shoot. The aim of this study is to describe some molecular changes in the vegetative embryonic shoot of Norway spruce during dormancy and to precise their relation to structural changes and to the function of embryonic shoot during both the endodormancy, ecodormancy, and during the dormancy-to-growth transition. For the molecular description of Norway spruce embryonic shoot we apply proteomic methods. We propose the following hypothesis: Every one of the dormancy phases is characterised by a specific set of proteins, and the modification of the set is related to the structural and ultrastructural changes in the embryonic shoot. The characterization of these proteins provides insights into the molecular mechanisms of bud dormancy regulation. We will present preliminary results of proteomic analyses of the dormant embryonic shoot which comprise the tissue location of selected proteins.

Supporting Agencies:
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1.25. Activity of antioxidative enzymes in *Zea mays* plants exposed to biotic and abiotic stress

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Both abiotic and biotic stresses induce or involve oxidative stress in plants. Antioxidant enzyme activity is a widely used marker of oxidative stress. The aim of the present study was to determine the activities of H_2O_2 scavenging enzymes in the leaves of *Zea mays* L. plants cultivated hydroponically in the growth chamber and exposed to abiotic (heavy metal) and/or biotic (insect biting) stress factors.
The plants were treated with 50 μM Cu for 24 h. Subsequently, they were transferred to the control nutrient solution and some of them were exposed to the holarctic plant bug *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridiae, Stenodemini) at a L4 larval stage for 7 days, whereas the others were left without any treatment. In addition, some plants were pre-treated with methyl jasmonate (MJ, 10 μM) for 24 hours prior to exposing them to the insects' sucking to check if the altered level of that signal molecule affects plant antioxidative response to the insect. The activities of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) were determined in the leaves of the control plants and those exposed to the insect, Cu, Cu+insect, MJ and MJ+insect. Moreover, H_2O_2accumulation in the leaves was visualized through histochemical staining. The highest level of CAT activity was detected in Cu-treated maize leaves not exposed to the insect. Increased APX activity was found in Cu- and MJ-treated plants both without subsequent exposure to the insect. The highest level of GPX activity was determined in plants exposed to *Trigonotylus caelestialium*. In general, MJ pre-treatment did not affect significantly antioxidant enzyme activities in the plants subjected to the insects; thus, involvement of the MJ-dependent signalling pathway in plant defence against insect attack under experimental conditions used was not found.

1.26. Presence of progesterone in leaves of spring wheat

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Steroids are present in living organisms as one of the most important groups of compounds. A large of number of steroid groups possess hormonal properties (brassinosteroids, ecdysteroids, progesterone, estrogens). Continuous research has led to new discoveries and the revising of existing information concerning the occurrence and the role of steroids, both in animals and plants. A very good example are the discoveries concerning progesterone - C-21 steroid until now known as regulator of reproduction of mammals. Presence of progesterone in plants was first reported in the late 1960s. According to our previous research conducted on wheat, progesterone stimulates plant generative development and receptors of this steroid in membrane and cytosol fractions are found (Janeczko and Filek 2002, Janeczko et al. 2008). Presence of progesterone in green parts of wheat has not been previously investigated so the aim of our study was to demonstrate the occurrence of progesterone in the leaves of wheat. Moreover changes of level of progesterone were studied in drought-resistant and susceptible cultivars of wheat (Katoda and Monsun) exposed to water deficit. Analysis were performed using ultraperformance liquid chromatography tandem mass spectrometry with to use on immunoaffinity purification stage during the extraction process (Simerský et al. 2009). Progesterone was found in leaves of wheat in range 1,16 to 1,82 ng/g F.W. Preliminary data shows that as a result of plant exposition to water deficit, level of progesterone increased in drought-susceptible cultivar by a third. In more tolerant cultivar amount of progesterone remained similar in drought-stressed plants and in watered control. In wheat was found free progesterone and progesterone conjugated with sugars. Occurrence and role of progesterone as plant metabolism regulator will be discussed.

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1.27. Response of plasma membrane H^+-ATPase to heavy metal (Cd, Cu) stress in cucumber roots

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The effect of heavy metals on the plasma membrane H^+-ATPase activity in cucumber seedlings roots was studied. Plants were treated with 10mM Cd or Cu for 6 days. Some of the plants after 3 days exposure to heavy metals were transferred to control conditions for another 3 days (acclimated plants). The hydrolytic as well as transporting activity of H^+-ATPase in the plasma membranes of cucumber root cells was increased in plants treated for 6 days with Cd or Cu and in acclimated plants. Estimation of transcript levels of PM-H^+-ATPase in roots indicates that the action of cadmium, involves the gene expression level. Moreover the Western blot analysis with the antibody against phosphothreonine showed that increased activity of PM-H^+-ATPase under Cd treatment could result from phosphorylation of the enzyme protein. However the stimulation of PM-H^+-ATPase activity in the case of Cu treated plants could partially result from increased activity of PM-NAD(P)H oxidoreductase. Moreover, under long term treated plants with heavy metals copper markedly changed the antioxidant enzymes activity. On the contrary, cadmium had no effect on those enzyme activities.
Taken together, these data indicated that cadmium and copper, in the long term experiment, in different ways modified the PM-H^+-ATPase activity. Alteration of this enzyme protein under Cd is due to genetic level and posttranslational modification (phosphorylation) of its protein. In the case of plants treated with copper-changing activity of PM-H^+-ATPase may be due to changes in redox homeostasis.

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1.28. The effect of phytoalexin from *Hippeastrum* on the pathogenicity of fungus *Phoma narcissi*

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Phytoalexins (PA) are antimicrobial substances synthesized by plants that accumulate at areas of pathogen infection. Despite many investigations, the exact role of such substances in the mechanism of resistance in plant disease remains undetermined. It was revealed that phytoalexin from *Hippeastrum x* hybr. Hort. was produced in bulbs both after mechanical wounding and infection by a fungus *Phoma narcissi* Aderh.
P. narcissi is recognized as pathogenic fungus of bulb plants. Since the ability of this fungus to infection depends on strain-specificity, the molecular genotyping as well as phylogenetic relationships of investigated strains were demonstrated. Moreover, the extracellular plant cell wall degrading enzymes of identified strains were analysed to differentiate their pathogenic aggressiveness in relation to the attacked plants. Finally, the effect of PA on *P. narcissi* mycelium growth *in vitro* and *in vivo* was studied. It was noted that growth of mycelium was suppressed by PA. Moreover, wounding of bulbs induces partial resistance to given strains, similar to that observed after exogenous PA application on intact bulbs.
The present findings suggest that differences in infection process depends on *P. narcissi* strains and rapid PA biosynthesis induces severe restriction on mycelium growth, at the early infection stage.

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1.29. Effects of selenium on cytochemical localization of superoxide anion and hydrogen peroxide in cucumber (*Cucumis sativus* L.) roots in response to exogenous ferulic acid

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Ferulic acid (FA), an autotoxin in root exudates of cucumber, exerts detrimental effects on plant growth by evoking physiological changes. It is known allelopathic compound which leads to generation of reactive oxygen species (ROS) in plant cells. The generation of ROS - superoxide anion ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) - is associated with normal plant biochemical processes, while their transient and intensified production is frequently early plant response to biotic and abiotic stresses. Recent evidence indicates that although selenium (Se) is harmful for plants in high concentrations it can exerts beneficial effects at low concentrations as an antioxidant and increase the tolerance of plants to oxidative stress. The aim of study was to determine the effect of applied selenium on ROS generation and cytochemical localization in roots of cucumber cv. Dar seedlings. We investigate whether selenium can modulate the effects of oxidative stress after FA treament. Experiments were carried out in water cultures. Se was added as 1, 5 and 10 µM sodium selenite ($Na_2SeO_3 \cdot 5H_2O$). Part of 11-day old seedling roots were treated with allelopathic agent (0,5 mM FA) for 1 and 3 hours.

Strong generation $\cdot O_2^-$ in roots treatment with FA by 1 h was observed using confocal microscope. The superoxide anion was detected using dihydroethidium (DHE). Selenium pretreatment at the concentration of 1 µM decreased the emission of yellow fluorescence indicating $\cdot O_2^-$ generation caused by ferulic acid, which is not observed in presence of Se at concentrations between 5 and 10 µM. Obtained results indicate that selenium may enhance tolerance of cucumber roots for FA and play the protective role in plants subjected to allelopathic stress by inhibiting $\cdot O_2^-$ generation.

1.30. Effect of soil drought on proline content in leaves of pea and yellow lupine

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Nowadays, soil drought is one of the major factor limiting crop yield. In legume plants drought reduces the yield by premature and insufficient filling the seeds and as result of rejection of flowers and young pods. Proline acts as an adaptive factor in water stress. As a low-molecular compound it may enable the maintenance of osmotic balance between cytoplasm and vacuoles, contribute to more efficient water uptake. Proline stabilizes the structure and synthesis of proteins and neutralizes toxic ammonia resulting in conditions of water stress.
The aim of the study was to determine the effect of soil drought stress on proline content in leaves of pea and yellow lupine. The material consisted of six varieties of pea (*Pisum sativum* L.) and yellow lupine (*Lupinus luteus* L.). Seedlings with 5-6 leaves were subjected to soil drought at 25% field water capacity for 2 weeks. Control plants and plants after drought period were watered at 70% field water capacity. In the 1st and 14th day of the drought, leaf relative water content (RWC) and endogenous proline content were determined.
The results showed that pea plants responded to the water stress by reduction of the RWC by nearly 3% on the 1st day of drought and by 7% on the 14th day, and in lupine plants 8% and 7%, respectively. Proline content in pea plants increased by *ca*. 11% on the 1st day of drought, and on the 14th day has nearly doubled (*ca.* 21%). Whereas, lupine plants on the 1st day of drought did not change endogenous amount of proline and on the 14th day showed very slight increase in proline content of about 2%. These results suggest that the pea is more sensitive to soil drought stress in comparison with yellow lupine.

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1.31. Different responses of tonoplast proton pumps in cucumber roots to cadmium and copper

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The vacuolar sequestration of heavy metals could be mediated by secondary antiporters energized by proton pumps: V-ATPase and V-PPase. Generally, it is assumed that the combined action of the two proton pumps with different energy source allows plants to maintain transport processes into the vacuole even under stressful conditions. However, their functional relation and relative contributions to ion storage and detoxification remain unclear.

Our earlier studies have demonstrated that 24 h-exposure of cucumber seedlings to 10 µM cadmium had no significant effect on the activities of both enzymes in roots. In contrast, V-ATPase was stimulated after treatment with 10 µM copper. To explain those differences, we examined tonoplast proton pumps in roots of seedlings grown under longer metal stress. Plants were exposed to 10 µM $CdCl_2$or $CuCl_2$ for 6 days (stressed plants) or for 3 days and then transferred to control conditions for next 3 days (acclimated plants). Both hydrolytic and proton pumping activities of V-ATPase increased in tonoplast isolated from roots stressed with Cu. Treatment of seedlings with Cd significantly decreased ATP-dependent proton transport. Functioning of V-PPase was modified by metals to a lesser extent than the functioning of V-ATPase. As the gene expression and immunoblot analyses indicated, observed changes in the proton pump activities were not due to the modification in the *CsVHA-A*, *CsVHA-c* and *CsVP* transcript levels or in the amounts of enzyme proteins.

Concluding, high activity of vacuolar ATPase induced in response to Cu exposure seems to play an important role in cucumber adaptation to copper stress. However, the mechanism by which Cu^{2+} ions acts on the V-ATPase complex has not been yet explained.

1.32. Association of protective proteins with dehydration and desiccation of orthodox, intermediate and recalcitrant category seeds

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Mature and dried seeds of broadleaves species that differed in desiccation tolerance were analyzed. The four species investigated were as follows: *orthodox* Norway maple (*Acer platanoides* L.); *intermediate* beech (*Fagus sylvatica* L.), *recalcitrant* sycamore (*Acer pseudoplatanus* L.) and silver maple (*Acer saccharinum* L.). We compared the appearance of dehydrin-like proteins in seedlots originating from cropping years that differed in weather conditions, which were monitored in detail during seed development. The experiments showed that three main dehydrin-like proteins with approximate molecular weights of 46, 35, and 23kDa were characteristic of all examined *Acer* species seeds. The three proteins were present in Norway maple seeds and were noted individually or together in the *recalcitrant* category seeds. The contribution of dehydrins and dehydrin-like proteins were determined in beech seeds during seed development, storage and germination, particularly the DHN44 and DHN26 proteins. The subcellular localization of dehydrins was studied in developing and mature beech seeds using immunochemistry and prediction servers. Experiments showed that dehydrins were detected within cytoplasm, nucleus, near the membrane structures or mitochondria. Moreover *in silico* analyses of *Fagus sylvatica* dehydrin proteins with accession numbers CAE54590.1 and CBY89194.1 in GenBank database pointed their possible roles according to water stress. Characterization of dehydrin protein expression, post-translational modifications and their possible roles in macromolecules protection in seeds is here discussed. The potential modulation of dehydrin-like protein expression by environmental factors such as developmental heat sum and rainfall is also examined. Moreover, the influence of water removal caused by seed drying and desiccation in seeds of the same genus, belonging to the *orthodox* and *recalcitrant* categories, is also explored.

1.33. Diversity of generative reproduction of *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. as the adaptation to different environmental conditions

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Deschampsia antarctica and *Colobanthus quitensis*, the only two indigenous vascular plants occurring in the Antarctic, are exposed to harsh environmental conditions. Thus, they are an interesting object of study on the adaptation of organisms to life in extreme environments. Our study conducted on specimens growing both under natural and greenhouse conditions, showed depending in relate to their reproductive strategy. Both plants produce two types of bisexual flowers – cleistogamous and chasmogamous. The former predominates in native conditions, characterized by low temperatures, strong wind and high humidity. The latter occurs on plants from greenhouse (stable conditions, temp. 20°C). Plants growing in the Antarctic are small, form compact and flat cushions and they have a great number of tiny flowers. Allocation of biomass in the greenhouse appears to be a bit different. Vegetative shoots grow intensively, forming long branches. On the other hand the number of flowers is small, only 2-5 on *C. quitensis* and on *D. antarctica* they appear extremely rare.
During germination test on *C. quitensis* seeds collected from the Antarctic (Feb-2010) and the greenhouse (June-2010), germinated only 15% material from natural conditions and 0% from controlled conditions. These findings in conjunction with literature reports show that the studied species create a seed bank. A similar result was obtained in earlier experiments conducted on *D. antarctica*. Caryopses did not germinate immediately after harvest, and to break dormancy was required a long-term cold stratification. The existence of the seed bank within the described species can be confirmed by their morphological features. Numerous reports describing the relationship between the creation of the seed bank and their morphology, indicate that the seeds actually creating them, are considerably smaller and have a compact shape. Such features have diaspores of *C. quitensis* and *D. antarctica*, whose the average weight of one piece is respectively 0,05mg and 0,22mg.

1.34. Comparative evaluation of copper, cobalt, cadmium and iron scavenging efficiency by *in vivo* and *in vitro* grown *Momordica charantia* using atomic absorption spectroscopy

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Phyto extraction, the use of hyperaccumulator plant species to scavenge toxic heavy metals from contaminated soils, is now considered as an emerging technique for cost effective and environment friendly detoxification. Present study was conducted to evaluate and compare the scavenging efficiency of *in-vivo* and *in-vitro* grown *Momordica charantia* for the uptake of copper, cobalt, cadmium and iron (the major components of inorganic contaminants and extremely harmful to the living beings (when exceeded to their threshold limits) using Atomic Absorption Spectroscopy. *In vitro* plants were cultured on a mixture of 2, 4-D (2.5mg/l) and NAA (2.0mg/l) in MS basal medium. Bioassays of these both type of *Momordica charantia* plants (field grown and *in vitro*) were subjected to Atomic Absorption Spectrophotometer for the analysis of their scavenging efficiency of the above mentioned elements, after 50 days of growth in soils contaminated separately, with each of the elements. The *in-vivo Momordica charantia* absorbed much lesser amounts of all the four metals i.e. 1.79ppm, 1.85ppm, 0.45ppm and 1.61ppm as compared to *in-vitro* plants which showed higher ranges of uptake, i.e. 3.09ppm, 5.39 ppm, 4.77 ppm and 6.29ppm for Cu, Co, Cd and Fe respectively, which was proportional to the metal concentration in the contaminated soil. Maximum uptake for all the four heavy metals was observed by the roots in all plants, due to localization of their ions in the apoplasm. Shoot nodes also showed most intensive heavy metal accumulation in the aerial region of the plant exhibiting their role in xylem - phloem transportation. High concentration of heavy metals in the soil showed a negative effect on growth of all plants influencing biochemical and physiological growth parameters. Increased accumulation in leaves, was followed by associated symptoms of toxicity. Toxicity symptoms appeared 15 days after the initiation of treatment. Chlorophyll concentration and activity of anti-oxidative enzymes, e.g. peroxidases and catalases, were found to be inversely proportional during the oxidative stress.

The data supported our postulation that that *in-vitro* grown hyperaccumulative species can be used as an effective tool of phytoremediation, for the removal of heavy metals through their rhizosphere scavenging action, from the contaminated lands on a wider scale.

1.35. Internal recycling of respiratory CO_2 in pods of *Pisum sativum* L.

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Bulky organs of plants such as fruit, stems and roots have special meaning for plant carbon balance. Because of the poorly permeable epidermis, they show high water use efficiency as well as high level of reassimilation of CO_2. The rates of net photosynthesis of pods were measured using Li-6400 portable photosynthesis system with conifer chamber which was large enough to enclose a single pod. At the same time, the inner concentration of CO_2 was measured in the pod space by means of a microelectrode. The measurements showed that the pods respond to the increasing intensity of the irradiation by reduction in CO_2 efflux. In the darkness, respiration *ca.* 20 µmol m^2 s^{-1} in pea pods was noted. Light compensation point of CO_2 efflux appeared at about 1300 µmol m^2 s^{-1} and the low net photosynthesis up to 1,0 µmol m^2 s^{-1}. At the same time the fluctuating concentration of CO_2 inside the seed chamber was measured. The highest values were noted after dark adaptation *ca*. 2,5 %. Along with the increase of light intensity, the concentration of CO_2 was falling down to the value of 0,6 % while being lightened at 2000 µmol m^2 s^{-1}. Afterwards the measurements of CO_2 efflux rate depending on the concentration of CO_2 in the range of 20 to 2000 ppm CO_2 were carried out. The rate of leakage of CO_2 was 4,5 µmol m^2 s^{-1} for 20 ppm CO_2 and achieved its compensation point at 600 ppm. Above these levels net photosynthesis increasing reached up to 6 µmol m^2 s^{-1} at 2000 ppm. Measurements of chlorophyll fluorescence in pods and calculation of reassimilated CO_2 in the seed chamber proved that the amount of CO_2 bound by the pods is very similar to the amount of CO_2 that is assimilated by leaves.

1.36. Secondary metabolites of *Arnica montana*, *A. montana* cv. Arbo and *A. chamissonis* and their influence on growth and anatomical structure of plants

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Arnica montana is a herbaceous perennial plant with valuable pharmaceutical and cosmetic specificity, wildly growing in moderate zone of Northern hemisphere. It is a protected plant (legal protection measures are now in place throughout much of Europe - species threatened over most of its natural range) in all countries with the exception of Spain and Romania.
There is an increasing demand for *Arnica* as an ingredient in medicinal physiotherapy and sports massage and in a growing range of cosmetics. Due to shortage in supply of flowers, roots and whole plants instead of *Arnica montana* it was proposed to use a species from North America *Arnica chamissonis* subsp. *foliosa* for pharmaceutical purposes. In Germany, in the 1980's, it was developed the only named variety of *Arnica montana* – Arbo. The main chemical constituents responsible for pharmacological properties of *Arnica* genus, are the sesquiterpene lactones helenalin and dihydrohelenalin, and their short chain esters. These are thought to be produced in the glandular trichomes that cover the surface of leaves, stems, flowers and seed coat. The second essential group of constituents are flavonoids and phenolic acids. The third type of component is essential oil. Content of the mentioned constituents in leaves of *A. montana*, *A. montana* cv. Arbo and *A. chamissonis* varies. Level of sesquiterepene lactones is the highest in *A. montana* cv. Arbo, then in *A. chamissonis* and the lowest in *A. montana*. In the studied species proportion of phenolic substances is adversely proportional to sesquterpenes and is higher in *A. montana* than in *A. montana* cv. Arbo, while the highest production of these substances was noticed in *A. chamissonis*.
Influence of the specific composition of secondary metabolites in species within *Arnica* genus on growth, propagation, biochemical features and anatomical structure will be discussed.

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1.37. Could osmopriming improve germination of rape seeds?

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Seeds germination is a very important stage in the ontogenetic development of plants. Several physiological and biochemical changes take place in seeds during germination. Seed germination is influenced by many abiotic factors such as temperature, humidity, salinity, which restrict or inhibit this process. One known way to increase seeds germination and seedlings vigor is conditioning. This action involves hydration of seeds in well-defined and controlled conditions sufficient to increase the metabolic activity but not enough to pierce the seed coat. Seed germination is characterized by three phases of water uptake. Conditioning treatment prolongs second phase preventing entry into the third phase. One way of seeds conditioning is osmopriming. During osmopriming seeds are exposed to osmotically active substances such as polyethylene glycol, sorbitol or mannitol.
In this work, rape (*Brassica napus* L. cv. Libomir) seeds were primed with -1.2 MPa polyethylene glycol (PEG 6000) for 7 days and then germinated on water for 72h. Our results showed that seed coat rapture occurred at 14 -15h of imbibition of osmoprimed seeds, while in not conditioned seeds at 22 - 24h of imbibition. The germination rate of osmoprimed seeds was greater than that of non treated seeds. The differences in kinetic of water uptake were observed between primed and unprimed seeds. Proteins of dry osmoprimed seeds were analyzed by two-dimensional electrophoresis 2D-IEF-SDS-PAGE in order to detect osmpriming induced changes in protein profile. Dry osmoprimed seeds and seedlings grown for 72 h on water showed higher activity of antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase as compared to non treated seeds and seedlings.
This study demonstrates that osmopriming improves germination of rape seeds.

1.38. Seasonal variation in germination of heteromorphic achenes of gallant soldier (*Galinsoga parviflora* Cav.)

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The gallant soldier (*Galinsoga parviflora* Cav.), a popular weed of root crops, grain crops and stubble fields, is an invasive species that easily colonizes new territories. It is a widely propagating and an increasingly aggressive species that poses a growing threat to crop production in Poland. *G. parviflora* is a native of South America, is a neophyte species introduced to Poland around 200 years ago. This annual plant produces heteromorphic achenes within each capitulum. We examined seasonal variation in germination buried in the soil of hetromorphic diasporas. Seeds (achenes) of *G. parviflora* were collected, stored in the laboratory and subsequently buried in the same area where they were collected, inside nylon bags. The bags were collected monthly for germination tests under continuous light at 10, 20 and 25°C.

Germination of achenes in the soil bank showed a seasonal pattern with two peaks of germination: in late spring (June) and autumn (October, November). In the first half of the year (till June) peripheral achenes germinated better or in similar level to central achenes, whereas after this period of time relationship changed: central achenes germinated better than peripheral ones. Central achenes germinated best in June at 20 and 25°C (70, 75%) and in October and November at 10, 20 and 25°C (68-88%). Peripheral achenes germinated best in June and in October and November at 20°C (56-68%). Nitrates affect germination was in January, February, March, May at 10 and in March at 20, 25°C in both types of achenes.

1.39. Chilling stress induces accumulation of raffinose family oligosaccharides in cucumber (*Cucumis sativus* L.) seedlings

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Cucumber seeds (*Cucumis sativus* L. cv. Polan F1) contain raffinose, stachyose and verbascose (raffinose family oligosaccharides, RFO) as reserve materials. During seeds germination RFO are gradually degraded, but they are still present in seedlings. After 5-7-days of germination all seedling parts: cotyledons, hypocotyl and root contained sucrose, monosaccharides (glucose and fructose), *myo*-inositol, galactinol and RFO (mainly stachyose and raffinose). Chilling stress induced by exposition of 7-day old seedlings (developing in climatic chamber at 14/10 h day/night photoperiod with 25/20°C day/night temperatures) to 5/0°C for 24-72 h induced accumulation of raffinose and stachyose in root, and at lesser extent in hypocotyls and cotyledons tissues. The elevated level of RFO decreased after seedlings transfer to control temperatures. Similar changes in the concentration were observed in the case of sucrose. After recovery seedlings were able to restore their growth without any visible damages, but the rate of seedlings growth was clearly delayed, as compared to control. Obtained results indicate on the protective role of RFO in cucumber seedlings resistance to chilling stress.

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1.40. Changes in free, microsome- and thylakoid-associated polyamine content induced by sorbitol and salt stresses by maze and bean

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Water deficit and salinity are one of the major abiotic stresses affecting plant agriculture worldwide. Most of the commercially important crops, including maize and bean, are water deficit sensitive. Polyamines have been considered like osmotic and salt tolerance modulators and biochemical indicators of these stresses. In the present study, we measured organ specific changes in the levels of free, microsome and thylakoid associated polyamines in maize and bean plants exposed for 24 h to osmotic and saline stresses. Results from these experiments showed a similar trend in both species concerning polyamine content: i. e., salt induced significant decrease of free spermidine and spermine in the roots. In osmotic stress conditions the levels of these polyamines fluctuated depending on the species. Putrescine levels were higher in studied organs of both species and under both stresses, only in the roots of salt treated bean its considerably decreased. We observed the significant decrease of all polyamines associated with the microsomes isolated from the leaves and roots of maize and bean growing in sorbitol and salt conditions, higher in ionic stress. Our data showed also the reduction of putrescine contents with significant decrease of spermidine and spermine levels in thylakoids isolated from the chloroplasts of maize and bean plants growing under both stresses. The received results indicate that cultivars of studied maize and bean plants belong rather to drought sensitive. Additionally the thylakoid and microsome bound polyamines seem to be a good markers to define plant stress tolerance.

1.41. The role of anthocyanins as a signalling molecules and non-enzymatic antioxidants in *Elodea* exposed to cadmium and manganese

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Foliar anthocyanins are known to play an important role as modulators of reactive oxygen signalling cascades involved in plant growth and stress response. The effect of exogenous anthocyanins from red cabbage on *Elodea (Egeria) densa* Planch. exposed to Cd or Mn was analyzed to study the defensive and stress-related signaling pathways. Incubation of plants for five days in 100 μM $CdSO_4$ or $MnSO_4$ induced strong oxidative stress resulting in production of reactive oxygen species (ROS) – $\cdot O_2^-$ - in the case of Mn ions and H_2O_2, lipid peroxidation in the case of both heavy metals. Concomitantly the activation of SOD, an increase of proline, anthocyanins and total soluble thiols (Cd only) concentrations was observed. Pre-incubation of plants with anthocyanin extract (100 mg/l) for 24 h resulted in accumulation of anthocyanins in tissues exposed to Cd or Mn. Exogenous anthocyanins strongly affected the plants treated by cadmium: level of oxidative stress and activity of antioxidant enzymes decreased in spite of increase in Cd content in tissues. Interestingly, exogenous anthocyanins significantly increased concentration of others non-enzymatic antioxidants such as carotenoids, proline and ascorbate. Mn concentration in the leaves pre-incubated with the anthocyanins and treated with the metal decreased. Peroxides, superoxides and lipid peroxidation also significantly declined. These findings indicate that anthocyanins influence the pro-/antioxidant balance in different heavy metals exposed water plants, but the mechanisms are metal dependent. Anthocyanins modulated signalling responses in Cd exposed *Elodea* by scavenging ROS directly by inducing biosynthesis of some cellular antioxidants. Anthocyanins and Mn formed stable chelates on surface of leaves.

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1.42. Mapping QTL for proline content in wheat under severe soil drought treatment

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Proline is the most common of compatible solutes accumulated in plant tissues in water deficit conditions. Evaluation of sensitivity or tolerance for drought stress in plant tissues could be possible by estimation of proline content (PC), amongst synthesized metabolites. Biochemical analysis of PC in flag leaves of CSDH (doubled haploid lines obtained from Chinese Spring x SQ1) mapping population plants, grown under water limited environment (severe drought, SD) and well water conditions (WW), revealed wide fluctuations between drought and control treatments. Quantitative trait locus (QTL) analysis was performed using single-marker analysis (SMA) and composite interval mapping approaches (CIM) in Windows QTL Cartographer V. 2.5. Mapping QTL for proline detected 7 major QTLs: 4 mapped for well watered conditions localized on chromosomes 5B, 5D (2 QTLs), 7A and 3 QTLs for severe drought (1D, 2A, 6A).

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1.43. Structure and function of cucumber metal transport proteins CsMTP3 and CsMTP4

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Metal Transport Proteins (MTP), designated also as Cation Diffusion Facilitators (CDF), constitute a phylogenetically ubiquitous family of membrane proteins generally believed to contribute in the homeostasis of a wide range divalent metal cations. Members of MTPs have been found in bacteria, yeast, animal and plant cells, so they seem to be useful in the engineering of hyperaccumulative phytoremediation systems. However, there is currently relatively little data describing the properties (function, localization, substrate specificity) of plant MTPs in detail. Here, we present the transcriptional profile of two cucumber genes encoding for proteins with features typical of MTP (CDF) proteins, that are homologous with *Arabidopsis thaliana* MTP3 and MTP4. Reverse transcription RT–PCR analyses demonstrated that *CsMTP3* was expressed predominantly in leaves, fruits and female flowers, whereas the transcript of *CsMTP4* accumulated in all tissues at different level. The expression of *CsMTP4* in roots was almost unaffected by heavy metals (Cd, Zn, Mn or Ni) after the short-term (5-24 hours) or permanent exposition of plants to 20 µM metals. On the contrary, the level of *CsMTP3* transcript was significantly increased in response to Cd suggesting that *CsMTP3* participate in the detoxification of leaf cells from Cd excess. The expression of *CsMTP3* was significantly down-regulated under Zn or Mn starvation, indicating that the gene encodes protein transporter with broad specificity to metals. In this work, the physiological function of both proteins in cucumber cells is discussed basing on their structure, subcellular localization and expression pattern under heavy metals.

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1.44. The effect of exogenous nitric oxide donor and sucrose on the synthesis mechanism and accumulation of genistein in yellow lupine embryo axes infected with *Fusarium oxysporum*

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Genistein is an interesting molecule with a broad spectrum of biological activity, which function in plants is determined, and is therefore a highly reactive free aglycone, which may contribute to the inhibition of infection and disease development during plant-pathogen interactions. The aim of the present study was to examine the effects of cross-talk interactions of the exogenous nitric oxide donor - sodium nitroprusside (SNP), and sucrose on the synthesis mechanism and accumulation of genistein in embryo axes of *Lupinus luteus* L. cv. Juno. At the same time it was verified whether the dialogue of these molecules can modulate the response of these axis to infection and development of the pathogenic fungus *Fusarium oxysporum*.
It was shown that in embryo axes of yellow lupine pretreated with 100 μM SNP and next cultured on a medium with sucrose high levels of genistein alone, 2'hydroxygenistein, the glucoside of 2'hydroxygenistein, wighteone and luteone are found in embryo axes non-inoculated and those inoculated with *F. oxysporum*, i.e. +Sn and +Si. However, it needs to be stressed that infection in tissues with a high level of sucrose (+Si) very strongly enhances the accumulation of these metabolites, including genistein. It results from determinations of phenylalanin ammonia lyase (PAL) – an enzyme initiating phenylpropanoid metabolism, that SNP pretreatment and exogenous administration of sucrose stimulated PAL activity in +Sn embryo axes, i.e. non-inoculated and cultured on a medium with sucrose, and infection additionally enhanced this activity. The highest PAL activity in +Sn and +Si tissues was recorded at 48h culture. The strong accumulation of genistein and other isoflavonoids observed within this study in embryo axes of yellow lupine is the result of the amplification of the signal coming from sucrose and nitric oxide.

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1.45. Role of the glutamine synthetase isoenzymes and Rubisco in drought stress

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Drought stress may have a considerable impact on the ecosystem and agriculture. Drought stress induces early leaf senescence. During this process, chloroplasts are degraded and photosynthesis drastically drops. The objective of this investigation was to look into the regulation of nitrogen and carbon metabolism during water deficit stress. GS isoenzymes are good markers of the plastid status (GS2) and the nitrogen metabolism (GS1). Tolerant and sensitive wheat (*Triticum aestivum* L.) genotypes were tested, which are widely used in agriculture. The amount of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and GS isoforms in leaves were measured during the grain filling period, as indicative traits that ultimately determine the onset and stage of senescence. The proteins was isolated by non-denaturing polyacrilamyde gel electrophoresis and the GS isoenzymes was identified by Western blot. The symptoms of senescence appear first on the oldest and finally on the youngest leaves. The sequentiality of senescence was disrupted in the sensitive varieties during drought stress. In the flag leaves, an untimely senescence appeared, earlier than in the older leaf levels. Total protein and Rubisco content decreased and the GS2 isoenzyme disappeared. These physiological parameters did not change in the tolerant varieties under drought as compared to the control, well watered plants, or only the gradient of senescence became steeper, indicating the acceleration of this process. Our results revealed the indicator role of GS in different abiotic stresses, which can be applied for characterization (classification) of wheat cultivars in terms of abiotic stress tolerance.

1.46. Adaptations of cells of arctic and antarctic plants to environmental stress

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Microscopic analysis of anatomical and ultrastructural features of polar vascular plants, representatives of families – Caryophyllaceae *(Cerastium alpinum, Colobanthus quitensis)* and Poaceae *(Poa arctica* var*. vivipara, Deschampsia antarctica)* revealed their unique adaptations to abiotic stress. Observations based on semi-thin sections, stained with toluidine blue and after PAS reaction, confirmed the xeromorphic (sclerophytic or succulent) cell structure features, such as thick walled epidermis cells covered with numerous secretory hair, stomata mainly on the adaxial side of the leaves, and within vascular bundles, narrow tracheary elements, which inhibit freezing of water in vessels. In response to salt stress and hypoxia the epidermis of Poaceae species forms vesicular cells, whose number and size depends on microhabitats. In plants constantly flooded with water, large groups of these cells and extensive intercellular spaces were simultaneously observed.
Ultrastructural observations of ultra-thin sections shows ergastic substances – considerable amounts of starch granules in chloroplasts, lipid materials associated with chloroplast membranes or within the cytoplasm and polysaccharide derivatives in the intercellular spaces. In stressful conditions the substances could serve as a readily available source of energy and a raw material for the biosynthesis of cryprotective compounds. Nuclei, likewise other cell organelles, form numerous, long protrusion, concavities or cytoplasm filled canals with organelles and vesicles. Increased surface of contact between a nucleus and cytoplasm elements as well as specific, strict adjacency indicate tight cooperation and an efficient signal transmission, which may be important for growth and development in harsh environmental conditions.

1.47. Isolation and characterization of ABA metabolism genes from *Pharbitis nil*

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Abscisic acid (ABA) is a naturally occurring sesquiterpene phytohormone present in all higher plants as well as in some mosses, green algae, fungi and bacteria. The level of ABA in particular plant tissue is determined by the rate of its biosynthesis and catabolism. Therefore, identifying all the genes involved in the ABA metabolism is essential for a complete understanding of how this hormone direct plant growth, development and stress responses. Previously, our physiological studies revealed that ABA has a significant role during flowering induction in model short day plant *Pharbitis nil*. For deeper investigation into this problem we present here the first report of the identification and description of two genes involved in biosynthesis and one gene for ABA catabolism pathway.
Using RT-PCR based methods we cloned three full-length cDNA sequences corresponding to NCED, ZEP and 8'-hydroxylase enzymes. Two identified cDNAs, *InNCED* (Gen Bank accession no. HQ64566) and *InZEP1* (HQ827173) are 2375 bp and 2213 long and have an ORF for 615 and 672 amino acids, respectively. The predicted protein sequences are highly homologous (80%) to 9-cis-epoxycarotenoid dioxygenase and zeaxanthin epoxidase from other higher plant. *InZEP1* cDNA encodes a putative chloroplast-imported protein of 73.4 kDa, sharing similarities with different monooxigenases and having a FAD-binding domain. The *InHx1* cDNA (HQ641567) is 1844 bp long and encodes protein with high sequence similarity to plant ABA 8'-hydroxylase, which catalyzes the first committed step in predominant ABA catabolism. Recent ongoing studies are focused on the analysis of the isolated genes expression level in the vegetative and generative *P. nil* organs. This research will give new insight into the regulation and site of ABA metabolism in relation to its physiological roles.

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1.48. Flavonoids and other phenolic compounds in response of barley (*Hordeum vulgare* L.) plants to drought

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Drought is one of the major abiotic stresses in agriculture. Phenolic compounds are important parts of plant response to abiotic stresses. The main objective of this study was to evaluate this chemicals on 9 cultivars of barley (*Hordeum vulgare* L.) originating from Europe and Syria. Tests were performed at different developmental stages. The effect of drought stress depended not only on the duration and intensity of water deficiency, but also on the developmental phase in which it began. Changes in profiles of phenolic compounds under different stress conditions were analyzed. The most accurate and fastest tool for this purpose is ultra-high pressure liquid chromatography (UHPLC). It is also useful for searching biomarkers pattern for drought tolerance. To handle and interpret the complexity of the data generated multivariate statistical processing was applied. UHPLC rows data had been mathematically pre-processed to reduce the effects of instrumental factors and then analysis of variance was used. Drought stress induced changes in various secondary metabolites, indicating differences between cultivars. Drought at seedling stage excited the most of statistical important changes in cultivar Sebastian (in comparison with control group), whereas drought at flag leaf altered most cultivar Cam B1. Simultaneously, MS/MS-UV-HPLC was used for qualitative analysis of phenolic compounds.
The results suggest that the effects are a stage-specific trait and change during the life cycle. Phenolic compounds in control group are mainly O-glycosides or C-glycosides of flavons: apigenin, luteolin, tricin and chrysoeriol. The sugars can be acylated with hydroxycinnamic acids. At drought at seedling stage, new compounds were observed: flavanon naringenin and its derivatives as well as malonylated compounds. At drought at flag leaf number of compounds with directly acylation of aglycon appeared. It is substantial to get to know the molecular mechanisms that cause such changes in phenolic profiles.

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1.49. Influence of heavy metal ions on the AOS generation, antioxidants level, phytochelatin biosynthesis and growth of *Pisum sativum*

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In last years we observe a gradual increase of heavy metal contamination in environment therefore, there is a need to investigation the responses of food crops to Cd, Cu. Zn and Pb toxicity. The aim of this work was to investigate the effects of Cd, Cu, Zn and Pb concentrations on the growth, nutrient composition, AOS generation, heavy metal accumulation and functioning of the antioxidative and detoxicative system in these plants. *Pisum sativum* plants were studied for the plant growth, heavy metal accumulation and mineral nutrients content under excess heavy metal conditions. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used for quantitative imaging of toxic (Cd, Pb) and essential (P, K, Ca, Mg, Cu) elements in *Pisum sativum* tissues. The highest heavy metals accumulation was found in roots, mostly in the epidermis, exodermis and endodermis zone. The most phytotoxic effects was observed for the pea plants treated with Cu and Cd ions, in this plants also were determined the most significant changes in the nutrients signal intensity.
During exposition to heavy metals were also determined significant changes in the level of ascorbate, glutathione and phytochelatins. In treated plants we observed increase of reduced form of glutathione and phytochelatin. While in pea cultivated with Cu and Zn analysis by HPLC ESI IT MS shown significant increase of thiols oxidative form, which may suggests their participant in antioxidative response.

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1.50. Are phototropins involved in *Arabidopsis* flowering?

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Plant flowering is controlled by several factors. One of them is light. Light quality and quantity as well as the relative lengths of day and night (photoperiod) influence the transition from vegetative to generative growth. It has been shown that plant photoreceptors, phytochromes, cryptochromes and ZTL/FKF1/LKP2 family, are involved in the regulation of photoperiodic flowering. We tested the role of another class of photoreceptors, phototropins, in this process. *Arabidopsis* WT Columbia and phototropin mutants were grown in 10hL/14hD photoperiod. *Phot1-5* mutants flowered up to 2 week earlier than WT under these conditions. They also had fewer rosette leaves than WT. In contrast, no differences between WT and *phot2* and/ or *phot1-5/phot2* plants were observed. The expression of two flowering-associated genes: *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) was tested every week starting from 3-week-old seedlings up to 7-week-old plants. In 3-week-old seedlings, the transcript level of *CO* was about 2,5 times higher in *nph1-5* than in WT, but they became comparable in older plants. The expression of *FT* was significantly higher in *phot1-5* plants in every time point tested. The largest difference was obtained at the 7th week: the *FT* mRNA level in *phot1-5* was 12 times higher than in WT.
Thus, the early flowering phenotype of *phot1-5* seems to be caused by changed expression of the flowering-associated genes. Our results suggest that phototropin2 is responsible for flowering induction only when phototropin1 is absent. Probably, PHOT1 acts downstream PHOT2 inhibiting the expression of *FT* gene.

1.51. Sucrose synthase and invertase activities in relation to phosphorylation status of *Vicia faba* root meristem cells during reactivation from sugar depletion

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In response to carbohydrate starvation of *V. faba* root meristems the control points block cell cycle. Its reactivation is possible after sucrose provision. Supplied sugar can be a source of energy only if sucrose metabolic and signaling pathways function efficiently. Inhibitors of protein kinases (6-dimethylaminopurine; 6-DMAP) and phosphatases (okadaic acid; OA) significantly disturbed metabolic regeneration, simultaneously interfering with the activities of sucrose metabolism enzymes: hexokinase and fructokinase (Polit and Ciereszko 2009).
In this study, it was investigated whether 6-DMAP and OA were involved in inhibition of cell cycle revival through interference with the activities of the sucrose-cleaving enzymes: sucrose synthase (SuSy) and invertase. In sugar-starved cells the activity of both enzymes decreased significantly. During sugar-regeneration of root meristems the invertase remained inactive, but SuSy activity increased. In these cells, the low level of polysaccharides, and relatively high soluble sugar content was observed. SuSy activity was induced more by exogenous sucrose than by glucose. However, sucrose-induced activity of SuSy was inhibited by OA, especially at the early stages of regeneration. Later, before the appearance of replication or mitotic activities, SuSy activity was not inhibited by OA. In cells treated with 6-DMAP no decrease in SuSy activity was observed. The results indicate that prolongation of regeneration (caused by OA), and marked decrease in the number of cells resuming proliferation, are correlated with the blockade of PP1/PP2A phosphatases activity, which contribute to the process of SuSy activation at the beginning of regeneration from sugar starvation.

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1.52. Effect of PLA2 inhibitors on ROS production in *Solanum* species treated with elicitor from *Phytophthora infestans*

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Activity of phospholipase A2 (PLA2), a key enzyme in lipid metabolism, were investigated in *Solanum* species and *Phytophthora infestans* interaction. We have compared PLA2 activity in response to an elicitor, culture filtrate (CF) derived from *P. infestans*, in non-host resistant *Solanum nigrum* var. *gigantea*, field resistant *Solanum tuberosum* cv. Bzura and susceptible *S. tuberosum* clone H-8105. To elucidate the contribution of specific forms of PLA2 to plant defence mechanism PLA2 inhibitors, haloenol lactone suicide substrate (HELSS) and p-bromophenacyl bromide (BPB), which discriminate between Ca^{+2}-independent PLA2 (iPLA2) and Ca^{+2}-dependent secretory PLA2 (sPLA2), were used. In plants, there are some evidence for the involvement of PLA2s activities in the generation of reactive oxygen species (ROS) by NADPH oxidase in response to elicitation. To ascertain a possible relationship between ROS production and PLA2activity, we have compared ROS production in response to CF in leaves preincubated with the inhibitors of PLA2.
We found that PLA2 activity increased in response to CF treatment in *Solanum* genotypes. Differences among the genotypes in the effects of each inhibitor on CF-induced PLA2 activity and on ROS production may reflect the diversity of PLA2 isoforms in plants. Contrary to BPB, the inhibitory effect of HELSS was observable mainly on CF-induced PLA2 activity, which suggests that iPLA2 participates in signal transduction in defence reactions. Various effects of the two inhibitors on PLA2 activity and ROS production suggest different contribution of sPLA2 and iPLA2 to modulation of defence reactions in the interaction between *Solanum* genotypes and *P. infestans*.

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1.53. Salt stress- and salicylic acid-induced programmed cell death in tomato leaves

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Programmed cell death (PCD) is an integral part of the plant development. It is important in the response to changing environments and it can be triggered by abiotic and biotic stressors. Salt stress and salicylic acid (SA) can induce PCD by triggering different signaling pathways. The addition of NaCl at 100-250 mM and SA at 10^{-3}-10^{-2} M to the hydroponic culture of tomato plants for 6 hours resulted in stomatal closure on intact leaves. At 100-250 mM NaCl and 10^{-3}-10^{-2} M SA decreased the maximal CO_2 fixation rate (Amax), and the initial slopes of the CO_2(A/Ci) and light response (A/PPFD) curves and relative electron transport rate (Rel. ETR). The inhibition of photosynthetic electron transport can contribute to the death of plants by increasing reactive oxygen species (ROS) production of chloroplast. NaCl and SA treatments increased the amount of ROS and H_2O_2content of leaf tissues which can induce the death of plants. The specific genes involved in cell death program were induced by NaCl and SA and they were analysed by RT-PCR. The expression of both the inhibitors (e.g., BAXInhibitor) and effectors (e.g., cysteine proteinases) of PCD were significantly enhanced at lethal concentrations of NaCl and SA. These results suggest that the PCD in these tissues can be triggered in spite of the high expression level of PCD-inhibiting genes. Signal transduction pathways induced by salt stress and SA have been compared in tomato cell suspension culture.

1.54. The effect of selenium on viability of recalcitrant *Acer saccharinum* L. seeds during desiccation

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Seeds, which belong to recalcitrant category, are sensitive to dehydration below a certain, relatively high threshold values of moisture level, or if they are stored in a hydrated state. Freshly harvested silver maple (*Acer saccharinum* L.) seeds were soaked in either sodium selenite (10 mg/L) or water for 6 h. After washing and air drying, seeds were desiccated at 22°C at a RH of 45- 50% RH to comparable water levels from 50 to 13%. Germination capacity was significantly higher in seeds treated with selenium and desiccated (from 50 to 40, 35 and 30% WC) than in water-soaked seeds. At 20% WC, the seeds from both treatments had low viability (approximately 20%).The electrolyte leakage and the MDA content were significantly lower in the embryonic axes of seeds soaked in selenite than in seeds soaked in water. It was also found that the activity of glutathione peroxidase (GPX) of embryonic axes from selenium-treated seeds that were not desiccated, or from seeds that were desiccated to 40% and 35% WC was significantly higher than that of non-treated axes. No difference in GPX activity was detected in cotyledons. This was confirmed by activity staining of GPX after native PAGE of proteins extracted from embryonic axes and cotyledons. An increase in glutathione reductase (GR) activity was also observed in embryonic axes of seeds treated with selenium and dried to 35 and 30% WC compared to non-treated samples.

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1.55. Cryopreservation of embryonic axes of orthodox, suborthodox and recalcitrant seeds of woody plants in relation to their desiccation sensitivity

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Cryopreservation in liquid nitrogen (LN, -196°C) allows safe and long-term conservation of many plant species. The objective of the presented research was to determine the possible damages of embryonic axes (EA) of the *orthodox* Norway maple (*Acer platanoides*), sycamore (*A. pseudoplatanus) recalcitrant* and typical *suborthodox* beech (*Fagus sylvatica*) seeds, during the step-by-step cryopreservation process. Their tissues can be damaged during desiccation and/or the LN-thawing cycle. Ice nucleation during slowly cooling rate (0.25 °C/min) was determined by differential thermal analysis (DTA). The non-freezable water was estimated when the tissues were desiccated below a water content (WC) at 26% *Fagus sylvatica,* 27% *A. platanoides* and 36% in *A. pseudoplatanus*. Damage to membranes during desiccation and cryopreservation was assessed based on electrolyte leakage and analysis of lipid peroxidation, as the concentration of its end product – malondialdehyde (MDA). Accumulation of MDA slightly increased when desiccation progressed, and a further increase was observed after freezing in LN. Desiccation alone slightly impaired plasma membrane integrity, while the increase in membrane breakdown was observed after pre-freezing to 40°C and then after cryo-freezing if WC was above 23%. The increase of electrolyte leakage above 50% as a result of desiccation was observed only in EA of *A. pseudoplatanus* below 20% WC. No substantial changes were observed in levels of particular fatty acids within the phospholipid fraction. Desiccation and cryopreservation caused an increased production of reactive oxygen species: superoxide anion radical ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2). The strong rise in $\cdot O_2^-$ production appeared during cryopreservation stress. The accumulation of AOS as a result of desiccation and freezing was associated with decrease in activity of guaicol peroxidase (POX). While the superoxide dismutase (SOD) activity initially increase during desiccation and then decrease in *A. pseudoplatanus* and it reached at the end of desiccation a value 13% higher in comparison with control. In turn during desiccation in EA of and after exposure to LN in both species activity of SOD decrease. Ultrastructural examination of *F. sylvatica* tissues desiccated to a WC of 21% and next frozen in LN, showed mild injury of the cell wall, cell membrane and nuclear envelope, but also mitochondrial swelling and coalescence of lipid bodies. Optimal survival of EA tissues after cryogenic stress was achieved when they were desiccated to a WC of 10% of *F. sylvatica* and *A. platanoides* and 15% of *A. pseudoplatanus*. EA of species subjected to variable levels of desiccation stress showed a minor decrease in viability and vigour, but after extreme desiccation, an increase in electrolyte leakage appeared, indicating a impaired membrane integrity.

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1.56. Circadian rhythm leaf movement of *Phaseolus vulgaris* and the role of calcium ions

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Legume plants, due to their distinctive botanical characteristics, such as leaf movements, physiological characteristics, such as nitrogen fixation, and their abilities to endure environmental stresses, have important roles in sustainable pastures development. Leaf movement of legume plants is turgor regulated, and osmotically active fluxes of ions between extensor and flexor of pulvinus cause this movement. To determine the role of calcium ions in circadian leaf movements of *Phaseolus vulgaris* L., a radiotracer technique experiment using ^{45}Ca ions were employed. Measurements were taken during circadian leaf movements, and samples were taken from different parts of the leaflet. The ^{45}Ca β-particle activity reduced from leaflet base pulvinus to leaf tip. The pulvinus had the highest activity, while the leaf tip had the lowest. By increase of the ratio of ^{45}Ca β-particle activity within flexor to extensor (Fl/Ex) the midrib-petiole angle, as an indicator of leaf movement, increased linearly during circadian leaf movement ($r = 0.86$). The ^{45}Ca β-particle activity of Flex/Ext ratio reduced linearly ($r = -0.88$) toward midnight. In conclusion, it was found that calcium ions accumulation is opposite to the fluxes of somatically active ions and water movement. Calcium ions accumulate at less negative water potential side of the pulvinus.

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1.57. Production and localization of reactive oxygen species in *Fagus sylvatica* L. seeds during storage

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The common beech (*Fagus sylvatica* L.) is one of the most important broadleaved species in European forestry. Beech is propagated by seeds, but seed set is irregular, with five to ten years between crops. Consequently, it is necessary to store the seeds. The accumulation of reactive oxygen species (ROS) is one of the main factors that affect the seeds tissues and cause the loss of viability during storage. Localization and production of ROS (superoxide radical $\cdot O_2^-$ and hydrogen peroxide H_2O_2) was performed in the embryonic axes and cotyledons of beech seeds. The seeds were stored at -10°C through 15, 13, 9, 3 years in controlled conditions. Observation of the superoxide radical and hydrogen peroxide localization was performed using the confocal microscope. The accumulation of $\cdot O_2^-$ and H_2O_2 was noted in part top of the embryonic axes. However in cotyledons ROS were spread on the entire surface. The level of superoxide radical and hydrogen peroxide was also measured using the spectrometric method. The results were consistent with the microscopic observations. The increase in the ROS level was spectacular in embryonic axes and correlated with extending time of seed storage. Beech seed viability was assessed on the basis of ability of the seeds to germinate. Seed germinability was the lowest in seeds that were stored for the longest time and characterized with the highest ROS level. In present study the involvement of production and localization of superoxide radical and hydrogen peroxide with the loss of germination capacity of beech seeds during storage is here discussed.

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1.58. Changes in NR activity and *Nia* genes expression after supplying of C and N metabolites in *Arabidopsis thaliana glt1-T* mutant

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The first and key enzyme responsible for proper incorporation of nitrate ions into organic compounds is nitrate reductase. The activity and expression of NR is tightly regulated by different factors. It is closely related to C and N metabolites. Sugars and organic acids are required as a carbon skeletons for incorporating of inorganic N. On the other hand, amino acids are involved in feedback regulation of nitrate assimilation. The regulation of NR depends on changes in *Nia* genes expression as well as rapid and reversible phosphorylation of enzyme protein.

In presented studies the NR activity and *Nia* genes expression were investigated after external supplying of selected carbon and nitrogen metabolites to *Arabidopsis thaliana glt1-T* mutant plants with disorder in nitrogen assimilation pathway. NR activity and expression were measured in roots and leaves after exposition of starved or NO_3^- induced plants to nitrate with addition of sugars (glucose or sucrose), amino acids (glutamine or glutamate) or organic acids (α-ketoglutarane). Addition of sugars into nitrate solution led to increase of NR activity and expression only in roots of WT but not in mutant plants. Also reduction of the level of phosphorylated enzyme was also observed in the presence of sugars. Stimulation of NR activity but not *Nia* genes expression was observed after α-ketoglutarane treatment. Activation of the enzyme was also present in *glt1-T* plants and it was even stronger then in WT. Amino acids, especially glutamine, decreased NR activity. Glutamate was less effective in WT, and in mutant plants it had slight stimulatory effect.

1.59. Hydroxyurea-induced replicational stress involves tankyrase localized at the nuclear pore complexes of *Vicia faba* nuclear envelope

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Tankyrase is a nuclear enzyme named poly(ADP-ribose) polymerase (PARP) involved in DNA damage signaling. Tankyrase was identified in the interaction with the telomeric protein TRF1 and located at human telomeres. PARP-2, one of the two members of the PARP-family, induces repair processes in response to DNA strand breaks (Smith and de Lange 1999, Malanga and Althaus 2005, Dregalla et al. 2010). Here, we show that PARP-2 is induced following DNA-replication stress induced by hydroxyurea (HU). In this thesis, we demonstrate that: (i) prolonged action of HU causes changes either in intracellular PARP-2 location or in their intensity, (ii) PARP-2 localizes at the nuclear pore complexes and locus near the telomeres, (iii) PARP-2 colocalizes with DNA-dependent protein kinase (DNA-PK).

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1.60. The impact of osmotic stress on metabolic heat production and proline content in pea and soybean genotypes differing in drought tolerance

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The aim of the work was to characterize the changes of the metabolic heat production and an accumulation of free proline in young leaves and roots during osmotic stress evoked by blocking the water uptake in roots by polyethylen glycol (PEG-6000) of 5% and 10% concentration, and to verify an applicability of isothermal calorimetry as a quick screening method for a determination of drought – resistance in pea and soybean genotypes. The effects of the osmotic stress on metabolic heat output of pea and soybean was measured by the isothermal microcalorimetry. The mentioned method is very interesting physiological view on the metabolic changes during the plant stress. Reached results from several measurements of metabolic flow heat in leaves and roots, as well as the accumulation of free proline in leaves, confirm the precedence role of metabolic changes which are signalizing drought. The water stress has increased the accumulation of free proline, especially in all pea and soybean genotypes. All plant reactions were contingent on a variety. Created model conditions allow determining indicators of the water stress activity tolerance in plant varieties.

1.61. Analysis of drought responsive proteins in barley (*Hordeum vulgare*) by 2-D gel electrophoresis and Maldi-Tof mass spectrometry

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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 stress, that strongly influences plant growth, development and 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 with control gene expression and signal transduction. Identification of these proteins is very important in plant breeding programs and can help to improve a yield under drought conditions.
The aim of conducted research was the analysis of changes in barley proteome under drought stress. Comparative analysis was studied by 2D PAGE and MALDI-TOF mass spectrometry. Changes in protein expression patterns in response to water deficit were monitored in two different genotypes of barley (Maresi and Cam B1/C1). Plants were grown in greenhouse for three weeks under controlled environmental conditions. After this time, plants were subjected to drought stress. Leaves and roots samples were harvested after 6 and 10 days of drought. Proteins for qualitative and quantitative analysis 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 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 MASCOT program. The list and the expression profile of drought related proteins are presented on the poster.

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1.62. Morpho-anatomical characterization of *Brachiaria* and *Panicum* species using elemental dispersive spectrophotometer analysis

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Viewing the images of microscopic areas through SEM only solves half the problem in an analysis. It is often necessary to identify the different elements associated with a specimen. This is accomplished by using the "built-in" spectrophotometer called an elemental dispersive x-ray spectrophotometer. SEM in conjugation with EDS (SEM/EDS) makes possible the quick resolution of tough analytical problems effectively, timely and economically. Present study was conducted to identify, evaluate and compare the elemental composition of the *Cenchrus* species present in Pakistan. The SEM/EDS of abaxial and adaxial epidermis of leaf samples was carried out. The abaxial and adaxial epidermal leaf samples were placed on stubs and after the gold coating put into SEM. The EDS detector was used for the quantitative and qualitative determinations of the elements present in the phytoliths of grasses. Especially the mass percentage of silicon was calculated in order to make a comparison between the different taxa. This examination indicates variations in leaf blade epidermal characters and EDS analysis of the phytoliths determines a new structure used as an aid in phytosystematic characterization. The quantitative analysis provides silicon as a new taxonomic character to distinguish different species of the genus *Brachiaria* and *Panicum*. The recent studies indicate that the systematic anatomical investigation can be very useful in delimiting species, genera, tribes and sub-tribes. The present studies also showed that EDS analysis of the grass silica bodies can be constructed for various purposes ranging from their taxonomical description to classification. Mainly in relation to anatomy, the present research presents a standard basis for grouping of grasses focusing on the grass silica bodies giving their specific organizational detail that helps in the identification of grasses. The present study is a step towards preparing a systematic inventory of grass phytoliths. EDS analysis (SEM) showed the elemental composition of phytoliths and a great variation was observed among the percentage of the elements between different species of the genus *Brachiaria* and *Panicum*. The technique of EDS analysis for grass taxonomy is used first time in Pakistan.

1.63. Root tip organization and ultrastructure in certain species of Fabaceae

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The structure of root tip and its relevance for the root development is widely studied in model plant *Arabidopsis thaliana*. In this species, root apical meristem is of the closed type, with storied arrangement of histogens' initial cells. However, this type of root apical meristem organization is not common to all angiosperms. The alternative open type is formed in numerous taxa and intermediary types are also known.
Root apical meristem spatial structure and cell ultrastructure was investigated by means of light and electron microscopy in three fabaceans: model plant *Medicago truncatula* and two species from Genisteae tribe, *Genista tinctoria* and *Lupinus angustifolius*. In all three species, root apical meristem is of open type. The ultrastructure of organizing center, histogens' initials (stem cells) will be presented. Also, the ultrastructure of root cap central gravity-sensing cells, as well as its outer secretory cells will be shown.

1.64. Distribution of cadmium and copper in *Cardaminopsis arenosa*, a plant growing on contaminated soils

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Plant species that accumulate exceptionally large amounts of heavy metals in their tissues are named hyperaccumulators. These plants have developed many protective mechanisms that enable them to survive under harsh environmental conditions. *Cardaminopsis arenosa*, a family member of Brassicaceae, attracts much interest because of its similarity to *Arabidopsis halleri*, a hyperaccumulator of zinc and cadmium. It is a common weed which in Poland occurs on calcareous heavy-metal polluted sites.
In this study two populations of *C. arenosa* were compared: one came from a zinc-lead waste heap in Bolesław (southern Poland), the other one was obtained from the area of Jagiellonian University, III Campus in Kraków. The objective of this study was to determine potential differences in cadmium and copper accumulation and distribution in these two plant groups and to find out what strategies have been adopted by the plants coming from the polluted areas helping them to survive. Moreover, we investigated the protective role of calcium ions in the destructive processes caused by heavy metals. Cadmium and copper concentrations were assessed in different parts of plants: green leaves, senescent leaves and roots. These measurements were done using Atomic Absorption Spectroscopy (AAS).
The concentrations of cadmium and copper were greater in roots than in green leaves, suggesting an exclusion strategy by metal immobilisation in roots. High concentrations of investigated metals were found in senescent leaves, suggesting a second strategy – detoxification of the plant by excision of leaves that have hyperaccumulated the toxic ions. These results demonstrate that *C. arenosa* can be considered as hyperaccumulator and potentially used in recultivation of the land polluted by heavy metals.

1.65. Dark-induced senescence of barley leaves involves mechanisms of programmed cell death

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Senescence is relatively slow cell death of tissues at the end of their life span. It involves the ordered disassembly of cellular components and allows for maximum recovery of nutrients from the senescing tissues for recycling to the parts of the plant that survive. Leaf senescence represents a genetically controlled developmental stage in the life of plants.
We found that dark-induced senescence of barley leaves is a highly regulated process that involves cessation of photosynthesis, disintegration of chloroplasts, breakdown of leaf proteins, loss of chlorophyll, removal of amino acids, internucleosomal fragmentation of nuclear DNA and significant chromatin condensation. Moreover, that degradation of compounds inside the senescing mesophyll cells may be due to autophagy. That all prove that dark-induced leaf senescence is a genetically defined process that involves mechanisms of programmed cell death.

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1.66. Global analysis of gene expression in seedlings' leaves of three inbred maize lines during cold stress

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In spite of several years of research, conducted by standard molecular biology methods, our knowledge concerning expression changes in maize response to cold is restricted to hardly few tens of genes, mainly connected with photosynthesis, carbohydrate metabolism and secondary metabolites (Marocco et al. 2005). New high throughput methods like microarrays give us ability to better resolve molecular mechanisms of maize response to cold.
Our group applied microarray technique in several projects, in one of them we focused on pattern of gene expression changes in chilling-treated leaves of seedlings of three maize inbred lines differing in cold tolerance. We used microarrays produced by Maize Oligonucleotide Array Project (www.maizearray.org) containing 48,000 probes. We conducted in-depth data analysis. Clustering gives us several gene groups, which expression changes could be connected with cold tolerance or cold sensitivity of inbred lines tested. We also conducted global analysis using Gene Ontology (GO) classification and more detailed analysis of relations among genes. For GO enrichment analysis we used Ontologizer program, we took into account GO tree structure by using "Parent-Child Union" correction (Grossmann et al. 2007), we used also FDR multiple comparison correction (p=0.05)(Benjamini and Hochberg 1995). Plots were generated with GraphViz program (www.graphviz.org). We present global analysis results for genes with changes of expression in cold unique for each of the inbred lines tested.

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1.67. Changes in phenolic secondary metabolite profiles in lupine leaves after infection with pathogenic fungus or elicitation with fungal toxin

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Profiles of isoflavone conjugates in leaves of narrow leafed lupine (*Lupinus angustifolius*) treated with anthracnose causing fungus (*Colletotrichum lupini*) were studied. Lupine plants were either sprayed with the *C. lupini* spore suspension or a solution of phytotoxic secondary metabolites produced by this fungus *in vitro.* The fungal toxin solution was also spotted on wounded leaves. LC/MS and GC/MS techniques were applied for profiling of the target isoflavone glycoconjugates and free aglycones. Some of these polyphenolic compounds play an important role in plant defence during interactions with pathogenic microorganisms and have been reported to be phytoalexins. Furthermore, we conducted experiments in which expression of selected phenylpropanoid pathway enzymes was measured.
Over twenty isoflavone glycosides were recognized in leaves of *L. angustifolius*. A number of them were isobaric or isomeric compounds. Profiles of the target secondary metabolites in leaves of control, infected and elicited plants differed substantially from each other. There were both qualitative and quantitative differences. Amounts of free isoflavone aglycones did not increase significantly after elicitation with the toxin, whereas quantities of these compounds were higher in plants infected with *C. lupini* spores. Differences concerning malonylated derivatives of aglycones and their isomers were also observed in the infected and elicited plants. Noteworthy differences were observed in the rate of plant response to various treatments. Whereas the increased isoflavone synthesis was observed within 12, 24 hours after phytotoxin elicitation. The first reaction to the spore infection was observed after three days, the strongest one between seven and eleven days. Another notable difference in plant reaction to both types of treatment was the exudation of prenylated isoflavones onto the leaf surface observed only after the spore infection. Most probably, various plant receptors recognize different signals during infection with fungal spore suspension or elicitation with the isolated fungal phytotoxin and that leads to differences in the plant response. These processes activate various enzymes and synthesis of different phenolic secondary metabolites may occur.

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1.68. *Botrytis cinerea*- induced changes in decarboxylation processes and malate content in C3 or CAM *Mesembryanthemum crystallinum* plants

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Interaction of facultative halophyte *Mesembryanthemum crystallinum* with nectrotrophic fungus *Botrytis cinerea* leads to the alteration in activities of NADP and NAD dependent malic enzymes and malate amount as quickly as 3, 24 and 48 hours post infection (hpi). Changes are observed in inoculated as well as in opposite leaves and also in leaves in the upper whorl in both C3 and CAM (Crasulacean Acid Metabolism) performing plants, indicating the induction of systemic resistance (SR). Moreover, the obtained results point out that defense mechanisms are related to decarboxylation processes in cytoplasm as well as in mitochondria. We suggest that the released CO_2 during decarboxylation plays an important role at the early stage of response of C3/CAM plants to biotic stress.

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1.69. Metabolite profiling in barley leaves (*Hordeum vulgare* L.) subjected to drought stress

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Drought is an important abiotic stress that limits agricultural production of different crop plants. It adversely affects plant growth and development contributing to crop yield loss. Reduced crop yield due to water deficit is related to the intensity, duration and timing of the stress. Therefore it is interesting to understand complex physiological and biochemical mechanisms underlying this abiotic stress tolerance.
Barley is an important cereal, grown for animal feed, human food and as a material for malting for alcohol production. In our preliminary project we observed metabolic responses to drought stresses for two barley (*Hordeum vulgare* L.) varieties, both being spring-type. Plants were subjected to 10-day long water deficit at the growth stage of three leaves. Leaf extracts were subjected to gas chromatography - electron impact - time of flight - mass spectrometry analysis (GC-EI-TOF/MS), which is common analytical technology allowing nonbiased and simultaneous analysis of a large number of small molecules in complex samples. Metabolites were identified on the basis of retention time and mass spectra registered for derivatives of standard compounds, commercial mass spectra library NIST and high resolution mass spectral database MassBank.
Exposure of plants to drought resulted in changes of level of different classes of metabolites. Barley plantlets subjected to the stress had increased intracellular concentration of amino acids (especially proline) and carbohydrates; also TCA cycle intermediates changed in comparison with the control. Such accumulation may be correlated with inhibited plant growth during the stress or being adaptive response to water deficit. In continuation of drought markers studies metabolite profiles in lines of a chosen barley mapping population will be subject of further research.

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1.70. Programmed cell death – a component of triticale resistance mechanism to infection with *Microdochium nivale* (Fr.) Samuels et Hallett

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Microdochium nivale is regarded as a serious winter fungal pathogen, caused loses up to 50-90% of autumn sawn cereals and grasses. Moreover, there are no efficient fungicides or another crop protection strategy against *M. nivale.* On the other hand, it was observed that maximal snow mould resistance effectively develops in cold-hardened plants of triticale. However, mechanisms leading to plant acclimation in such biotic interactions are not well known.

We have assumed that leaf tissues of winter triticale can use the mechanisms of programmed cell death (PCD) to restrict *M. nivale* growth and development. We confirmed this hypothesis by TUNEL/DAPI staining and direct detection and quantification of PCD at the single cell level. Higher amount of apoptotic cells in hardened plants of resistant variety indicates that mycelium, but not fungal extract can induce reactions similar to hypersensitive responses. Hydrogen peroxide (H_2O_2) content and expression analysis of *CAT1*(*Ta*) (primer designed according to *Triticum aestivum* gene), *CAT1*(*Sc*) (primer designed according to *Secale sereale* gene), *APX1* and *PR4* were performed. We found that H_2O_2 can be involved in the mechanism of PCD. Additionally, H_2O_2 may oxidize molecules used in direct defence against fungal pathogen in resistant plants, and it can be also a signalling molecule during infection. Moreover, the obtained results indicate that cold induced the expression of *CAT1*(*Ta*), *CAT1*(*Sc*) and *APX1*are the key expression markers for the induction of cold-mediated resistance to *M. nivale*.

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1.71. Changes in the expression of *Medicago truncatula* full-size ABCG transporters upon treatment with selected plant hormones

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Changes under environmental stimuli, plasticity of plant metabolism and developmental processes often rely on the availability and distribution of endogenous signaling molecules – plant hormones. Hormone signaling in legume plants has been reported to regulate the nodulation process. In particular, balance between auxin (IAA) and cytokinins in root cortex and in pericycle determines whether the development of lateral roots or nodules will be initiated. It has been proposed that Nod factor perception at the root epidermis increases local production of cytokinins and the latter are transported to cortical cells (Oldroyd 2007). The mechanism of such translocation remains elusive. A major function fulfilled by ATP-binding cassette (ABC) proteins is membrane translocation of a wide range of unrelated molecules. Recent data point to full-size ABCG transporters as important players that influence IAA transport/homeostasis and IBA (indole-3-acetic acid) sensitivity, which affect many aspects of primary root development (Ruzicka et al. 2010).
Previously, we identified 20 genes, which code for full-size ABCG proteins in *Medicago truncatula,* a model legume plant (Jasinski et al. 2009). Here we address a question how their expression in root tissues is modulated by hormones like auxins and cytokinins. We found out that the expression pattern of two of them is particularly influenced by hormonal treatment.

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1.72. Characterization of cDNA encoding phytocystatins in generative and vegetative organs of winter triticale

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Phytocystatins are plant endogenous inhibitors of cysteine endopeptidases of papain (C1A) and legumain (C13) families. Seven different cDNAs encoding triticale phytocystatins: *TrcC-1-5*, *TrMDC* and *TrHvC* have been obtained. In all the deduced amino acid sequences of identified inhibitors the presence of conserved domain containing the G and W residues, the sequence of QxVxG and the sequence of LARFAV characteristic for plant inhibitors, have been noted. Moreover, the sequence of TrMDC contains an additional carboxy-terminal extension. The phytocystatins TrcC-1-5 and TrHvC belong to inhibitors group with low molecular weight (from 12 to 16 kDa) while deduced amino acid sequence of *TrMDC* probably encodes a protein with molecular weight about 23 kDa. Phylogenetic analysis of the deduced amino acid sequences of identified inhibitors with other cereal phytocystatins allowed the classification of the inhibitors to group A. According to research performed by Abraham et al. (2006) the members from this group can regulate the activity of the endogenous cysteine endopeptidases both in vegetative and generative tissues and control activity of exogenous enzymes from pests and pathogens. Our research conducted so far show that cDNAs encoding *TrcC-1-5* are present in developing and germinating triticale seeds (*TrcC-1-4*) while the cDNAs of *TrMDC* and *TrHvC* are identified in leaves and roots of triticale seedlings. The presence of seven different triticale phytocystatins in the tissues of vegetative and generative organs could indicate the participation of these inhibitors in the regulation of development and plant defence mechanism.

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1.73. Concomitant activation of jasmonate and cyclic AMP response pathways is required for phytoalexin synthesis after mechanical wounding of *Hippeastrum* bulbs

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Phytoalexins (PA) are antimicrobial substances synthesized by plants that accumulate at areas of wound. It was revealed that mechanical injury of *Hippeastrum* x hybr. scales leads to formation of PA, that makes the damage tissue become red. Little is known about the downstream signaling events activated by mechanical wounding. Thus, the aim of our study was to investigate the role of adenosine 3',5'-cyclic monophosphate (cAMP) in wounding-induce responses.
As a consequence of bulb wounding increase in cAMP level was observed, that reached the maximum at the 48th hours. Application of adenylyl cyclases (enzymes response for cAMP synthesis) inhibitors reduced the level of PA whereas application of adenylyl cyclase activator or pure cyclic AMP accelerated phytoalexin synthesis. These results show that cAMP accumulation was restricted to wounded tissue and was necessary to phytoalexin synthesis.
It is known that jasmonates (JA) are involved in wound signaling. In our experiment influence of JA to cAMP level was investigated. Incubation with 2-(4-isobutylphenyl)-propionic acid – jasmonate biosynthesis inhibitor decreases the level of cAMP and at the same time reduced the concentration of PA. Reverse effect was observed when coronatine (an analog of methyl-JA) was added on scales. The fact that observed elevation of cAMP occurs as a consequence of JA appearance may lead to conclusion that both compounds play a role in the mechanism of PA biosynthesis and further resistance to infection in the areas of wound.

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1.74. ROS homeostasis and biometric analysis in various tree species as tools for selection of the best species for phytoremediation of habitats polluted with heavy metals

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Soil pollution with heavy metals is a major threat to the environment. Because of their toxicity, it is necessary to search for cheap and environment-friendly methods of their immobilization. One of the possible methods is dendroremediation. However, it is crucial to select tree species that are most suitable for afforestation of such areas. In the present study, we compare the response of various species and cultivars of *Populus*, *Alnus*, and *Salix* to the stress associated with pollution of the soil collected from the buffer zone of copper works. Concentrations of heavy metals (Cd, Cr, Fe, Zn) in the polluted soil were significantly higher than in the soil collected from areas free from industrial pollution (control), and markedly exceed critical concentrations for lead and copper. Plant condition was assessed on the basis of the tolerance index, biomass production, and morphometric parameters of roots. Results of analyses of changes in concentrations of reactive oxygen species (ROS), lipid peroxidation, and levels of antioxidants, revealed differences in adaptation of the selected species and cultivars to stress conditions. Qualitative and quantitative variation was detected in the analysed parameters. The observed trends were sometimes completely reversed. Moreover, we showed that the response to the stress caused by heavy metal is much stronger in roots than in aboveground parts. This is probably due to the accumulation of Pb and Cu. Our results indicate that among the compared trees, *Alnus glutinosa* (L.) Gaertn. is most favourable for remediation of areas directly affected by copper works. It also seems that biometric analyses combined with complex biochemical investigations help to determine which species is most tolerant to phytotoxic concentrations of heavy metals in the soil.

1.75. The comparison of *HVA1* and *SRG6* genes expression profiles in seedling and heading stage in a spring barley forms during drought treatment

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Drought is considered to be the main environmental factor causing lower crop productivity, including that of barley. Drought tolerance in this crop is highly correlated with the expression of two genes: *HVA1* (*Hordeum vulgare aleurone 1*) and *SRG6* (*stress-responsive gene 6*). A research was conducted in seven cultivars of spring barley drought-treated in seedling and in heading stage. The level of *HVA1* and *SRG6* gene expression during drought stress were carried out by real time PCR method. In the present study an increase in *SRG6* gene expression in seedlings compared to the later stage was showed. Differentiation of *HVA1* gene expression between growing stages in drought was noticed.

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1.76. The expression of the late blight resistance gene *Rpi-phu1* after the pathogen challenge

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Phytophthora infestans causes potato late blight that, including yield loss and chemical protection, costs € 5.2 billion annually. Since, the first half of the twentieth century, when eleven resistance genes (*R1-11*) from *Solanum demissum* were discovered, a lot of new R-genes have been identified in wild species of plants. Among them there was also the *Rpi-phu1* gene, identified in *S. phureja* and mapped on potato chromosome IX. The *Rpi-phu1* gene was transferred into cultivated potato gene pool using a series of interspecific crosses, first at the diploid, and then tetraploid level. The aim of the ongoing experiments is to investigate expression pattern of the *Rpi-phu1* gene in the noninfected and infected plants, with different isolates of *P. infestans*. The poster presents the preliminary results of the experiments where *Rpi-phu1* plants (potato clone 04-IX-21) and susceptible plants of cv. Craigs Royal were inoculated with *P. infestans* isolates (MP324, MP828, MP1162). Before inoculation and 1, 3, 5 days after inoculation samples were taken from all plants. Relative expression of *Rpi-phu1* was measured in three biological and technical replications. The expression level of the *Rpi-phu1* gene changed after contact with pathogen. We plan to investigate the expression pattern of *Rpi-phu1* gene depending on genotype and age of plants, length of a day, and genotype of pathogen.

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1.77. Molecular cloning, characterization and expression of adenylyl cyclase (HpAC) gene from *Hippeastrum*

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It is well know that adenylyl cyclases catalyse the conversion of ATP to cAMP and pyrophosphate. The occurrence of 3'-5'-cAMP in plants has been established, however in contrast to the well documented situation in the animal kingdom, the activity and physiological role of AC in higher plants are quite obscure. The only annotated and experimentally confirmed AC in plants is a *Zea mays* pollen protein generating cAMP with a role in polarized pollen tube growth.
In the light of our interest in studying the role of cyclic AMP and the intracellular signaling processes to improve stress tolerance in *Hippeastrum*, we have cloned cDNA that represents a putative member of the adenylyl cyclase gene family in plants. Using the RACE-PCR method we isolated and characterized *HpAC1* (accession no. HM991704.1), which is 979 bp long and coding a 206 amino acids peptide with a theoretical molecular mass of 23,07 kDa. Predicted amino acid sequence of HpAC1 is highly homologous to plant ACs. qRT-PCR analysis shown that *HpAC1* mRNA was detected in all tested tissues, including vegetative and generative organs. The highest amount of *HpAC*transcript was observed in ovary and root, and the lowest in pollen. We also investigated the involvement of *HpAC1* in responses to wounding and pathogen attack of *Phoma narcissi.* The observed changes in *HpAC1* transcript level after mechanical wounding and infection suggest that adenylate cyclase can be involved in response to both abiotic and biotic stresses.

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1.78. Role of ethylene in plant survival strategy of adverse environment conditions

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Our preliminary experiments, conducted on a transgenic tobacco plants with decreased ability of ethylene synthesis due to introduction of *1-aminocyclopropane-1-carboxylate deaminase* (*ACCD*) gene, led us to conclusion, that ethylene stress might participate in defence mechanism against high irradiance via stimulation of unspecific peroxidases (class III). Moreover, our data gave evidence that interaction between ethylene and SA could be quite different under normal conditions and under stress. Under normal conditions ethylene, at least partially, may stimulate SA level, whilst under HL prevent its accumulation.
Further experiments performed on tobacco WT plants revealed that in control light regime diminution of ethylene level had no influence on the level of hydrogen peroxide production, as well as on the appearance of oxidative damage (MDA). In contrary, higher level of ethylene led to increase of peroxidase activity in associate with decrease of hydrogen peroxide production and increase of lipid peroxidation. In high light stress conditions low ethylene level caused a considerable oxidative injury. In this conditions addition of exogenous ethylene increased activity of peroxidase did not affect neither the level of hydrogen peroxidase nor lipid peroxidation. Obtained data suggest that elevated H_2O_2 level might be beneficial for plants as a part of plant strategy to prepare for survive in unfavorable conditions. Moreover, the level of ethylene seems to be negatively correlated with the level of H_2O_2, which may make a different consequences under normal and stress conditions.

1.79. Could we modify a plant response to Fe-deficiency and Fe-excess?

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Nicotianamine (NA), a non-protein amino acid synthesized by nicotianamine synthase (NAS), as a chelator of metals is a key element participating in mineral nutrition of plants and homeostasis of heavy metal ions, mainly Fe^{2+}, Fe^{3+}, $Zn^{2+}$and Cu^{2+}. Considering the role NA plays in plants, we expressed *AhNAS2* gene from *A. hallerii* under its own promoter in tomato to evaluate its potential use to improve a plant response to Fe-deficiency and/or Fe-excess. Breeding of plants able to cope with low/high metal level in the soil is a challenging task for plant science.

Transgenic and wild-type plants were exposed to iron deficiency and excess (0.1 μM and 100 μM Fe in Knop's medium, respectively), and their tolerance, as well as Fe and Zn accumulation, were evaluated. Our studies indicate that expression of *AhNAS2* under its own promoter in tomato modifies a plant response to Fe-deficiency and Fe-excess, and that this response depends on the developmental stage. Seven-day old transgenic plants exposed to low Fe displayed less symptoms of Fe-deficiency – had green leaves whereas wild-type had yellow leaves. However, when 17-day old plants grown on control medium were transferred to Fe-limiting conditions, transgenic plants appeared to be more sensitive. On the other hand, results indicate that transformation leads to better management of excess iron. Seven-day-old transgenic plants exposed to high Fe displayed no symptoms of Fe-excess and were bigger than wild type. Thus, results suggest that contribution of NA to deficiency and excess of Fe involves different mechanisms.

Examination of Fe and Zn accumulation showed that *AhNAS2* induced modifications of partitioning of both metals, specific for a given treatment. These modifications, however, do not justify alterations in a plant responses to low/high iron described above. It suggests that intracellular distribution and alteration of Fe availability account for detected phenotypes.

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1.80. Analysis of *APETALA3* gene expression in basal angiosperms: a case study - *Magnolia* L. genus

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The origin and development of flower, the characteristic structure of angiosperms, has recently became one of the most widely studied problems in modern evolutionary and molecular plant biology. The result of these studies is an ABC model, which explains how the genes regulate the identity of subsequent structures within the flower. The assumption of this model is that interaction of three classes of homeotic genes: A, B and C is sufficient to define phenotypes of flower parts: sepals, petals, stamens and carpels.
The aim of this work was to investigate the functioning of ABC model in *Magnolia* genus. The perianth of magnolia flowers is composed of three threefold symmetry whorls. Above the perianth, numerous generative parts: stamens and carpels wind up in spirals on an extended flower receptacle. It is generally accepted that the perianth is undifferentiated. The theoretical basis for it, in the context of the ABC model, is the hypothesis of shifting boundary of B class gene expression towards the outermost flower whorl. This, however, would not explain morphological differences between the most external and internal perianth whorls we have noted in some magnolia species, e.g. *Magnolia acuminata, M.hypoleuca, M.liliiflora*. Their presence suggests the absence of B class gene expression in the outer whorl. Using various techniques of molecular biology we try to address the dilemma of the presence (or absence) of B class gene expression in the outermost whorl, using *M.acuminata* as an example. In the first step a gene has been isolated and sequenced from genomic DNA, which is now confirmed to be a homologue of B class gene *APETALA3* from *A.thaliana*.

1.81. Characterization of the initial phase of heat stress response in Arabidopsis

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The initial phase of the heat stress (HS) response is aimed to prevention of the acute damage. Upon HS, transient expression of heat stress factor *HSFA2* was detected in apex and leaves, together with expression of dehydration associated transcription factor *DREB2B* (15-45 min after HS initiation). Short transient increase of stomata conductance found after temperature elevation indicated stimulation of leaf transpiration. Stomata opening is positively affected by plant hormones cytokinins (CKs). HS response was associated in leaves with a mild, temporary increase in levels of bioactive CKs, in spite of the fact that expression of genes for CK biosynthetic enzymes isopentenyl transferases (especially *IPT3*) started decreasing immediately at stress conditions. Transcripts for CK degrading enzymes - cytokinin oxidases/ dehydrogenases decreased as well, reaching the minimum after 30 min of HS.
In apex, bioactive CKs were maintained at the very early phase of the response. The expression CK biosynthetic genes was down-regulated. The activation of CK signalling was indicated by stimulation of the expression of genes for CK receptors (*AHK3, 2* and *4*), as well as for positive regulators of CK signal transduction, type-B response regulators (*ARR10* and *ARR12*), which exhibited mild elevation for the first 15 minutes. In case of negative regulators, type-A response regulators (*ARR8, ARR9*), fast decrease was observed. CK signalling exhibited the second maximum after 2 h, which might indicate plant adaptation to supraoptimal temperature.
Response in roots differed significantly depending whether only leaves or the whole plants were exposed to HS. In the former case, transient stimulation of CK biosynthesis and signalling was observed in roots after 30-60 min. Our data indicate an important physiological role of CKs in the HS response.

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1.82. *Arabidopsis arenosa* from calaminous population shows microevolutionary physiological adapation to calaminous waste heap environmental conditions

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Mining and metallurgy of polymetalic calamine ores in the northern part of Upper Silesia region of Poland since the 13th century resulted in existence of many degraded areas with high heavy metal contamination and other specific environmental condition as low water capacity of the soil, strong insolation and wind, high calcium and low nutrient content. On these habitats developed the special plant communities, typical for metal-rich soils. Plants of this habitats shows adaptations to these conditions, and at least part of these adaptations is genetically fixed. The aim of presented studies was to show the differences in growth rate, efficiency of light phase of photosynthesis and heavy metal uptake between plants from populations of *Arabidopsis* (syn. *Cardaminopsis*) *arenosa* from calaminous and non-polluted habitats. Plants from both populations were cultivated from seeds on garden soil and native heavy metal-rich substrate from calamine waste heap localized in Bukowno near Olkusz, where seeds of calaminous population has been collected, too.
Plants from calaminous population showed much better tolerance calamine soil stress conditions than plants from unpolluted population. It manifested in faster growth and better parameters of photosynthesis. Additionally, they absorbed significantly lower amounts of Zn and Pb in comparison to normal plants, when grown on calaminous substrate, whereas the opposite situation were observed on garden soil with low Zn content. These results clearly demonstrate the microevolutionary physiological changes in calaminous population of *Arabidopsis arenosa*.

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1.83. An inventory of genes encoding for nitrate transporters NRT1 in cucumber

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Nitrogen is a key limiting factor for plant growth and crop productivity. For most of the cultivated crops nitrate (NO_3^-) is the major source of nitrogen. Recently, it has been shown that the proteins responsible for nitrate uptake and distribution within plant tissues belong to three different families: nitrate transporters of NRT1 and NRT2 families and CLC channels. A few members of these three families from *Arabidopsis thaliana* have been characterized in detail showing that they ensure the proper supply and provision of nitrates to plant cell and cell organelles. However, a global view of all nitrate transporters in *A. thaliana* and their homologs in other plants is still lacking. The availability of NRT and CLC sequences in other plants is still limited due to the lack of the full genomic resources. The recent release of the cucumber genome sequence (Huang et al. 2008) has allowed us to make the first inventory of all cucumber genes encoding for NRT1 proteins. The cucumber whole-genome shotgun reads were queried with *Arabidopsis* nucleotide sequences using Blastn to select *NRT1* cucumber homologs. We have identified 13 homologs of *Arabidopsis NRT1* genes and analyzed the expression of all *CsNRT1* in different organs of cucumber at two different stages of development (1-week-old seedlings and 8-week-old flowering and fruitful plants).Two of the newly identified *CsNRT1s* were specific for flowers and fruits whereas the transcripts of remaining 11 genes were clearly detectable in vegetative tissues. The complete analysis of genomic structure and organ expression of *CsNRT1s* as well as the phylogeny and predicted topology of proteins encoded by these genes are presented in this work. The results provide a strong basis for further functional studies of cucumber *NRT1*.

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1.84. Identification and expression analysis of H^+-ATPase genes in *Cucumis sativus* L.

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Plasma membrane H^+-ATPase generating the proton gradient across outer membrane of plant cells plays a crucial role in regulation of many physiological processes. In several species, H^+-ATPase is encoded by a multigene family, in *Cucumis sativus*, only two isoforms (CsHA2 and CsHA3) have been described until now. Based on the cucumber genome sequence we identified and characterised eight novel genes encoding H^+-ATPase in this plant. The phylogenetic analysis of H^+-ATPase proteins revealed the representatives of three subfamilies among five subfamilies known in other plants. Each of these groups included a few representatives. Such an arrangement of *CsHA* sequences indicates that gene duplication occurred in cucumber similar to other plant species. The expression profile of *CsHAs* in cucumber organs during different developmental stages has confirmed that genes clustered in subfamilies I and II (*CsHA4, CsHA8, CsHA9* and *CsHA10*) shared widespread expression but with different intensity, while genes belonging to subfamily IV (*CsHA5, CsHA6, CsHA7*) were expressed only in specific parts of flower. Furthermore, distinct differences in *CsHAs* expression in roots and leaves of plant grown at various stress conditions as well as in the presence of hormones were observed.

Taken together, our data demonstrated that H^+-ATPase genes simultaneously expressed in the same organs could be probably required for the maintenance of basal physiological function, what was confirmed in unequal regulation of *CsHA* genes at transcriptional level under certain conditions. While proton pumps genes that were presented only in selected organs probably were involved in more specific physiological processes in plant. These results also indicated that H^+-ATPase gene duplication could be intended to ensure protein activity at the high level in every cell or in each environmental conditions.

1.85. Changes in chloroplastic metabolism under salinity in glicophytic and halophytic plants

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In order to investigate the mechanisms of protection against salinity operating in halophytic plants**,** we compared several components of photosynthetic electron transport (PET) in glicophytic *Arabidopsis thaliana* and halophytic *Thellungiella halophila* plants. Glicophytic and halophytic plants were irrigated for 7 days with 0**.**15 and 0**.**3 M NaCl, respectively. A significant decline of actual quantum efficiency of PSII in *A.t.* was associated with increased NPQ value. For*Thellungiella* no change in Y(II) and NPQ was detected. In control plants**,** PQ pool was considerably higher in *T.h.* than in *A.t.* Additionally, in both species PQ pool was highly reduced. Among the most prominent effects of salinity, a strong reduction in the PQ pool of *A.t* was observed. In contrast, in *T.h.* only a slight reduction of PQ pool was detected. Analysis of PSI quantum yield, Y(I), in *A.t.*revealed a similar decline due to salinity as that of Y(II). This was associated by an increased donor side limitation of P700. Whilst, in *T.h.* quantum efficiency of PSI was very high in control plants and remained unchanged after salinity treatment. No limitation from the donor side of PSI, and very slight from the acceptor side, was detected in *T.h.* leaves. Altogether, these data suggest that ability to maintain a large PQ pool and high intensity of PET around PSI may be a prerequisite for the resistance against salinity in *T.h*

1.86. Jasmonates and the red pigment formation in mechanically wounded scales of *Hippeastrum* x hybr. hort bulbs

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Wounding results not only in breaking tissue continuity, but also in opening the way for the invasion of pathogenic organisms. It is well established that plants can react to wounding and infection by inducing the formation and accumulation of defence compounds (e.g. phytoalexins). Studies revealed that various mechanically wounded organs of *Hippeastrum* produce a red pigment that prevents penetration of injured tissues by *Phoma narcissi.* The chemical nature of the red pigment is not fully determined but it probably belongs to a class of oxidized flavan(s). Because there are many data indicating that jasmonates play a crucial role in phytoalexins accumulation, we investigate their effect on formation of the red pigment in wounded scales of *Hippeastrum* x hybr. hort. bulbs. Mechanical damage of the scales of *Hippeastrum* x hybr. hort. bulbs leading to the formation of a compound that makes the wounded tissue become red is preceded by an increase in the methyl jasmonate (JA-Me) level. Application of 2-(4-isobutylphenyl)propionic acid – a jasmonate biosynthesis inhibitor – decreases the level of endogenous jasmonates and, at the same time, decreases the ability of plants to produce the red color pigment.
Our results indicate that jasmonates are involved in the response to mechanical stress in *Hippeastrum* and participate in the defence reaction consisting in the production of the red color pigment with properties similar to phytoalexins.

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1.87. Involvement of MAP kinase signaling pathway in response to hydroxyurea treatment in *Vicia faba* root meristem cells

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Cell cycle progression is regulated by many proteins that constitute sophisticated metabolic pathways. A great number of relationships between those proteins and the presence of specific sensor kinases provide response to genomic stress which includes activation of cell cycle checkpoints in order to ensure DNA integrity. Multidimensional character of these mechanisms leads not only to cell cycle arrest but also, if necessary, brings about activation of diverse genes and trigger DNA repair processes. Activation of ATM/ATR sensor kinases may be caused by various chemical agents (e.g. hydroxyurea, an inhibitor of ribonucleotide reductase) which in turn cause cell cycle arrest and trigger DNA repair processes (independently of cell cycle phase) as a result of DNA lesions.
Obtained results indicate that apart from activation of mechanisms ensuring cell cycle arrest in response to hydroxyurea treatment, global transcription activation was also observed. Changes in transcription level seem to be the effect of DNA damage, but not replication inhibition in S-phase per se. Chromatin decondensation required for transcription is correlated with the induction of histone H4 Lys-5 acetylation. By using SB 202190, an inhibitor of p38 MAP kinase, it is shown that in plants this kind of response is modulated, at least in part, by MAP kinase signaling pathway, probably p38- like protein. Performed research indicates that MAP kinases may be involved in response to DNA lesions and the potential upstream factors to p38-like protein could be ATM/ATR kinases.

1.88. Genetically encoded algorithm regulates photosynthesis, foliar hydrogen peroxide and seed yield efficiency in Arabidopsis

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In his theory on natural selection of species, Darwin proposed that all living organisms strive to transfer their species-specific traits to their progeny (Darwin 1859). Accordingly, all cellular and organismal processes are subordinated to this biological imperative. In this context, photosynthetic efficiency, growth, water use efficiency and seed yield are key traits for the plants Darwinian fitness. *EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1)*, *PAD4 (PHYTOALEXIN DEFFICIENT 4)* and their negative regulator *LSD1* (*LESION SIMULATING DISEASE 1*) were previously studied with regard to cell death dependent defence response, root hypoxia and light acclimatory response in *Arabidopsis*. Here, we demonstrate that the regulatory role of LSD1 on EDS1/PAD4 depends on growing conditions and is far less tight in the field compared to the laboratory. Moreover, our results indicate that such distant and outwardly unconnected parameters as photosystem II maximum efficiency (*F'*v/*F'*m), water use efficiency (WUE), SA/H_2O_2 cellular homeostasis, plant size and seed yield (*Y*S) are computed and regulated by the same biological nonlinear quartic function similar to the cellular automation (Peak et al. 2004, Szechyńska-Hebda et al. 2010, von Neumann and Morgenstern 1944) that depends on LSD1, EDS1 and PAD4. Using this algorithm *Arabidopsis* plants perform complicated computations that aim at optimizing cellular processes to reach the best possible plant Darwinian fitness.

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1.89. The influence of seedlings chilling on glutathione content, CAT and POD activity in broccoli plants

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Studies on the response of plants to various abiotic stresses indicate a similar mechanism for adaptation to unfavorable environmental conditions. Chilling of plants before planting results in better adaptation to a variety of stresses. The aim of three-year study was to determine the relationship between the storage of broccoli seedlings at 2°C in the dark for week one and two and the level of antioxidant constituents in them respectively, such as glutathione, activity of catalase and peroxidase. It was attempted to determine how long the reaction to low temperature treatment might last in plants.
After two weeks of seedlings chilling a significant increase of glutathione content in leaves was observed. During the vegetation in the field the effect maintained in the leaves up to the stage of flower heads forming. At harvest, the highest content of glutathione was demonstrated in broccoli flower heads from seedlings which were chilled for two weeks (in relation to those chilled for one week and non-chilled). One week of cooling did not have a definite effect on the content of glutathione in leaves. Peroxidase activity in broccoli seedlings increased in each year of study due to cooling time. In the case of catalase, changes were not so clear. In most cases after one week of chilling CAT activity decreased, after next week - an increase was observed. In harvesting term, activity of both enzymes in the leaves and flower heads were quite varied, due to the year of study.
It is still unknown if changes arising under the influence of cold stress in the early stages of plant growth lasts during vegetation in the field. Results of this experiment suggest that in the case of glutathione such effects can be expected, but not in the case of catalase and peroxidase activity.

1.90. HVA1 and *SRG6* expression in leaves of drought-treated barley (*Hordeum distichon*) may be regulated by different signals

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Drought tolerance in barley is highly correlated with the expression of two genes: *HVA1* (*Hordeum vulgare aleurone 1*) and *SRG6* (*stress-responsive gene 6*). Though their role in the mechanism of drought response in barley has been confirmed in transgenic plants, the regulation pathways of these genes' expression have not been sufficiently studied. We used four barley genotypes of different drought tolerance to establish and compare the expression profiles of *SRG6* and *HVA1* genes and to associate them with the the possible physiological and biochemical reaction signals of barley to water deficit. Both genes studied were expressed to a greater extent in drought tolerant genotypes. *HVA1* expression was induced only when the leaf water potential decreased significantly. Under conditions of soil water deficit higher ABA amounts coming from roots were connected with the increased accumulation of *SRG6* but not *HVA1* transcript in leaves. The expression of both genes appears to be down-regulated by light. H_2O_2- mediated signal from roots was not involved in the regulation of the processes studied here. In drought-treated barley plants, *SRG6* gene expression seems to be regulated mainly by ABA transported from drought-treated roots, whereas the induction of *HVA1* expression requires water deficit *in situ*.

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1.91. Symplasmic communication during androgenesis and somatic embryogenesis

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In recent years cell-to-cell communication has been under thorough investigation. Cell structures enabling this kind of communication are termed plasmodesmata. They are channels that span cell walls of neighbouring cells thus plasmalemma, cytoplasm and endoplasmic reticulum are continuous creating the symplast. Micro- and macromolecules can move through plasmodesmata which affects plant development. Regulation of shoot apical meristem (SAM) formation, hair root emergence, cotton cell elongation, secondary root creation are just a few examples of this influence. Because of significant changes that occur in plant body, an embryogenesis is an ideal system to examine correlation between symplasmic communication and differentiation of plant tissues and organs.
In the present study HPTSA, LYCH and caged fluorescein was used to monitor diffusion via the symplast in androgenic embryos of *Hordeum vulgare* L. and somatic embryos of *Arabidopsis thaliana* L. Heynh. and *Daucus carota* L. Embryos of all mentioned species are single symplasmic units at a globular stage of development. At the following stages of embryogenesis symplasmic isolation occurs. Embryos of *H. vulgare* at a transitional stage and embryos of *A. thaliana* and *D. carota* at a heart stage are divided into two symplasmic subdomains: apical and basal. It is due to lack of fluorochrome diffusion between cells of an apical and basal pole. Moreover, during further development the symplasmic isolation refers to embryo organs. Tissues also become isolated from each other. At the beginning a protodermis and a few cell layers of a ground meristem are isolated from the rest of embryo cells. The symplasmic isolation of tissues proceeds during embryogenesis, so that three subdomains can be distinguished: the protodermis, the ground meristem and the procambium.

1.92. Phenotypic plasticity in *Arabidopsis thaliana* WT plants expressed in ontogenetically changing phyllotaxis and vascular architecture

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In the analysis of phenotypic effects of mutations it is important to remember how flexible plants are in regulating their development. Phenotypic plasticity of a model plant *Arabidopsis thaliana* has been investigated with regard to the pattern of vascular connections, related to the pattern of lateral organ distribution. Two batches of plants grown in the same conditions have been used. One was Columbia ecotype, world-wide known as control in molecular genetic studies, another was the ecotype obtained from the seeds collected from the wild population found in the field near Dobroszyce, Poland. In both cases three different states of phyllotaxis have been identified: one was the main Fibonacci, another distichous and third, most exotic, was the Lucas state, first time reported for this plant. Until now the main Fibonacci pattern, was regarded as exclusive for *Arabidopsis*. In our material it was always present in vegetative rosette and usually was propagated in the main axis of inflorescence. Transition to flowering sometimes induced the emergence of ephemeric Lucas pattern. This change in phyllotaxis invariantly occurred with the reversal of initiation order. For the Columbia ecotype it was a rare situation whereas in the Dobroszyce plants it was surprisingly frequent. The distichous state was mostly present in the weak axillary shoots of the inflorescence. An analysis of accompanying vascular patterns has shown that the number of vascular sympodia in the rosette was usually 8. In the inflorescence it was lower: 5 in case of Fibonacci or 7-4 in case of Lucas pattern. In lateral branches of inflorescence it was variable from 2 to 5. Geometric size of the shoots did not reflect directly their anatomic diameter and the number of vascular bundles visible on the cross-section did not unequivocally indicate the number of sympodia. High degree of variation in phyllotaxis and in vascular architecture in *Arabidopsis* WT plants shows how careful should be the interpretation of phenotypic effects resulting presumably from mutations.

Session 2
Emerging role of plant cuticle in plant development and defense

Plenary lectures

Biosynthesis, assembly and regulation of the cutin polymer in reproductive organs

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The cuticular layer plays multiple roles in plants including the regulation of epidermal permeability and the protection against insects and UV light. It also functions in development as for example in the prevention of post-genital organ fusion and pollen-pistil interactions. Generation of cuticular components in epidermal cells involves 2 major biosynthetic pathways, namely, the synthesis of cutin monomers and aliphatic wax components. In recent years we dissected several aspects of the cuticle assembly including those related to transport, cutin polymerization and the transcriptional regulation of metabolic pathways. Members of a small clade of ABC-type transporter genes have been characterized that are involved in the transport of components from the epidermis through the plasma membrane to the cuticle construction site. The data provided evidence that these proteins function in the transport of wax and/or cutin constituents and possibly suberin (chemically similar to the cutin polymer). We showed that a member of the BAHD family of acyltransferases (termed DCR) is required for incorporation of the most abundant monomer into the polymeric structure of the Arabidopsis flower cutin. In terms of transcriptional regulation, we are conducting in-depth characterization of the *SHINE (SHN)/WAX INDUCER (WIN)* clade of transcription factors that control the cutin biosynthesis pathway. Arabidopsis plants silenced for all three *SHINE* genes were examined for alterations to gene expression using microarrays and two dozen putative target genes were identified. Taken together, this work provides insight to the molecular and metabolic basis for cuticle assembly in both vegetative and reproductive plant organs.

Cuticle, an essential component of systemic immunity in plants

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Systemic acquired resistance (SAR) is a form of immunity that provides protection against secondary infections and involves the generation of a mobile signal at the site of primary infection, which translocates to distal tissues and activates defense responses. We show that the plant cuticle is an essential component of the SAR process. Our results demonstrate that the cuticle is specifically required for the processing and/or perception of the mobile signal in distal tissues. Genetic mutants defective in cuticle formation are fully competent in generating the mobile SAR signal, but are unable to induce SAR in response to this signal. For example, mutants defective in acyl carrier protein 4 (ACP4), long chain acyl-CoA synthetases (LACS2 and LACS9), and proteins involved in cuticular wax biosynthesis or trichome formation, all contain impaired cuticles and are unable to induce SAR. Furthermore, physical removal of the cuticle in wild-type plants also compromises their SAR-inducing ability. We also show that glycerol-3-phosphate (G3P), a highly conserved primary metabolite and an essential component of plant glycerolipids, is a critical mobile inducer of SAR. Genetic mutants defective in G3P biosynthesis cannot induce SAR, but do so in response to exogenous G3P. We show that a G3P derivative is translocated to distal tissues and this requires the lipid transfer protein, DIR1. Conversely, G3P is required for the translocation of DIR1 to distal tissues, which occurs via the symplast. This and the fact that *dir1* plants accumulate reduced G3P in their petiole exudates suggest that the cooperative interaction of DIR1 and G3P orchestrates the induction of SAR in plants.

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The complex role of the cuticle in plant development and defense

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A critical step during the evolution of terrestrial plants was the development of a semi-permeable cuticle that at the interface between the plant and its environment, controls the diffusion of water, communication between plant cells of different organs and the interaction of the plant with other organisms. The cuticle is organized in distinct layers. The cuticle proper below the epicuticular wax layer consists of the aliphatic polyester cutin as main structural component as well as intracuticular waxes. The cuticular layer, found below the cuticle proper, is rich in cutin and polysaccharides and forms a continuum with the cell wall. In recent years *Arabidopsis thaliana* has been explored as model system for studying the biosynthesis of cutin and the different roles this polyester in plant development and plant-pathogen interactions. Our current understanding of the formation of cutin as well its role in organ fusion formation and resistance to the fungal pathogen *Botrytis cinerea* will be discussed.

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Poster presentations

2.1. Differences in protodermal cell wall structure in zygotic and somatic embryos of *Daucus carota* (L.) cultured on solid and in liquid media

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The ultrastructure, cuticle and distribution of pectic epitopes in outer periclinal walls of protodermal cells of *Daucus carota* zygotic and somatic embryos from solid and suspension culture were investigated. It appeared that analyzed features of the cell wall were different in all types of embryos. Lipid substances (cuticle) were present as a continuous layer only at the surface of zygotic and somatic embryos from solid medium. Somatic embryos from suspension culture were devoid of cuticle. The ultrastructure of the outer periclinal walls of protodermis of embryos was similar in zygotic and somatic embryos from solid culture. At the surface of somatic embryos fibrillar material was observed. In zygotic embryos, in cotyledons and root, pectic epitopes recognised by JIM5 antibody were observed in all cell walls. In hypocotyls of these embryos pectic epitopes were not present in the outer periclinal and anticlinal walls of the protodermis. In somatic embryos cultured on solid media distribution of pectic epitopes recognised by JIM5 was similar to that described for their zygotic counterparts. In somatic embryos produced in suspension culture, pectic epitopes recognised by JIM5 were detected in all cell walls. In the cotyledons and hypocotyls, a punctate signal was observed outside of the protodermis. Pectic epitopes recognised by JIM7 were present in all cell walls, in all embryos, independent of embryo organs. In zygotic embryos this signal was punctate while in somatic embryos from both culture systems was uniformly distributed. Additionally, in embryos from suspension cultures, a punctate signal was detected outside the surface of cotyledon and hypocotyl. These data are discussed in light of current models for zygotic and somatic embryo development.

2.2. Are *Ornithogalum umbellatum* lipotubuloids territory of cuticle synthesis?

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The site of synthesis and the way of transport of cuticle components to the epidermis surface are among the issues raising doubts and controversies. A cuticle consists of cutin composed of highly polimerised fatty acids – C16 and C18 as well as waxes containing very long fatty acids (VLCFA). Cutin forms matrix which is insoluble in fat solvents while waxes (soluble in fat solvent) saturate this matrix and form on its surface an epicuticular film which protects aerial organs against water loss. Both cuticle components are hydrophobic while they have to go through hydrophilic plasmolemma and polysaccharide wall. It is supposed that so called transporters contribute to this. Spanish scientists believe that fatty polyhydroxyacids are capable of spontaneous formation of *cutinsomes* which are spherical units constituting cuticles. They are able to cross the walls and fuse to form procuticles. Thus further research on cuticle is necessary.
The results of our previous experiments made us undertake this studies. It was observed that *O. numbellatum* lipotubuloids which synthesized lipids – incorporated ^{3}H-palmitic acid, whose presence was evidenced by autoradiographic grains, aggregated over this structure, after some time labeled the cuticular layer, and were insoluble in organic solvents similarly to cutin. Some grains leaving lipotubuloids during non-radioactive postincubation disappeared after lipid extraction. They might have been waxes. We put forward a hypothesis that lipid synthesized in lipotubuloids were the precursors of cutin and waxes. A question arises whether they become components of cuticle in lipotubuloids or whether are only the source of lipids. Our research with the use of the immunogold technique and autoradiography aim at solving this issue.

2.3. Biochemical analysis of cutin in *Arabidopsis thaliana* by FT-IR microspectrometry

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The plant cuticle is a lipidic outer extracellular matrix layer of the epidermis that covers most organs of the shoot as a protective diffusion barrier. The main structural component of the cuticle is cutin, a polyester formed of fatty acids and hydroxy fatty acids and their derivatives as well as glycerol and low amounts of phenolics. The composition of cutin on different plant species is routinely analyzed by gas chromatography/mass spectrometry (GS/MS) after depolymerisation and necessities the pooling of large quantities of material (milligram-gram scale). Therefore, only the average quantities of cutin on different organs of *Arabidopsis* are known.

Fourier transform infrared spectroscopy (FT-IR) allows the determination of the chemical composition of samples. As biological samples can be chemically complex, infrared spectra can be rather complicated. Thus, the analysis usually focuses on identifying alterations in the infrared spectra between different samples. The conjunction with optical microscopy, *i.e.* FT-IR microspectroscopy, could support biochemical analysis of plant material in micro scale: little fragment of tissue is required to obtain spectrum, biochemical information comes from predefined area of sample and no special plant material treatment is required.

Aliphatic esters, the main components of cutin layer, have got characteristic IR spectrum. The presence of the most explicit spectral features is connected with ν(C=O) at 1734 cm^{-1}, symmetric and asymmetric ν(C-H) vibrations at 2850 and 2920 cm^{-1} of fatty acid chains. The intensity of listed peaks was used to study the polymer composition of wild type *Arabidopsis* and several mutants involved in cutin formation. The results are compared to these obtained by GS/MS analyses.

2.4. Changes in triterpene content of cuticular wax layer in relation to *Vaccinium vitis-idaea* leaf age

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Triterpenes are common constituents of foliar cuticular wax, however, their content varies to a great extent not only with plant taxon but also geographic location, environmental conditions and the stage of plant development. This research examines the content and composition of triterpene compounds of chloroform-soluble cuticular waxes of young, immature, current-year leaves and old, previous-year leaves of *Vaccinium vitis-idaea* collected in Finland and Poland in August and December 2010. Qualitative and quantitative analysis was carried out using GC-MS/FID technique. Young leaves sampled in August contained comparable amounts of triterpenes, accounted for 13-14% of total mass of extracted waxes. The main groups were triterpene monols and isomeric acids, ursolic and oleanolic; however, some significant differences in triterpene profile were detected. In leaf wax of Finnish plants, triterpenols were the most abundant with fernenol as the most prevalent compound (25% of all triterpenes). In Polish plants, the major triterpene was ursolic acid (48% of all triterpenes), whilst fernenol was not detected and instead taraxasterol predominated among occurring triterpenols. In December, the level of triterpenes in Finnish plants reached 15.1% of wax mass, whereas it decreased in Polish plants to 9.7%. In old leaves, triterpene content was relatively high in August (in Finnish plants 15.4%, in Polish 10.7%) and afterwards it decreased markedly in December to 9.3% in Finnish, and 6.9% in Polish plants. Triterpenes, with their various biological activities, might protect the leaves against the pressure of biotic agents, such as insect herbivory and fungal pathogeny, which are important in summer (the latter also below the snow cover). In winter, other classes of wax lipids, like hydrophobic long-chain aliphatics, are more essential for avoiding frost damage. Thus, the observed pattern of seasonal changes of wax composition of *V. vitis-idaea* leaves may reflect the strategy of plant survival imposed by climate and environmental conditions.

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Strasburger Day – cellular level

Eduard Strasburger: founder of modern cell biology

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Eduard Strasburger is certainly the most important Polish/German botanist. Improving microscopic techniques which he studied as a student in the Julius Sachs's laboratory in Bonn, he revolutionized the understanding of nucleus and cell division (karyokinesis and cytokinesis). Even ten years after discovering division of the nucleus and formation of the cell plate, his results were criticized as artifacts produced by his new microscopic techniques, tissue fixation and staining. Nevertheless, on the basis of his results, Eduard Strasburger came to the conclusion that differences between plants and animals might be much smaller than thought at that (and even at present) time. Some of the Strasburger's central topics, the process of cytokinetic cell plate formation by vesicle trafficking (Dhonukshe et al. 2006) and the transport of water in trees just by physical forces (Zimmermann et al. 2002), are still matter of discussions and hot debates.

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Session 3
The vesicle or there and back again

Plenary lectures

At the crossroads of membrane traffic and ion transport

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Throughout the life cycle of plants, from germination to senescence, cell elongation and swelling of cells regulates a variety of important functions. Balancing turgor pressure and delivery of membrane material facilitates and fine-tunes these processes; however our understanding of the underlying mechanisms remains scant.
Work from our lab suggests that the plasma membrane SNARE SYP121 interacts with some of the Kv-like K^+-channels altering their gating behaviour. Interestingly, the domain that is involved in the binding of the channel is not the promiscuous SNARE-domain that facilitates SNARE-core-complex formation, but a short N-terminal sequence. This sequence shows homology to S/M-related binding sites which themselves are important cofactors that regulate the SNAREs ability to form SNARE-complexes that enable vesicle fusion.
In vitro and *in vivo* analysis included split-ubiquitin, bimolecular fluorescence complementation, heterologous expression and electrophysiological analyses to demonstrate physical interaction and impact on K^+ transport. Subsequent studies have since indicated a wider scope to the SNARE-channel interactions and analysis of the peptide domains essential for these interactions now offer important clues to possible mechanisms that may coordinate inorganic solute uptake with membrane expansion for cell growth and volume control.

A close look on plasma membrane dynamics

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Lateral diffusion of proteins and lipids in the plasma membrane provides the physical basis for a number of key biological processes, which rely on the formation of protein multimers or clusters such as signal transduction and exo-endocytosis.
In order to capture the dynamic nature of such processes, the distribution and mobility of membrane components need to be recorded with a spatial and temporal resolution established fluorescence microscopic techniques such as widefield or confocal microscopy cannot provide. The detection of single molecule fluorescence, in turn, which also forms the basis of some super-resolution microscopy techniques, is able to deliver the required spatio-temporal precision. The application of the single-molecule fluorescence toolkit to investigations on plant cells will be presented.
By using single-molecule microscopy as an accessory, additional quantitative information can be obtained from fluorescence images, which have been recorded on standard widefield or confocal platforms. The valuable information of local protein concentration, for example, is usually acquired, but rarely quantified, with every recorded fluorescent image. A simple calibration procedure will be presented, which allows extending the dynamic range of standard fluorescence microscopy techniques to the quasi-single molecule level.

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Regulation of cell elongation through vesicular trafficking

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As providers of structural rigidity and regulators of cell shape and growth anisotropy, cell walls are of essential importance for plant growth and development. Cell wall biosynthesis and deposition processes remain poorly known but have been shown to be linked intimately with the endomembrane system. Through vesicular trafficking, the endomembrane system directs the distribution of cell components, such as cell wall polysaccharides or synthesizing enzymes, to their target destinations via vesicle formation, relocation and fusion with target membranes. In this study, we are investigating the involvement of vesicular trafficking in cell wall formation and thus cell elongation using chemical genetics in *Arabidopsis thaliana*. We have developed high-throughput screens for selecting small molecules that alter the components of cell wall synthesis mechanisms and regulatory gene networks. The aim of this study is to identify new trafficking pathways and most likely proteins involved in cell wall biosynthesis and deposition.

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Endosomal trafficking in plant immunity

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Cell surface receptors of plant cells constitute recognition sites to detect invading pathogens and to active defenses. Arabidopsis FLS2 encodes the receptor kinase for bacterial flagellin (flg22) and is required for immunity against a broad-spectrum of potentially pathogenic bacteria. Upon flg22 perception FLS2 accumulates at plasma membrane microdomains and is internalized. Although receptor trafficking became a focus of research in the past years, there is largely nothing known about downstream molecules and regulatory components of receptor endocytosis. I will present results from our current research, which addresses the identity of the FLS2 endosome, molecular components regulating FLS2 endocytosis, and the interception of FLS2 endocytosis and flg22 signaling. These results provide good evidences for a role of late endosomes/multivesicular bodies in plant immunity. Remarkably, markers for late endosomal compartments also cluster around the haustoria, pathogen feeding structures projected inside plant cells in the interaction with filamentous *Hyaloperonospora arabidopsidis*.

To genetically dissect endosomal trafficking in plant immunity, we applied quantitative high throughput confocal imaging and identified mutants with altered levels of late endosomal compartments, as detected with the fluorescent biosensor GFP-2xFYVE. While the mutants *fel2*, *fel4* and *fel9* display a high increase in GFP-2xFYVE compartments, they are reduced in *fel5*, which occurs in a cell-type specific manner. I will present quantitative high throughput confocal imaging in plants and will discuss characterization of the *fel* mutants. These mutants are likely affected in endosome trafficking as revealed by chemical interference. Altogether, these novel membrane trafficking mutants allow us to better understand the molecular mechanisms underlying the subcellular rearrangements in plant-pathogen infections.

The vesicle or there and back again

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ER exit sites (ERES) are discrete domains of the ER characterized by local accumulations of dimeric COPII proteins. ERES and their relationship to the Golgi apparatus are fundamentally different in mammalian and higher plant cells. In higher plants, ERES and Golgi stacks are tightly associated with one another and move together as a unit over the surface of the ER. Retrograde traffic from the plant Golgi apparatus is mediated by COPI vesicles, but the location of their fusion with the ER is not known. To identify ER import sites (ERIS) we have screened for an ER-localized SNARE whose expression is not disruptive for the early secretory pathway. Fluorescently tagged SYP72, fulfills this criterion and produces discrete punctae on the ER. As markers for ERES we have used the COPII coat protein SEC24, as well as the 6 kDa viral protein (VP) of *tobacco etch virus* (Lerich et al. 2011). SYP72 and ERES/Golgi signals are of similar size, but are not spatially related to one another. However, after Golgi immobilization through latrunculin B, SYP72 and the ERES/Golgi signals show a perfect colocalization. Immunoflourescence with SYP72 antibodies confirm the distribution of endogenous SYP72. Inhibition of retrograde COPI traffic through expression of the ARF1-GTP (Q71L) mutant interrupts the cycling of p24-RFP causing it to accumulate in stationary structures which also coaligned with SYP72 signals. These observations indicate that ERIS and ERES are both located immediately adjacent to Golgi stacks, and that the SYP72 signals represent *trans*-SNARE complexes which first become visible after Golgi stacks have moved on. As a consequence, bidirectional ER-Golgi transport normally only takes place when Golgi stacks are temporarily sessile.

References:
Lerich A, Langhans M, Sturm S, Robinson DG (2011) Is the 6 kDa tobacco etch viral protein a bona fide ERES marker? *J. Exp Botany* (in press).

Supporting Agencies:
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Poster presentations

3.1. Immunolocalization and visualization of a lytic vacuole compartment in syncytia induced by cyst nematodes in Arabidopsis and tomato roots

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Plant-parasitic cyst forming nematodes are serious pests of all economically important crops. They induce a specific feeding site called a syncytium in host plant roots. Characteristic feature of the syncytium is condensation and proliferation of its cytoplasm, hypertrophy of nuclei and general increase of organelles numbers. Central vacuoles typical for differentiated plant cells become replaced by a system of numerous small vacuoles and vesicles. Very little is known about their origin and functions in syncytia. Using specific antibodies directed against γ-TIP protein and Neutral Red dye, immunolocalization and visualization of a lytic vacuole compartment was performed in syncytia. Tonoplast Intrinsic Protein (γ-TIP) is a marker for tonoplast of the lytic vacuoles whereas Neutral Red is a suitable probe for visualization of these organelles. The lytic vacuole compartment was examined in syncytia induced by *Heterodera schachtii* or *Globodera rostochiensis* in roots of *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*), respectively. Two-step immunolocalization in conjunction with fluorochrome (FITC) or colloidal gold labelling was performed. Using fluorescence and TEM microscopy γ-TIP protein was detected in membranes surrounding the most of vesicles present in syncytia induced by both nematodes. Confocal laser scanning microscopy indicated that Neutral Red was also accumulated in small syncytial vacuoles and vesicles.

3.2. Proteoliposomes as a model of aggregating chloroplasts' membrane

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Chloroplast membranes – unique assemblies of lipid, protein and pigment molecules – accommodate all light-harvesting and energy-transducing functions. In higher plants thylakoid membranes are differentiated into grana and stroma regions, also known as stacked and non-stacked regions, respectively. Various agents, including stress agents, change the proportion of stacked regions. The mechanism of membrane aggregation are not yet well-known. A good model of origination of the stacked regions is modulation by concentration of magnesium ions.

An attempt was made to characterize aggregation of artificial systems of liposomes and to compare it with the native thylakoid membranes. Proteoliposomes containing chloroplast membranes' lipids (MGDG, DGDG, PG) and incorporated LHCII (isolated from spinach) were used as a semi-lamellar system for a multi-method study by infrared spectroscopy – FTIR, confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) imaging. The topographic images obtained by means of AFM microscopy as well as 3D CLSM images of aggregated, in presence of magnesium ions, proteoliposomes revealed structures very similar to the grana membranes of higher plants. Spectroscopic data showed the type of protein-protein and lipid-protein interactions during the stacking.

Examination of the type of interactions observed in an artificial, less complicated system, makes mechanisms of specific thylakoid membrane *in vivo* organization foreseeable.

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3.3. Unraveling the exocyst complex in plant cells

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Exocytosis is a fundamental process in all eukaryotic cells and is indispensible for cell growth and development that require delivery of components to the plasma membrane or to the extracellular environment. Exocytosis is localized process that occur preferentially at specific domains. Exocytotic vesicles are tethered at the plasma membrane by the octameric exocyst complex. All proteins in the complex have been conserved during evolution and and exocyst complex is functional in plants (Hála et al. 2008). Here we show localization of exocyst subunits in living plant cells and unravel some of protein-protein interactions between exocyst subunits using microscopy approaches.

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Hála M, Cole R, Synek L, Drdova E, Pecenkova T, Nordheim A, Lamkemeyer T, Madlung J, Hochholdinger F, Fowler JE, Žárský V (2008) An exocyst complex functions in plant cell growth in Arabidopsis and tobacco. Plant Cell 20: 1330-1345.

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3.4. Expression and localization of calreticulin during pollen-pistil interactions in *Petunia hybrida* – genetic and immunocytochemical studies

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Calreticulin (CRT) is a highly conserved Ca^{2+}-binding reticuloplasmin that is involved in Ca^{2+} storage and quality control of N-glycosylated proteins in the ER. The plant CRT shares the same molecular structure identified for its animal homologue and seems to have similar functional properties. Based on the patterns of CRT gene expression and protein localization, there are several proposed functions for plant CRT. The best documented are for roles in cell proliferation, signal transduction, regeneration, gravistimulation, and stress/pathogen attack response. CRT has also been suggested to be involved in reproductive events in plants. In the studies reported here, the expression pattern of CRT was determined in relation to pollination and subsequent stages of pollen germination and tubes growth through the *Petunia* pistil. The expression profile of CRT was obtained by Northern blot analysis of total RNA with the full-length *Petunia* CRT cDNA and by protein blotting with the anti-maize CRT antiserum. The results showed highest levels of CRT transcripts and protein in mature unpollinated pistils and during first few hours after pollination, when pollen germinated and tubes grew throughout the stigma. At that time, an exudate was detected on the stigma surface and in the stigma glandular zone. Immunolocalization of CRT confirmed the presence of CRT in the stigma and in germinating pollen and elongating tubes. Accumulation of the protein in these cells was correlated with the increased level of exchangeable Ca^{2+}. The obtained results indicate that CRT could regulate secretory events and Ca^{2+} sequestration during pollen-pistil interactions.

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3.5. A chemical genomic approach to identify genes implicated in vesicular trafficking of cell wall biosynthetic enzymes

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The chemical genomics approach uses small molecules to modify or disrupt the function of specific proteins. It provides a novel avenue for rapid and effective dissection of biological mechanisms and gene networks in ways not feasible with mutation-based approaches. Vesicular trafficking is an essential cellular process driving the distribution of cargos within cells and maintaining subcellular structure. It basically underlies all cellular functions and can be modulated by both developmental and environmental signals. Moreover, cell wall plays a central role in plant development such as the regulation and control of growth anisotropy and cell shape. Understanding cellulose synthesis and deposition is therefore essential for understanding plant growth, development and evolution. The biosynthesis and deposition of cell wall component rely on vesicular trafficking, which involves vesicle formation, transport and fusion with target membranes. Despite the tremendous importance of this process, our knowledge on the underlying mechanism and the regulatory networks is very limited. In this study, we have developed a pollen-based high-throughput screen to select chemicals as inhibitors of pollen germination, a process absolutely dependent on intact vesicular trafficking. Using cellulose biosynthesis enzyme GFP-tagged, on a secondary screen, we are dissecting a network of genes controlling vesicle trafficking involved in cell wall biosynthesis. By this approach, we expect to obtain novel regulators of cellulose biosynthesis, which will be instrumental to further dissect mechanism of plant development.

3.6. Around the plant exocyst

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Exocyst is heterooctameric protein complex involved in vesicle tethering near the plasma membrane during the process of exocytosis. It is linking vesicles movement along cytoskeleton with their fusion with plasma membrane done by tSNARE proteins. Existence of exocyst is common for all eukaryotes however only plants possess more than one paralogs of some subunits. It is believed that abundance of the exocyst genes is associated with its more diverse functions in plant cells. Functionality of exocyst is controlled by plant small GTPases (e.g. from ROP family) and probably some other factors.
Our goal is to confirm known interactions of exocyst complex with other proteins from exocytic pathway and to find upstream and downstream partners of the exocyst complex in plants.

3.7. Capacity for somatic embryogenesis in culture of *Arabidopsis thaliana pin2* and *pin7* mutants

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Somatic embryogenesis (SE) is an obvious experimental evidence of totipotency and is used as a model system for studying the mechanism of de-differentiation and re-differentiation of plant cells. In our studies SE was induced during in vitro culture of immature (in late cotyledonary stage) zygotic embryos. We observed a few somatic embryos formed in direct way, but in general somatic embryos were formed from callus tissue through an indirect route, so we investigated auxin distribution in embryonic callus, since auxin local maxima is necessary for *Arabidopsis thaliana* embryogenesis. To achieve this aim, we used transgenic plants expressing reporter genes (*GUS*, *GFP*) driven by a synthetic *DR5* promoter, an auxin response promoter. To achieve auxin gradient, PIN-efflux system is required. PIN proteins are expressed in different parts of the plant and are almost universally required for all aspects of auxin-related plant development, including embryogenesis. During zygotic embryogenesis there are expressed PIN1, 3, 4, 7 proteins. In our research, we investigated the capacity for SE of *eir1* (Columbia), *agr1* (Landsberg erecta), which are *pin2* mutants, and *pin7* (Columbia). They displayed drastic inhibition of SE, although *eir1* and *agr1* had an increased ability to produce callus, as compared with wild type plants. These results provide evidence, that besides their key role in controlling many different aspects of *Arabidopsis thaliana* zygotic embryogenesis, *PIN2* and *PIN7* genes are also essential for in vitro somatic embryogenesis induction.

3.8. Biochemical approaches to reveal the functions of presenilins in *Arabidopsis thaliana*

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Intramembrane proteolysis is an ancient mechanism to control cell metabolism, differentiation and development in diverse organisms, from bacteria to higher plants and humans. Presenilin (PS) forms a catalytic core of γ-secretase complex. The proteolytic activity of the complex is crucial in Alzheimer disease etiology. Many substrates of human presenilins are identified, for example Amyloid Precursor Protein, Notch receptor, cadherins. The other proteins of the complex are: nicastrin (NCT), anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2). All complex components contain at least one transmembrane domain.

Although all γ-secrestase components are present in plant genomes, there is little information about plants presenilins and even complex existence. The only one published paper ascribes for presenilin the role which is protease-independent, and related to cytoskeletal organization in *Physcomitrella patens*. What is interesting, the amino acid sequences of animal and plant presenilins are similar, but the γ-secrestase substrates identified in *Homo sapiens* are not similar to any plants proteins.

Biochemical and microscopic approaches will be used to find out the true function of plant presenilins. To elucidate the putative similarity in proteolytic activities of human and *Arabidopsis thaliana* γ-secretase complexes, the recombinant form of human γ-secrestase substrates, C100 and N100, will be produced and utilized for biochemical assays using membrane fractions derived from wild type *Arabidopsis* and *psn1*/*psn2* mutants devoid of presenilins. The pull-down technique will enable us to identify potential substrates, interacting proteins and to confirm previous results on the interactions within the complex. PEN2:GFP from plants stably overexpressing this construct will be used as a bait. Newly identified protein-protein interaction would be verified by FRET FLIM. Subcellular localization of human presenilins fused with fluorescent proteins will be investigated in *Arabidopsis*. In this way evolutionary conservation of presenilins trafficking and similarity in subcellular localization will be checked.

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3.9. Structural and functional analysis of γ-secretase complex in *Arabidopsis thaliana*

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Presenilin is an intramembrane protease that handles proteolysis inside the hydrophobic environment of diverse membranes, including plasma membrane, ER and Golgi. It was discovered in humans by linkage with Alzheimer disease. In animals, presenilin is a nine-pass transmembrane catalytic core of a multiprotein complex - γ-secretase. The complex contains three additional proteins: nicastrin, PEN-2 and APH-1. Complex seems to be involved in many processes, like amyloid processing, NOTCH signaling, cell adhesion or cytoskeleton dynamics. In plants, the function of presenilin is completely unknown. The only research made on *Physcomitrella patens* indicated the putative role of presenilin in cytoskeleton organization. What is even more interesting this function seemed to be independent from protease activity.
To identify potential functions of presenilins in plants, the functional genomics study was initiated. Here, the presence of homologues of γ-secretase components in *Arabidopsis thaliana* and their subcellular localization were investigated. Sequence analysis indicates that all components of γ-secretase complex are present in *Arabidopsis* genome. Number and organization of transmembrane helices in plant homologues was analyzed and was shown to be conserved for all γ-secretase subunits. For the subcellular localization of γ-secretase subunits, genetic constructs were made with gene of interest fused with fluorescent protein (GFP or RFP). Constructs were introduced to *Arabidopsis* protoplasts and leaves. Co-localization analysis with marker lines was used to confirm localization of particular complex subunits. To generate data of the complex function the γ-secretase components were co-localized with each other. In a series of experiments, attempts are made to show that γ-secterase complex is present in plants and to demonstrate its potential interacting partners. It will give the possibility to compare function of this unique protease in both plant and animal models.

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3.10. Long-distance symplasmic transport in wood – underestimated role of xylem parenchyma cells

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Long-distance transport in angiosperms is proceeded in two different pathways. The majority of the molecules, such as water and solutes is transported apoplasmically via unliving tracheary elements. The second route is performed symplasmically via plasmodesmata of living axial and radial parenchyma cells. Symplasmic transport is less-intensive but it seems to be more precisely controlled and specialized than apoplasmic. Long-distance symplasmic pathways have been investigated in dormant and active cambium of 2-4 year-old branches of *Acer pseudoplatanus*. Several types of symplasmic tracers differing in molecular weight were applied through the vascular system using various loading methods. Tracers firstly moved in the apoplast, where were especially visible in the vessels. Then tracers were transported to the paratracheary parenchyma cells abutting the vessels, probably via endocytosis. After getting into the symplast, tracers were spreading between xylem parenchyma cells, firstly in the axial direction, and then also radially, via xylem rays to the cambium and phloem regions. Tracers of low molecular weight freely moved in the symplast of the wood. Contrary, transport of larger molecules (about 4kDa) was precisely controlled. Tracers of high molecular weight were retained in the apoplast. Their distribution in the symplast was restricted mainly to the paratracheary parenchyma cells, however in few cases they were also visible in the symplast of xylem rays. Based on these results, the model of long-distance symplasmic transport in angiosperm wood, its main direction and limitations, is proposed. Specifically, this model stresses the possible role of paratracheary parenchyma cells and the significance of endocytosis in the molecules transfer from apoplast to symplast.

3.11. Calreticulin and its transcripts synthesis during pollen germination and pollen tube elongation in *Petunia hybrida* – fluorescent *in situ* hybridization and immunocytochemical studies

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In angiosperm sexual reproduction, pollen tubes elongate from the stigma through the stylar transmitting tract to the ovary to deliver non-motile male gametes for fertilization. Investigations performed since the 1960s have documented that Ca^{2+} play a fundamental role in pollen germination and tube growth, but the mechanism of regulation Ca^{2+} homeostasis in the male gametophyte is still unknown. Calreticulin (CRT) is a highly conserved Ca^{2+}-binding protein that is involved in Ca^{2+} storage and molecular chaperoning in the ER of eukaryotic cells. In all animal and plant cells where it has been investigated, CRT has been found in the ER and its expression level appears to be related to the abundance of the ER membranes. Consistent with a role of CRT as a Ca^{2+}-binding/buffering protein, there are some indications that this protein may be involved in pollen germination and tube elongation. The *in vitro* studies reported here confirmed the presence of CRT mRNA and protein in *Petunia hybrida* pollen and pollen tubes. The strongest signals detected were associated with aperture regions of germinating pollen and sub-apical zone and peripheral cytoplasm of elongated tubes. The results have also revealed a co-localization of two investigated molecules with 18S rRNA at the cells' regions enriched in rough ER (RER). Therefore, the Ca^{2+}-binding/buffering activity of CRT may be involved in modulation of Ca^{2+} homeostasis which seems to be critical for pollen germination and polar tip-growth of the pollen tubes. Furthermore, data presented here suggests that the RER is a site of CRT translation.

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3.12. Protein-lipid interactions in membrane trafficking in the plant cell

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Most of the proteins which are implicated in the process of endocytosis have the possibility to interact with phosphoinositides (PIPs) localized in various membranes of the cell. In the plant cell diverse subcellular compartments are equipped with the specific PIPs e.g. PtdIns3P is present in the membrane of endosomes, PtdIns(4,5)P_2 in the plasma membrane, and PtdIns(4)P_2 in the plasma membrane or Golgi apparatus.
The goal: To assess the PIPs-binding properties of selected six *Arabidopsis thaliana* proteins, potentially implicated in the process of endocytosis.
Results: Using fat blot procedure, it was found that domains responsible for PIPs binding (PX, PH, FYVE, ENTH) of the selected proteins, preferentially bound the following PIPs:
-FYVE (Praf 1; At1g65920)- PtdIns4P and with less affinity Ptdns5P and PtnIns3P
-FYVE (Praf 4; At1g76950)- PtdIns4P and with less affinity Ptdns5P and PtnIns3P
-PH (Praf4; At1g76950)– PtdIns(3,5)P_2, PtdIns4P, and with less affinity Ptdns5P, PtnIns3P, PtdIns(4,5)P_2
-ENTH (SlaA; At4g02650) - PtdIns4P, and with less affinity PtdIns(3,5)P_2 and Ptdns5P
-PX (PXS; At4g32160)- PtnIns3P
-PX(PXX; At1g15240)- binding without specificity
-PX(PXC; At3g15920) - PtnIns3P, PtdIns4P
Conclusions:The results indicate clearly that all of tested proteins are able to interact with phosphoinositides. Most of the proteins showed the biggest affinity to PtdIns4P what can suggest their potential ability to interact with plasma membrane or Golgi apparatus. FYVE domain from Praf 1 and Praf4 preferentially bound PtdIns4P, and this result questions the overall opinion that FYVE domain specifically binds PtnIns3P!

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Session 4
Plant cell wall and adaptations to biotic and abiotic stresses

Plenary lectures

Pectin in defense against pathogens

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The majority of fungi and bacteria need to breach the plant cell wall to gain access to plant tissues and, once inside, to release nutrients to sustain their growth during the invasion process. The extensive degradation of the cell wall is a typical feature of necrotrophs that secrete high quantities of cell wall degrading enzymes (CWDEs). The correlation between cell wall disassembly, CWDEs and disease symptoms has been well defined and, in general, most virulence-associated CWDEs are involved in pectin digestion. Pectin is present in the primary cell wall and intercellular spaces of higher plants. Due to its cohesive and interacting properties pectin is critical for tissue integrity, porosity and accessibility to CWDEs. Enzymatic depolymerisation of pectin weakens the cell wall and exposes other polymers to degrading cellulases and hemicellulases. Pectin is synthesized in the Golgi and released *in muro* as a highly methylesterified form and soon thereafter de-esterified by pectin methylesterase (PME). De-esterification is the pre-requisite for subsequent pectin degradation by pectic enzymes such as polygalacturonases and pectate lyases. PMEs have also been shown to play a role in plant response to fungal and bacterial pathogens, nematodes as well as in the systemic spread of tobacco mosaic virus. PME activity is regulated by specific proteinaceous inhibitors (PMEIs) that were discovered in kiwi fruit and subsequently identified in Arabidopsis, pepper, broccoli and wheat. We have stably over-expressed PMEI in plants and demonstrated that it causes a decrease of PME activity and a subsequent increase of pectin methylesterification. This limits plant diseases caused by fungal and bacterial pathogens.

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Plant innate immunity at the cell wall and beyond

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Plant cell walls constitute the first barrier that some types of pathogens, such as necrotrophic fungi, must overcome to colonise plant tissues. The traditional view of the cell wall as a passive barrier has evolved to a new concept that considers the wall as a dynamic structure that regulates both constitutive and inducible plant defence responses. The activation of plant innate immune system is triggered by microbe-associated molecular patterns (MAMPs) from the pathogens, but also can be regulated by damaged-associated molecular patterns (DAMPs) that are molecules released from plant cell walls upon pathogen infection. In line with this putative function of the cell wall in innate immunity, we have identified novel regulators (ELK2, AGB1 and ER (Llorente et al., 2005, Sánchez-Rodríguez et al., 2009) of *Arabidopsis* resistance to necrotrophic fungi that may also be involved in the control of cell wall integrity/architecture. To further characterize the function of cell wall on the regulation of immune responses we have performed a biased resistance screening of 100 putative/ characterized primary/secondary *Arabidopsis* cell wall mutants. In this screening we have identified 20 mutants with altered susceptibility/resistance, compared to wild type plants, to at least one of the following pathogens: *Plectosphaerella cucumerina* (necrotrophic fungi), *Ralstonia solanacearum* (vascular bacterium) and *Hyaloperonospora arabidopsidis (*biotrophic oomycete). Expression analyses of immune response genes in the selected mutants have revealed the complexity of the regulation of the defense responses in these mutants. These data together with those obtained from the characterization of the cell wall structure/ composition of the selected mutants suggest a putative interconnection between cell wall structure/composition and resistance/susceptibility to pathogens. These results will be used to build a cell wall topology map correlating specific wall modifications with plant resistance to particular type of pathogens.

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Oral presentation

Vesicle transport and cell wall remodelling in plant cells response to Pb

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Tip growing protonemata of *Funaria hygrometrica* (Hedw.) were used as model for studying effects of Pb exposure (1000µMPb, 4h) on plant CWs. The experiments showed that plant CW was markedly remodelled. The most striking difference was the appearance CW thickenings (CWT) containing high amount of low-esterified pectins (up 40% - JIM5 epitope; JIM5-P) which were absent from the tip CW of control protonemata and are known as the compound able to bind Pb^{2+}. In fact extremely large and numerous Pb deposits were observed within CWT. Hence, CWTs appear to be a very important repository for Pb^{2+} in protonemata cells. Recent experiments with poplar seedlings (*Populus tremula x P. tremuloides*) treated with Pb, at similar concentration and exposure time as *Funaria*, showed an analogous reaction. In the root tip cells of poplar seedlings CWTs occurred. They were abundant in JIM5-P and contained numerous, large Pb deposits.
What could be the intercellular mechanism of CWTs formation? Both, in *Funaria* protonemata and in *Populus* roots, together with CWTs formation the intensive vesicle transport of JIM5-P and Pb was observed. The Vs carrying these two compounds were accumulated around the CWTs - almost certainly participating in CWTs formation. The experiments with Brefeldine A, carried out in *Funaria*, showed that marked amount of JIM5-P was intensively internalized from existing CWs. Together with JIM5-P internalization - Pb also entered the vesicles.
Taken these facts together we can conclude that in response to Pb plant CW are actively and intensively remodeled what results in the CWTs formation. Abundant in JIM5-P CWTs are able to accumulate especially large and numerous Pb deposits. Thus, CWTs seems to be a special, additional plant cell compartment for Pb sequestration. Hence, such a plant cell response to Pb could be a step of more general tolerance strategy to this metal.

Poster presentations

4.1. Detection of cellulase activity in the secondary cell walls of interfascicular fibers in Arabidopsis

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Plant cellulases are the enzymes active on non-crystalline cellulose and sometimes also on other glycans. They are either secreted enzymes involved in cell wall restructuring or they may be associated with the plasma membrane, possibly playing an important role in the cellulose biosynthesis. Their function in the cell wall is poorly understood. Direct *in situ* analysis of cellulase activity in the cell wall is difficult because of the lack of specific substrates. Therefore, we tested three resorufin- labeled substrates: Glc-Res, Glc_2-Res and TCB-Res in order to verify whether they can be used for *in situ* localization of the cellulase activity in cell walls. These substrates give a fluorescent signal only after the hydrolysis of the bonds between glucose and resorufin (Ibatullin et al. 2009). Therefore we can observe the enzyme activity not only *in situ* but also in real-time. Experiments were conducted on cross sections through the basal parts of the inflorescence shoots of Arabidopsis, in the region of the interfascicular fibers in the wild type and in cellulase mutants. The obtained results indicated that two of the compounds: Glc2-Res and TCB-Res, were substrates for cellulases, the latter one being more specific because it is not, or to much lesser extent, hydrolyzed by other glycosidases. Furthermore, our experiments showed that the activity of cellulases is specifically localized in the secondary cell walls, detected as the fluorescent signal forming a ring at the inner side of the wall close to the plasma membrane. These substrates may find wide application in studies of the structure and modifications of the cell walls.

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4.2. Photoassimilate translocation in nitrogen-fixing root nodules of *Medicago truncatula*

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In higher plants, sucrose, major transport from photoassimilated carbon, is transported from source organs to sink organs via the phloem. Root nodules induced by nitrogen-fixing bacteria represent strong facultative sinks. The process of biological nitrogen fixation requires a high level of sucrose supply. Phloem unloading and post-phloem transport of sugars can proceed symplastically via plasmodesmata or apoplastically via plasma membrane sugar transporters. It is not clear which kind of route – symplastic or apoplastic in the cells of indeterminate root nodules is in favour. It is already known that during nodule organogenesis the symplastic connection between plant phloem and nodule initials is created.
To understand how sucrose is transported and incorporated into metabolism in nodules of *Medicago truncatula*, 6(5)carboxyfluorescein diacetate solution was introduced to the abraded surface of the basal leaflet. 6(5)carboxyfluorescein diacetate (6(5)CFDA) is a phloem-mobile tracer and it is widely in use to study symplastic continuity in leaves and stems. It presumes to monitor sap translocation in real time, during short- or long-term experiments.
Freehand cross- and longitudinal-sections of nodules were prepared and observed with a confocal laser scanning microscope. Firstly, CF-associated fluorescence appeared in uninfected cells. After 2hr fluorescence in infected cells was detected. Autofluorescence of uninfected cells was also revealed. The results indicate that pathway flow of assimilates between uninfected and infected cells in nodules of *Medicago truncatula* is considered to be symplastic. Uninfected cells may mediate as a pathway to the infected cells.

4.3. Small molecules in the pre-invasive defence of model Brassicaceae species

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One of the evolutionary conserved responses of flowering plants to pathogen attack involves biosynthesis and secretion of secondary metabolites. Model plant *Arabidopsis thaliana* accumulates upon infection tryptophan derived indole-type metabolites, including cell wall bound indole-3-carboxylic acids and soluble phytoalexin camalexin. Our recent study revealed a pathogen triggered pathway for metabolism and secretion of tryptophan-derived indole glucosinolates, which is critical for pre-invasive defence against a number of fungal and oomycete pathogens. In this study we investigate the conservation and diversification of the pathogen-inducible tryptophan-derived metabolism in close and distant *A. thaliana* relatives by metabolic profiling. We substantiate the observed species-specific metabolic patterns by the presence or absence of candidate ortholog genes encoding enzymes involved in tryptophan metabolism in accessible genomes of *A. thaliana* relatives. Our metabolic survey reveals a surprising conservation of the pathogen-triggered IG metabolic and secretory pathway between the tested plant species, suggesting an ancient and important function of this metabolic branch in Brassicaceae pre-invasive defence responses. In contrast, I3CA and camalexin biosyntheses appear to be clade-specific innovations within the conserved framework of pathogen-inducible tryptophan metabolism and represent relatively recent manifestations of the plant-pathogen arms race.

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4.4. A correlative study of H_2O_2 generation induced by copper and mercury observed under light, electron and confocal laser scanning microscopy

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Copper is redox active metal which in opposition to mercury is an essential micronutrient for plants. Excess of copper is cytotoxic, as it participates in ROS generation via Fenton-type reaction. It is widely known that mercury triggers disturbances in cellular metabolism but its influence on ROS generation is poorly understood. We used root nodules as a model system, because this organ has high level of metabolic activity and cell differentiation in the degree of development and function.

The present work describes changes in H_2O_2 accumulation in response to monthly stress caused by copper (16 mg/l $CuCl_2$) or mercury (6 mg/l $HgCl_2$) in *Medicago truncatula* root nodules. H_2O_2 accumulation viewed with a light microscopy was detected by the use of diaminobenzidine (DAB). Our results indicate that H_2O_2 accumulates mainly in meristem, young symbiotic tissue and around vascular bundles after Cu^{2+}-treatment, and in cortex and young symbiotic tissue under Hg^{2+}-treatment.

Electron microscopy observations with the use of $CrCl_3$ show H_2O_2 accumulation after Cu^{2+}-treatment in walls of infection threads, in cell walls and peribacteroid membranes. In contrast to the Cu^{2+}, Hg^{2+}-treatment results in distinct H_2O_2 accumulation within meristematic cell walls, which is not indicated by DAB- method and subsequent light microscopy observation.

Dichlorofluorescein diacetate was used as a probe for the intracellular localization of H_2O_2 with a confocal laser scanning system. The main result of this observations is increased accumulation of H_2O_2 in response to Hg^{2+}-treatment in the part of bacteroid tissue located in the neighbourhood of nodule cortex. So, our results revealed different patterns of H_2O_2 accumulation in root nodules in response to copper or mercury-treatment.

4.5. The effect of cadmium ions on the organization of microtubule cytoskeleton in roots of soybean seedlings

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The microtubule cytoskeleton is a structure, which takes part in most important processes in plant such as growth and cell division. However, still little is known about response of microtubule cytoskeleton to stress factors, particularly to heavy metals including cadmium. In the present study, the growth rate and viability measured by triphenyltetrazolium chloride (TTC) reduction assay in roots of soybean seedlings (*Glycine max* L. cv. Navico) were investigated. The obtained results demonstrate inhibition effect of cadmium ions on root growth and viability in range of 20-200μM Cd^{2+} after 48h treatment. The effect of cadmium ions on the microtubule cytoskeleton organization and γ-tubulin distribution was detected by immunocytochemical methods with monoclonal (α-tubulin) and polyclonal antibodies (γ-tubulin). Cadmium exposure resulted in significant reduction of mitotic activity of root cells treated with increasing concentrations of metal ions. Moreover, the organization of microtubule arrays were severely disturbed in interphase cells as well as cells during division, especially in the root cortex area. The observed changes were connected with significant decrease in the levels of both α-tubulin and γ-tubulin as revealed by Western blot analysis. The studies will be continued to recognize mechanisms responsible for microtubule cytoskeleton disturbances in soybean roots exposed to cadmium ions.

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4.6. Changes in cell wall composition and gene expression during somatic embryogenesis of *Arabidopsis thaliana*

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Somatic embryogenesis (SE) provides a good system for the investigation of processes by which somatic cells change their fate. The cell wall is a highly dynamic structure that is constantly remodelled during cell growth, division and differentiation. Many of the molecular markers of SE as well as of changes, which correspond with the cell differentiation/dedifferentiation, have been found in the cell wall. The aim of the studies was to describe spatio-temporal patterns of different genes expression and of the pectin, AGP and LTP epitopes distribution during SE in order to answer the question if there is any correlation of these patterns with changes in the cell fates.

Analysis was performed on explants of *Arabidopsis thaliana* (L.) Heynh., Columbia ecotype and transgenic lines: *LTP1::GUS* and *FAC1::GFP-GUS*. Explants of different days from a 21-day culture were taken for the histological and immunohistological analysis. The following antibodies were used to detect various epitopes of the cell wall components: JIM5, JIM7, LM19, LM5 for the pectin epitopes, JIM4, JIM8, LM2, JIM16 for the AGP epitopes and atLTP1 for the LTP epitopes. Gene expression was analyzed with the use of the standard method for GUS activity detection.

The observations proved that the expression of the analyzed genes changed both spatially and temporally during SE and the higher expression was present in the cells engaged in SE. Moreover, the obtained results provide evidence that in the SE system of *Arabidopsis thaliana* the events associated with the changes of cell fates include: a) appearance of AGP, pectin and LTP epitopes in explant cell walls and cytoplasm, and b) changes in the composition of the cell walls correlated with different developmental stages of cells.

4.7. Appearance of cell-wall associated red pigment(s) in stressed callus cells of *Mammillaria multiceps* (Cactaceae)

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Cell walls of higher plants contain, besides the major components (cellulose, hemicellulose, pectin), a large amount of other substances, whose profile varies depending on the conditions. Meanwhile, findings of colored compounds in the cell walls of vascular plants are uncommon, whereas bryophytes are known to accumulate pigments in this site.

We have been observed the appearance of bright reddish pigment(s) in callus cells of *Mammillaria multiceps* (Cactaceae). The pigment(s) appeared in response to stress, both biotic (a fungal invasion) and abiotic one (a transfer to liquid media/fresh solid media– a mechanical and/or an osmotic stress). A light microscopic examination showed the pigment(s) to be located in the cell wall. A variety of solvents of different polarity (water, alcohol, acetone, chloroform, toluene, hexane etc.) failed to extract the substance(s). However, it can be easily washed off from the cell walls by water-saturated phenol, dimethyl sulphoxide and dimethylformamide.

The chemical nature of the pigment(s) remains unknown. Some recently discovered in cell walls of vascular plants pigments related to anthocyanidins or anthocyanidin-like compounds. However, as generally believed, species of order Caryophyllales (including cacti) cannot produce this class of pigments. We hypothesize that the pigment(s) may belong to the poorly studied class of natural polymeric substances – phlobaphenes. Phlobaphenes are considered as red water-insoluble polymeric flavonoids and they, as believed, can accumulate in the cell wall. Moreover, the similar accumulation of pigments in a cell wall in response to fungal disease was observed earlier for sorghum mesocotyl; this species is known to produce phlobaphenes therewith.

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4.8. Modifications of sugar metabolism in response to leaf wounding in Arabidopsis

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Wounding is a common stress, which could be caused by pathogens, wind and hail. Defence mechanisms include local or systemic response and genes induction, which change plant's metabolism (León et al. 2001). Plant's response includes transduction of signals *via* e.g. oligosaccharides, systemin, jasmonic acid and ethylene. Wounding induces respiration, affects carbohydrates metabolism (Łukaszuk et al. 2010, Lafta and Fugate 2011). The aim of study was to determine the influence of mechanical wounding on carbohydrates content and activity of key enzymes in sugar metabolism.
Arabidopsis thaliana L., wt plants and hormonal mutants (*ups 1-1*, *rcd1-1, ein 2,*seeds supplied by TAIR), were grown 4-6 weeks in growth chamber. Measurements were made in 2 and 24 hours after injury of leaves. Total soluble sugars, sucrose, glucose and starch content decreased in response to wounding (after 4 w.); glucose content increased in plants cultured 5 - 6 weeks. Activity of sucrose synthase and invertases: cell wall, vacuolar and cytoplasmic was dependent on age of wounded plants. Activity of cell wall invertase increased in 24 h after leaves injury, however activity of sucrose synthase decreased.
Results indicate important role of sugar metabolism enzymes in plant's response to mechanical wounding. Sucrose after hydrolysis could play an important role as a substrate in e.g. cell wall synthesis as well as molecule in signal transduction.

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4.9. The mechanism of wavy cell wall development in pavement cells of camphor tree *Cinnamomum camphora* T. Ness & Eberm.

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Cinnamomum camphora exhibits high degree of phenotypic plasticity especially in shaping its epidermal cells. The expansion of leaf blade surface and the development of vascular tissue is coupled with increasing undulation of epidermal cell walls. Different leaf blades exhibit varying degrees of cell wall undulation, which might be the light dependent effect. Epidermal phenotype is also influenced by the signals coming out of the vascular tissue because the shape of epidermal cells positioned above the veins is different than that of the cells located above the mesophyll.

Two basic types of growth: isometric (diffuse) and multifocal (differential) often follow one another when regularly spaced new centers of tip growth emerge during cell expansion. In the result the initially straight walls become undulated and the isodiametric cells become puzzle-shaped.

Contrary to free living cells where differential expansion of some parts of the cell wall is possible because some other, intervening parts are constricted, the development of lobed epidermal cells poses challenging question of simultaneous expansion of two adjacent walls of two neighboring cells. Curving inward, concave surface of one of them represents bulging outward, convex surface of its neighbor in exactly the same location. Both walls attached one to another have to expand simultaneously. There is no possibility that one is restricted and the other free to expand as suggested by some authors based on distribution of diffuse versus bundled form of F-actin across the wall. Our studies confirm different state of actin on both sides of the lobe. Immunocytochemical analysis of distribution of pectin and xyloglucans in epidermal cell walls is under way.

4.10. Effect of adaptogenic preparations on H^+-ATPase function in plasmalema and tonoplast of corn root cells under salt stress condition

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Salt stress hardness for plant organisms is caused by osmotic and ionic homeostasis disturbance and toxic effect of Na^+ which is the main cation of soil salinized salts. Plant cell is not need in Na^+ whose accumulation injuries its metabolitism. Na^+ efflux from cytoplasma outside and to vacuolar space is realized by secondary active Na^+/H^+ antiporters supported energetically by primary H^+-pumps function. H^+-pump mechanism in plasmalemma is represented by H^+-ATPase of E-P-type whereas two H^+-pumps ones in tonoplast by V-H^+-ATPase and H^+-pyrophosphatase. Plant salt tolerance can be improved by transfere of genes coded powerful Na^+/H^+ antiporters or their regulatory proteins. But a considerable corn salt tolerance can be reached too with help of synthetic bioactive preparations. The aim of present experiments was to research Methyure and Ivine effect on H^+-ATPases activities under salinty condition.
7-days corn seedlings (hybrid Desna CW) grown Hoagland solution have been exposed on 0.1 M NaCl during 1 or 10 days. Preparations were used by seed soaking in their 10^{-7} water solution during a day. Membrane preparations have been isolated from roots by centrifugation. Hydrolytical activity of H^+-ATPases was assayed by Pi measuring and H^+-transport by fluorometric method.
It was found that NaCl exposition decreased hydrolytical activities of both H^+-ATPases but increased their transport ones. Preparations caused a twice fall of hydrolytical activity of plasmalemma H^+-ATPases at 1-day NaCl exposition. However it was restored partly at 10 days salt exposition, especially in Methyure variant. On the contrary, preparations intensified transport activity at both salt expositions. H^+-ATPase answers on praparations using were rather like but transport ones on them in tonoplast was more high than in plasmalemma. Obtained results showed that adaptogenic effect of these preparations, especially Methyure under salt stress conditions is connected with transport activity intensification of primary H^+-pumps.

4.11. Early events in nitric oxide and reactive oxygen species generation in tomato suspension culture in response to *Botrytis cinerea*

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Botrytis cinerea causes grey mold diseases in a broad range of plant species and is one of the most comprehensively studied necrotrophic plant pathogens. Plants have developed both constitutive and inducible barriers for defence against pathogen attack and this plant defence is activated through specific host signalling mechanisms. Some of the inducible defences have been shown to depend on the concerted action of reactive oxygen species (ROS) and nitric oxide (NO), their enhanced production can be involved in cell wall modification, defence signalling, hypersensitive response (HR), or can be directly toxic to pathogens. Despite elaborate research studies, the biochemical and genetic bases of resistance to *Botrytis* are still not fully understood; the role of ROS and NO in plant defence against the pathogen remains controversial.

In this study we compared *B. cinerea* infection development and viability of tomato cell suspensions differing in susceptibility to the pathogen, as indicated by Evans blue staining and successive addition of fluorochromes: acridine orange (AO) and ethidium bromide (EB). Parallelly changes in superoxide, hydrogen peroxide and nitric oxide generation in the cultures were compared using NBT and DAB staining and microscopy detections for $\cdot O_2^-$ and H_2O_2, respectively as well as NO detection by CLSM systems following biochemical, quantitative measurements of ROS and NO. S-nitrosothiols (SNO) content and lipid peroxidation (MDA) were also measured during the interaction.

4.12. Cell wall regeneration by protoplasts of *in vitro* recalcitrant plant species from monocot and legume

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Despite extensive studies, regeneration of plants from protoplasts, especially among monocotyledonous and leguminous species, has succeeded only in a few cases. One of the reason which prevent development of protoplasts may be changes in composition and structure of regenerated cell wall.
Two ornamental monocots: *Asparagus densiflorus* 'Sprengeri' and *Hyacinthus orientalis* 'Anna Lisa' as well as two legumes: *Lathyrus sativus* 'Derek' and *Lupinus luteus* 'Mister' were used as plant material. Protoplasts were enzymatically isolated from asparagus according to Pindel (2002), from hyacinth according to Pindel and Lech (2002) and from lupin according to Wiszniewska and Pindel (2009). Grass pea protoplasts were isolated from leaves of *in vitro* seedlings, incubated overnight in a mixture of enzyme containing 1% cellulase, 0.5% macerozyme and 11% sorbitol. Protoplasts were cultivated in liquid media of different basic composition and with the addition of various growth regulators.
The first divisions occurred in hyacinth protoplasts after 2-3 days of culture, and in asparagus and lupins ones after 7-10 days. However, after next few days of cultivation further subdivisions were not observed. There was no mitotic activity detected, only 'budding' of grass pea protoplasts. In connection with lack or distortions of divisions, the rate and course of cell wall regeneration were analysed by using Calcofluor White.

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4.13. Reversible cell wall deformation in response to humidity – a case study of scarious involucral bracts of *Helichrysum bracteatum* capitulum.

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Scarious involucral bracts surrounding capitulum of *Helichrysum bracteatum* undergo profound reversible deformation in response to changes in humidity: they bend outward from the capitulum centre in a dry state, or inward – when wet. We aim at recognizing the specific morphological features of the bending portion of the bract, at the organ, cellular, and subcellular levels, that enable such profound reversible deformation.
We compare cells in the bending region of the bract in terms of their morphology, wall composition and structure, as well as the wall thickness in the hydrated and dehydrated state. We also quantify the bract surface deformation measuring changes in the bract curvature and deformation of individual cells. All the cells from the bending bract region are dead and their walls are lignified. Inner parenchyma cells are closely packed and have thin walls. Epidermal cells on the adaxial side have thickened outer periclinal walls, while on the abaxial side there are two layers of cells with uniformly thickened walls. If the latter are in contact with water the bract bends inward and the cells undergo dramatic size and shape changes. This deformation is strongly anisotropic: they contract in transverse direction and expand - in longitudinal.
Scarious bracts of *H. bracteatum* are an especially valuable object for studies of xerochastic/ hygrochastic plant organ movements since the cells driving these movements are easily available, being on the organ surface, and bracts retain their ability to deform long after they have been dissected from the capitulum.

4.14. Nostoc symbiosomes in *Gunnera* – *Nostoc* symbiosis

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Gunnera is the only angiosperm genus known to enter symbiotic association with *Nostoc* cyanobacteria capable of fixing nitrogen. Infected gland cells of *G. mannicata* and *G. magellanica* are tightly packed with *Nostoc* filaments. These filaments become encapsulated with a sheath of plant cell wall-like material surrounded by host plasmalemma. This encapsulation is apparently continuous with the host cell walls and surrounds all *Nostoc* cells – vegetative as well as heterocysts and is produced by the macrosymbiont. The vegetative *Nostoc* cells are surrounded by their own cell wall located between two membranes. The colony is ensheathed by an outer layer of cyanobacterial envelope placed outside the outer membrane. In symbiosis the number of heterocysts significantly increase. The envelope of heterocyst is much thicker than these of vegetative *Nostoc* cells. It shows presence of the laminated and homogeneous layers external to the normal cyanobacterial cell envelope. Heterocyst envelope is tightly suppressed to wall-like material which encapsulated *Nostoc* filaments. In contrary, the vegetative *Nostoc* cells envelope does not fit tightly to these wall. This close contact between *Nostoc* heterocyst envelope and *Gunnera* cell wall-like material seems to support the exchange between both symbionts. The *Gunnera*-cyanobacterial associations are intracellular (Bergman et al. 1992), but these observations did not show cell wall outgrowing surrounding *Nostoc* filaments.

References:
Bergman, B., Johansson, C., Soderback, E. 1992 The *Nostoc–Gunnera* symbiosis. Tansley Review, no. 42. *New Phytologist* **122**, 379–400.

4.15. Phytotoxic effects of cyanamide on tomato (*Lycopersicon esculentum* L.) root growth – alterations in hormone balance and expansins expression

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Cyanamide (CA) is one of the allelochemicals reported as a natural product of hairy vetch (*Viccia villosa* Roth.) which is utilized as a winter cover crop in orchards and fields but its mode of action in plants is still unknown.
The scope of investigation was to determine some mechanisms of phytotoxic effect of CA on tomato (*Lycopersicon esculentum* L.) root tip cells division and root elongation growth. Tomato seeds were germinated and after radical protrusion transferred to water and 1.2 mM CA aqueous solutions. The root length, mitotic index and root cell size in each root zones (light microscopy), hormone concentration - auxin (indole-3-acetic acid (IAA)) (ELISA test) and emission of ethylene (gas chromatography) were determined. Expansins gene expression (semi-quantitative RT-PCR) and expansins protein content (Western-blot) in tomato roots were also analyzed. Inhibition of roots growth has accompanied by disturbances of root tip cells division, drops frequencies of mitosis and reduces roots meristematic zone. CA has also induced transient increase of ethylene emission and overaccumulation of auxins. High level of plant hormones has been correlated with modifications of expansins gene expression and protein content.

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4.16. Aluminum-induced rapid changes in apoplast of pea (*Pisum sativum* L.) root nodules

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Plant cell-wall remodelling is a fundamental aspect of the *Rhizobium*-invasion process (Brewin, 2004) and infection thread (IT) formation is crucial step in the establishment of endosymbiosis. Aluminum (Al) accumulated in the root apoplast modifies cell wall composition and properties. In this work pea (*Pisum sativum* L.) root nodules were used to investigate changes in the nodule structure and apoplast protein extensin content induced in response to short Al stress. Pea plants were treated with 50 µM of $AlCl_3$ for 2 h or 24 hrs. The distribution of extensin in the nodule apoplast was examined using specific monoclonal antibody (LM3).
Al induced rapid alternations in IT formation and function. The development of large and strong branched ITs surrounded by thick walls was observed. Exposure to 50 µM $AlCl_3$ for 2 and 24 hrs resulted in abundant occurrence of extensin in the cell walls of nodule cortex and in IT matrix. Microscopic observations showed that Al.: (1) changed nodule zonation, (2) caused the increase of thickened of the cells walls, (3) inhibited IT growth and expansion, (4) caused disturbances in bacterial release from ITs, and (5) induced the increase in extensin content in the nodule apoplast. Al makes the cell wall thick and rigid, thereby inhibiting the growth of infection threads and development of pea nodules. The possible role of extensin IT growth under Al stress was investigated.

References:
Brewin, N.J. 2004. Plant cell wall remodeling in the *Rhizobium* - legume symbiosis. Critic Rev Plant Sci 22: 293-316.

4.17. Localisation of arabinogalactan proteins in newly-reconstructed cell wall during early stages of protoplast culture – preliminary studies

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Numerous important crops, especially among monocotyledonous and leguminous species, are regarded as strongly recalcitrant or even almost unresponsive to protoplast culture. In case of limited regeneration potential a deep understanding of cellular events connected to the protoplast recalcitrance is essential. Apart from investigation of cell wall reconstruction and cytoskeleton rearrangements, an interesting area of research is the role of some cell wall components in the induction of morphogenetic activity in cultured protoplast-derived cells (Wiszniewska and Pindel, 2010). In this regard, the stimulation of differentiation processes in plants has been attributed to the presence and function of arabinogalactan proteins (AGPs) (Rumyantseva, 2005). These molecules are bounded both to the plasma membrane and cell wall and their alternated conformation may be a marker of cell identity or a signal for adjacent cells.
With a view to investigate the function of AGPs in protoplast culture, studies on their localisation in protoplast-derived cells have been undertaken in recalcitrant plant species: yellow lupin (*Lupinus luteus*) 'Mister' and two ornamental monocots: aparagus (*Asparagus densiflorus*) 'Sprengeri' and hyacinth (*Hyacinthus orientalis*) 'Anna Lisa'. Cell walls were stained with 0.01% β-D-glucosyl Yariv reagent at the early stages of cultivation and examined for the presence of AGPs in relation to diversified culture conditions. It was also intended to detect the relation of AGPs presence in the new cell wall and changes in cell nucleus. For that purpose, nuclei of protoplasts were stained with DAPI and observed in fluorescence microscope at λ=365 nm.

References:
Rumyantseva N.I. 2005. Arabinogalactan proteins: involvement in plant growth and morphogenesis. *Biochemistry (Moscow*) 70(10): 1073-1085.
Wiszniewska A., Pindel A. 2010. Komórkowe aspekty regeneracji w kulturach protoplastów łubinu żółtego (*Lupinus luteus* L.). *Biotechnologia* 2(89): 18-22.

4.18. A chloroplast Deg1 protease performs *in vitro* cleavage of the PSII proteins in *Zea mays*

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The chloroplast Deg1 protein is an ATP-independent Ser protease peripherally attached to the lumenal side of the thylakoid membrane. It contains a trypsin-like protease domain at the N-terminus and one PDZ domain which is responsible for substrates recognition and for complex assembly at the C–terminus. Its exhibits both the chaperone and proteolytic functions. Deg1 of C3 type plant *Arabidopsis thaliana* performs a proteolytic cleavage of the photodamaged D1 protein of the photosystem II reaction centre, the photosystem II extrinsic subunit PsbO and the electron carrier plastocyanin. Recently, it has been shown that Deg1 assists the assembly of the photosystem II, probably by interaction with the D2 protein of the PSII reaction centre. Our recent studies has revealed that Deg1 protease from *A. thaliana* may be responsible also for the degradation of the other photosystem II subunits CP26 and CP29 and PsbS protein. There is nothing know about function of Deg1 in C4 plants. *Zea mays,* the C4 plant is most cultivated crops all over the world and in which two distinct cell types, mesophyll (M) and bundle sheath (BS), cooperate during photosynthesis. In maize, mesophyll chloroplasts are structurally similar to those in C3 plants, while BS ones lack grana. Here we present the results of Pull-Down approach combined with immunoblotting and *in vitro* digestion of *Z. mays* thylakoid proteins by *Zea mays* Deg1 showing similarities and differences between substrates pool of Deg1 proteases derived from C3 and C4 plants after high light photoinhibitory treatment. Our results promote us to postulate a novel role of Deg1 in the acclimation processes to high light including photoinhibition and state transitions in both type of plants.

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Parnas Day – molecular level

Jakub Karol Parnas the outstanding Polish biochemist

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Jakub Karol Parnas was born in 1884 in Mokrzany, a small village in Galicia, which at that time belonged to Austro-Hungarian Monarchy. As a result of the WWI, Galicia became part of Poland. Following the Soviet-German agreement this land was turned over to the Soviets. From 1941 to 1944 the land was occupied by Germans and by now it is a part of Ukraine. Parnas studied chemistry at the universities of Berlin, Strasburg, Zurich and Munich where in 1907, he received the Ph.D. He was associate professor at Strasburg University in1913 and professor of physiological chemistry at Lwow University (1920-1941).
Lwów (in Polish) or Lviv (in Ukrainian) or Lemberg (in Yiddish) was a very special place. It was the Polish city built on Ukrainian land. In thirties of the last century, the Lwów population comprised of 52% Poles, 30% Jews, 17 % Ukrainians and small percentage of other minorities, like Germans, Chechs, Armenians. At that time Lwow was not a peaceful city. Ukrainian Nationalist struggled for free Ukraine and claimed that Lwów should be the Ukrainian city. Jews fought against the increasing wave of the anti-Semitism. Poles tried to maintain the *status quo*. Agents of German Nazi and Soviet Communist were also present trying to destabilize the situation in the city.
The Parnas' laboratory was an unique place where in a friendly atmosphere, young Polish, Ukrainian and Jewish scientists studied glucose metabolism in Vertebrate skeletal muscle cells. In order to follow the fate of the phosphate residue in glucose metabolism, it was necessary to employ the radioactive phosphor ^{32}P which had been successfully used in Parnas laboratory. The quintessential discovery of Parnas and his collaborators was: the breakdown of glucose results in ATP production.

A Time Line of the Discovery of Glycolysis

- hexokinase — Meyerhof 1927
- hexoisomerase — Cori, Cori 1936
- *1,6-phosphofructokinase* — *Ostern, Guthke, Terszakovec 1936*
- aldolase — Meyerhof, Lohmann, Schuster 1936
- triosephosphate-isomerase — Meyerhof, Lohmann, Schuster 1936
- GAPDH dehydrogenase — Warburg, Christian 1939
- 1,3PDG kinase — Bücher 1942
- 3-phosphoglycerate mutase — Meyerhof, Kiessling 1935
- enolase — Meyerhof, Kiessling 1935
- *pyruvate kinase* — *Parnas 1934*

Two enzymes of glycolysis have been discovered by Parnas and his collaborators. Ostern, Guthke and Tershakowec discovered phosphofructokinase and Parnas himself discovered pyruvate kinase. The results of their work were highly appreciated and for many years glycolysis was also termed as Embden-Meyerhof-Parnas pathway.

The good time for Parnas and his collaborators has ended in 1939, when the WWII began. Lwow was occupied by Soviets and became a part of the Soviet Union. Initially, Parnas was respected by Soviet authorities and even became the member of the Soviet Academy of Science and was allowed to continue his research. In 1941, Germans invaded Soviet Union and Parnas was forced to move to Ufa in the central part of Soviet Union.

After the WWII, Parnas wanted to go to Polish Republic but Soviet authorities did not allow him to leave the Soviet Union. Instead, in 1947, he was arrested and shortly after, he died in Moscow prison. The circumstances of his death have never been elucidated.

Parnas' students and collaborators were pioneers of biochemistry in Poland. Baranowski and Meybaum-Katzenelebogen in Wrocław, Mozolowski in Gdansk, Mochnacka and Heller in Warsaw. Polish biochemists still remember Jakub Karol Parnas as the outstanding biochemist and the great man and consider him as the Founder of the Polish School of Biochemistry.

Session 5
RNA metabolism in plants

Plenary lectures

Analysis of alternative splicing in plants

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Alternative splicing (AS) is one of the mechanisms which are involved in the regulation of gene expression and also in expanding the protein complexity. Despite its role in important biological processes, like development and stress response, its mechanisms have remained largely unexplored in plants. Alternative splicing is prevalent in humans with up to 95% of intron containing genes producing mRNA isoforms in different tissues and under various environmental conditions. In plants, however, their significance for gene expression was grossly underestimated due to relatively low EST sequence coverage. I will present results from an analysis of RNAseq data which we have conducted on normalized cDNA libraries.
In the past years we have analysed the Arabidopsis SR protein family which are important for spliceosome assembly and alternative splicing. SR (Ser/ Arg) proteins are a family of splicing regulators which contribute significantly to splice site selection and are equally important for constitutive and alternative splicing. SR proteins are evolutionary conserved phosphoproteins with one or two N-terminal RNA-recognition motifs and a C-terminal domain rich in arginines and serines. We have studied several SR genes for their expression patterns, post-transcriptional regulation, and overexpression and knockout phenotypes. These analyses have shown that expression patterns of Arabidopsis SR genes are subjected to tight spatiotemporal and environmental control. Accordingly, mis-expression of SR proteins has affected various aspects of plant development and environmental response. Changes of gene expression by deregulation of SR proteins have been analysed by gene chip microarray technology. These data were complemented by results of a recently established AS RT-PCR panel, a system of monitoring changes in alternative splicing in about 300 genes in Arabidopsis.

Highlights of plant RNA decay

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Apart from RNAi other RNA metabolic pathways in plants are still relatively poorly understood. However, the last decade brought new developments in this field, including identification of major players, such as the exosome, decapping enzymes, deadenylases and the XRN family of exonucleases. Although these enzymes and their auxiliary factors most likely play analogous roles in general and specialised RNA decay pathways as their yeast and human counterparts, their deciphering was impeded by a high degree of redundancy between different mechanisms. Our work focuses on the function of nuclear factors in Arabidopsis, the LSM complex, activator of RNA decapping, and XRN 5'-3' exonucleases, to understand their contribution to the nuclear RNA surveillance in plants. We have established that the LSM2-8 complex functions not only in pre-mRNA splicing as a core component of the U6 snRNP, but also in degradation of a subset of pre-mRNAs. However, the mechanism targeting these particular substrates for nuclear decay is not clear.
In turn, the analysis of nuclear XRN2 and XRN3 proteins revealed their involvement in the polyadenylation-mediated degradation of pre-rRNA processing intermediates. In addition, lack of these nucleases results in accumulation of cryptic intergenic transcripts located in the vicinity of highly transcribed loci. These RNAs may be generated by transcriptional read through of coding genes or by pervasive transcription of intergenic regions. The increase of cryptic transcripts, albeit of different origin, has been recently reported for exosome mutants, the major 3'-5' exonucleolytic machinery.
It appears that XRN exonucleases are another key player in the RNA surveillance pathways cleaning up after widespread, promiscuous transcription in plant cells.

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The role of miR393 and RNA decay in plant development

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RNA silencing refers to recently discovered RNA dependent processes involved in controlling the expression and integrity of eukaryotic genomes. It includes major mechanisms that coordinate development, responses to biotic stresses, defence against pathogens or, that control genome stability. Small RNAs (e.g. microRNAs, siRNAs or piRNAs), 19-40 nucleotides in length, are the core component of these processes. They carry sequence-specificity for function of effectors. Several proteins involved in these processes have been identified. However, little is known about how these processes are themselves regulated in time, in space or in response to developmental and environmental cues.
We have characterized an Arabidopsis RNA silencing pathway that involves miR393 and that functions in hormonal and developmental regulation. We are now aiming to elucidate the general principles underlying the regulation of this RNA silencing pathway by RNA decay components. I will present our recent insights into this layer of regulation.

Oral presentations

Ultrastructural distribution of RNA polymerase II in egg cell of *Hyacinthus orientalis* L. before and after fertilization

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Spatial distribution and the level of total pool RNA Pol II (CTD domain, 4H8 antibodies), hypophosphorylated Pol IIA (H14 antibodies) and hyperphosphorylated Pol IIO (H5 antibodies) was characterized using immunogold techniques in the egg cell and in the zygote of *H. orientalis* L. Pattern of the nucleus labeling in the egg cell before and after fertilization was compared with the pattern of somatic cells nuclei. Ultrastructural analysis indicated different chromatin organization in this cells. *H. orientalis* somatic cells have reticular nuclei whereas the egg cell chromatin was decondensed. In the zygote when second nucleolus was observed the chromatin organization was changed and formed of different sizes condensed clumps.
In electron microscopy CTD domain (total pool) of RNA Pol II in egg cell was localized mainly in the area of uncondensed chromatin and in the interchromatin regions. Amount of the grains of colloidal gold in this cell was lesser than in the nucleus of somatic cells. The intense labeling was observed after fertilization. In the zygote rich pool of the RNA Pol II was localized in the perichromatin regions and lower accumulation of the labeling was visibled in the interchromatin regions. In egg cell RNA Pol IIO occurred at the periphery of dispersed chromatin masses, as well as in the areas between them whereas very low labeling of RNA Pol IIA was observed. A significantly increase in Pol IIO and Pol IIA levels was located in the zygote. Gold grains were seen in mainly perichromatin regions and in the chromatin masses. The levels of 4H8, H14 and H5 antibodies labeling in the zygote nucleus was similar to the observed in somatic cells nuclei.

Sm proteins as a molecular switch triggering the formation of Cajal bodies during diplotene of first meiotic division

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Small nuclear ribonucleoproteins (snRNPs) play fundamental roles in pre-mRNA processing in the nucleus. The biogenesis of snRNP includes a sequence of events that occurs in both the nucleus as well as the cytoplasm. Despite, rich biochemical information of cytoplasmatic assembly of snRNPs, little is known about the spatial organization of snRNPs in the cytoplasm. In the cytoplasm of the larch microsporocytes, a cyclical occurrence of bodies containing small nuclear RNA (snRNA) and Sm proteins was observed during diplotene of first meiotic division. Immunofluorescent technique, fluorescence *in situ* hybridization (FISH) and were used to show localization of Sm proteins and snRNA. Correlation between the occurrence of cytoplasmic snRNP bodies (CsBs), the level of Sm proteins, and the dynamic formation of Cajal bodies were observed.
Larch microsporocytes were used for these studies. These cells characterized by a high degree of synchronization during anther development and a long meiosis, especially diplotene lasting about 5 months, in which periodical increases and decreases in transcriptional activity occur. In designing experiments, the authors took into consideration the differences between the nuclear and cytoplasmic phases of snRNP maturation and a hypothesis about the direct participation of Sm proteins as a molecular switch triggering the formation of Cajal bodies during diplotene of first meiotic division.

Poster presentations

5.1. Identification and preliminary characterization of *AtTCP4* homologue, the miR319 targeted gene, in *Pharbitis nil* seedlings.

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MicroRNAs (miRNAs) are small (21 nucleotides) noncoding RNAs that are key post-transcriptional controllers of gene expression in both plants and animals. MiRNAs regulate gene expression by guiding the cleavage of target mRNAs or attenuating the translation of their target genes. Many miRNAs that are conserved throughout flowering plants target transcription factor genes that control various aspects of development. However, very little is known about the cross talk between miRNA signals and hormones. Several of miRNAs in turn modulate the response to hormones, such as miR159-regulated *GAMYB* (*GIBBERELLIC ACID MYB*) genes. In contrast, miR319 and its target genes *TCPs* (*TEOSINTE BRANCHED/CYCLOIDEA/PCF*) control biosynthesis of the hormone jasmonic acid. The *TCPs* constitute a plant-specific group of transcription factor genes. A subset of class II *TCP* genes contains a miR319 binding site including *TCP2*, *TCP3*, *TCP4*, *TCP10*, and *TCP24*. *AtTCP4* coordinate two sequential processes in leaf development: negatively regulated leaf growth and positively regulated leaf senescence. Additionally, miR319a targeting of *TCP4* is critical for petal and stamen development in *Arabidopsis*. The aim of this work was to identify homologue of *AtTCP4* gene and examined its expression in *Pharbitis nil* (*Ipomoea nil*). We isolated the *InTCP4* cDNA from *I. nil* cotyledons and observed its expression levels in various organs of plants. The identified sequence contains TCP domain and nucleotides complementary to miR319. Its nucleotide sequence shows a significant similarity to the cDNA members of *TCP* transcription factor family, *LANCEOLATE* from *Solanum melongena*, *CYCLOIDEA* from *Lycopersicon esculentum* and *AtTCP4.* We found that identified gene is strongly expressed in cotyledons of *I. nil* seedlings. Transcript is also present in petals, stamens and leaves. These results may suggest potential role of *InTCP4* in regulation such processes as its homologues in other plant species.

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5.2. Micro RNAs in barley - their precursors and genes

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Micro RNAs (miRNAs) are a class of small, non-coding RNAs, usually of 21 nucleotides long which control gene expression by targeting the cleavage of the complementary mRNA or by inhibiting their translation. The sequence of single-stranded miRNA precursor (pri-miRNA), which is a product of RNA polymerase II activity, allows to form double-stranded hairpin structure (pre-miRNA) which contains miRNA. This phenomenon, as well as high conservation of some miRNAs among different plant species made it possible to predict *in silico* potential precursor and mature miRNA sequences in *Hordeum vulgare* (Dryanova et al. 2008; Kantar et al. 2010).

We decided to characterize the structure of miRNA genes and their transcripts for ten selected miRNAs: miR159, miR166, miR168, miR171, miR397, miR1119, miR1120, miR1121, miR1122 and miR1126 in barley Rolap cultivar. The majority of miRNA genes studied contains introns – to date only 3 miRNAs: miR397, miR1119 and 1121 are intronless. All identified introns are U2-type and their number differs significantly from one to five. Generally, miRNAs and miRNA* sequences are found within the first exon of intron-containing genes. So far the only exceptions are miR1126 and miR1122 located in second and sixth exon, respectively. Surprisingly, miR1122 is encoded within the last exon of protein-coding gene suggesting a possible novel mechanism of posttranscriptional gene expression regulation in plants. Most of the transcripts are heterogenous at their 3' ends resulting in different polyadenylation forms. We also detected an alternative 3' splice site event during pri-miRNA171 maturation indicating the potential mechanism of miRNA level regulation via alternative splicing.

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5.3. Cloning and molecular analysis of a *gibberellin 20-oxidase 3* gene expressed specifically in *Pharbitis nil* seedlings

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Gibberellins (GAs) are a class of phytohormones involved in the regulation of plant growth and development. The enzymes responsible for biosynthesis of these hormones are gibberellin 20-oxidases (GA20oxs). They are encoded by small genes families and the expression of *GA20oxs* genes is regulated by environmental and developmental signals.
A PCR technique enabled us to obtain a new *GA20ox* cDNA from *Pharbitis nil* designated as *PnGA20ox3* (GeneBank accession no. HM017849). The full length*PnGA20ox3* mRNA is 1578 bp long and contains an open reading frame of 1118 bp coding for a putative protein of 372 aa. The deduced amino acid sequence possesses three conserved domains characteristic for all GA20oxs in different plant species. The PnGA20ox3 protein shows the highest level (80%) of sequence identity to *Nicotiana tabacum* NtGA20ox and *Solanum dulcamara* SdGA20ox.
An investigation of *PnGA20ox3* transcriptional activity shows that during the 16h-long inductive night, the amount of studied transcript decreases in cotyledons of 5-day-old seedlings in comparison with control conditions. An extra application of exogenous ethylene gives similar effect. In hypocotyls, apexes and petioles the level of *PnGA20ox3* transcript is comparable with the exception of roots, where the mRNA abundance is clearly the lowest. The changes in gene expression are also observed in different vegetative organs during 5 days of seedlings development under various light conditions. Obtained results of *PnGA20ox3* gene research will lead us to better understanding of the gibberellin pathway in *Pharbitis nil*.

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5.4. Expression of the *DOF3.1* gene during somatic embryogenesis induced *in vitro* in Arabidopsis

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The Arabidopsis gene encoding *DOF3.1* transcription factor was studied to reveal its spatial and temporal activity during morphogenic processes induced *in vitro*. *In planta* the gene is active during zygotic embryogenesis and its expression is restricted to a quiescent centre (QC) of embryonic root.
Immature zygotic embryos (IZEs) at late cotyledonary stage of development were used as explants and alternative morphogenic pathways were induced, somatic embryogenesis (SE), shoot organogenesis (ORG) and seedling development, in response to different culture media applied. Real-Time qPCR was performed to evaluate *DOF3.1* expression pattern during the studied morphogenic pathways. Moreover, a transgenic line with a *GUS* reporter gene under control of*DOF3.1* promoter was used to study spatial and temporal promoter activity in response to IZE treatment with auxin. In addition, the capacity for SE was evaluated in culture of two mutant lines (*dof3.1* and *dof3.1*/amiR*DOF5.2*) with significantly decreased *DOF3.1* expression.
The results indicated that in somatic embryos, similarly to zygotic ones, the gene activity was found restricted to QC of a root and during SE induction the expression of *DOF3.1* was detected in hypocotyl parts of the cultured IZEs. Given that these IZE regions are not involved in SE or ORG, the activity of *DOF3.1 in vitro* indicated by Real-Time qPCR analysis seemed to be not related with morphogenic processes induced *in vitro*. In contrast, mutant analysis showed a significant decrease (above 50%) of SE efficiency in *dof3.1* in comparison to control Col-0 culture. Thus, the observed negative influence of dof3.1 mutation on embryogenic capacity of explant tissue remains to be elucidated.

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5.5. Identification and characterization of barley microRNAs genes and their precursors

Andrzej Pacak, Katarzyna Kruszka, Agnieszka Stefaniak, Aleksandra Świda-Barteczka, Hanna Kijak, Renata Michałowska, Łukasz Sobkowiak, Artur Jarmołowski, Zofia Szweykowska-Kulińska

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MicroRNAs (miRNA) are generated from subsequent semi-products: primary microRNA (pri-miRNA), precursor microRNA (pre-miRNA), and miRNA/miRNA* duplexes. MiRNAs bind to complementary sequences within target messenger RNAs resulting in gene silencing. Despite the crucial role of miRNA molecules in plant development, organ formation and responses to various stresses, the structure of miRNA genes, their transcripts and expression regulation is still very limited, especially in crop plants. For instance there are only few papers describing bioinformatically identified microRNAs and their precursors in barley (Dryanova et al. 2008, Kantar et al. 2010). Here we present our new experimental data on several miRNA gene structures, their transcripts and expression pattern during barley development. All experiments were done on doubled haploid line of *Hordeum vulgare* derived from crosses of cultivars Roland and Apex (Rolap).

Using 3'- and 5'-RACE and Genome Walking techniques we amplified and sequenced precursors for following barley microRNA genes: miR156, miR169, miR393, miR399, miR408, miR444, miR1436 and miR1439. So far we have identified introns in 5 out of 8 analyzed genes. MicroRNA156 gene contains at least two introns of classical U2 type. MicroRNA169 has typical U2 type branchpoint (CTAAC), however intron terminal dinucleotides are non-canonical. MiR399 and miR408 contain introns of unusual type: non-canonical terminal dinucleotides and no classical branchpoint. Interestingly, in miR169 gene intron disrupts miRNA* sequence suggesting possible role of miRNA169 expression regulation by splicing events. Moreover, in miR169 transcripts we observed different polyadenylation isoforms. These isoforms may acquire different secondary structures with alternative miRNA* molecules. Detailed information on miRNA genes and their transcripts will be presented and discussed.

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5.6. A role of evolutionary conserved U12 intron in plant nuclear *cap binding protein* (*CBP20*) genes

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It is known that binding the nuclear cap-binding complex (CBC) to the 5' cap is crucial for the proper mRNA maturation and transport. CBC is a nuclear complex composed of two cap-binding proteins: CBP20 and CBP80. The *CBP20* gene structure is highly conserved across land plants from *P. patens* and *S. moellendorffi* to higher plant *O. sativa*, *A. thaliana* and *N. tabacum*. The gene contains always seven introns with the fourth intron belonging to U12 class. The U12 intron divides the gene in two parts: one that encodes the core domain containing RNA recognition motif and the second one that encodes tail domain containing NLS signal. In all investigated plant *CBP20* genes first four exons coding the core domain have always the same length whereas exons coding the terminal domain differ considerably in length.
To answer the question why the presence and location of U12 intron is conserved in plant *CBP20* genes we prepared constructs representing *CBP20* mini-genes and its mutated full versions. Mini-gene constructs containing 4th and 5th exons from *A. thaliana CBP20* gene and U12 introns derived from different plants and differing in length (from 134nt to 2733nt) were transfected to tobacco mesophyll protoplasts and splicing was analysed. Our preliminary results show that the longer the U12 intron the more efficient splicing was observed.
Additionally we prepared five constructs containing *A. thaliana CBP20* gene sequence in which U12 intron was replaced by U2 one and U12 intron was moved to different locations within the gene. These constructs were introduced into *A. thaliana cbp20* T-DNA insertion mutant. Our preliminary results show that mutated *CBP20* genes generate additional alternatively spliced isoforms containing retained intron and/or longer/shorter exons (because of alternative 3' and 5' splice site selection). These data suggest that U12 intron in the proper position in plant *CBP20* genes is necessary for correct pre-mRNA splicing.

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5.7. Identification and characterization of genes specifically expressed during the development of male thalli and antheridia in the dioecious liverwort *Pellia endiviifolia* sp. B

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In lower plants, like bryophytes, sex determination is manifested in the gametophyte generation by the production of egg- and sperm-forming gametangia, in many species on male and female individual gametophytes. RDA-cDNA was performed to search for genes specifically expressed in the male thalli of dioecious liverwort *Pellia endiviifolia* species B. Four genes were selected. These are *PenB_TUA1* coding for an α-tubulin family protein, *PenB_ Raba1/11*coding for a Rab family protein, *PenB_HMGbox* coding for an HMGbox family protein and *PenB_MT* coding for an unknown transcript containing an ORF of 295 amino acid residues in length. *PenB_TUA1* and *PenB_Raba1/11* are expressed in male thalli, regardless of whether they develop antheridia or not. *PenB_HMGbox*and *PenB_MT* are exclusively expressed in the male thalli producing antheridia. Moreover, two genes *PenB_TUA1* and *PenB_Raba1/11* are encoded only in the male genome of *P. endiviifolia* sp. B. Our studies show for the first time the specific contribution of identified genes in the liverwort male gametophyte development. In higher plants properly regulated specific types of α-tubulin and Rab family proteins activity are essential for tip-focused membrane trafficking and growth of the male gametophyte. Thus they are pivotal to reproductive success of these plants. HMGbox family plant proteins display a binding preference toward DNA, this can increase the structural flexibility of DNA, promoting the assembly of nucleoprotein complexes that control DNA-dependent processes including transcription. Our results show that genes connected with the gametogenesis processes in higher plants already have their potential counterpart genes in liverworts – the oldest living land plants.

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5.8. Subcellular localization of *Arabidopsis thaliana* XRN-family proteins

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Two families of eukaryotic 5'-3' exoribonucleases, cytoplasmic XRN1 and nuclear XRN2/RAT1 function in the degradation and processing of several classes of cellular RNAs. In contrast, in *Arabidopsis thaliana*, there are no XRN1-type enzymes, but three XRN2/RAT1 homologues, nuclear AtXRN2, AtXRN3 and cytoplasmic AtXRN4. AtXRN2 has a role in ribosomal RNA processing, whereas AtXRN4 contributes to degradation of the 3' products of miRNA-mediated mRNA cleavage. Despite strong similarity and partial redundancy in rRNA processing, the major cellular roles of AtXRN2 and AtXRN3 are distinct and the essential function of AtXRN3 is still unknown. To understand the bases for these differences we established precise cellular localisation of AtXRN2- and AtXRN3-GFP fusion proteins transiently expressed in *A. thaliana* protoplasts. Our data show that while both proteins locate to the nucleus, only AtXRN2 is enriched in the nucleolus, consistent with its function in rRNA processing and with partly separate function of these two homologous nucleases.

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5.9. The RNA surveillence pathways in plants development: flowering

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The precise regulation of RNA metabolism has crucial roles in plants developmental processes such as the control of flowering. Dynamic changes in gene expression are generally based on the balance between RNA transcription and degradation and recent analyses underline the role of RNA decay related to surveillance pathways. RNA quality control is responsible for recognition and degradation aberrant and potentially harmful transcripts. One important surveillance mechanism, NMD (nonsense mediated decay), promotes degradation of mRNAs with prematured termination codons. Plants mutants defective in NMD machinery were shown to display altered flowering (*upf1-5*) or seedling-lethal phenotypes (*upf3-1)*. Interestingly, components of RNA metabolic pathways, LSM5 and UPF1 participate in ABA (abscisic acids) hormone signaling network that coordinates stress response and is involved in many developmental processes. However, the information integrating RNA surveillance pathways is still fragmentary.
To investigate the role of RNA quality control in plant physiological processes, especially flowering, we examined *Arabidopsis thaliana* mutants in RNA metabolism, with the focus on NMD, with respect to changes in flowering, on both physiological and molecular levels. Plants with defects in mRNA splicing (*sad1/lsm5, lsm8*) or decay (*lsm1, xrn4*) showed altered flowering time. Consequently, northern analyses of RNA levels for chosen markers known to regulate the timing of flowering, such as *FT*, *SOC1*, *LHY*and *FLC*, support physiological observations. These data, underscore the importance of investigating correlations between RNA and developmental pathways.

5.10. Identification and preliminary expression analysis of *InOPR3* gene from *Pharbitis nil* (*Ipomoea nil*)

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OPR3 (12-Oxophytodienoate reductase 3) belongs to a small family of related flavin-dependent enzymes regulating biosynthesis of jasmonic acid – one of the plant hormones that controls growth and development. Generally, jasmonates are involved in fruit ripening, production of viable pollen, root growth, tendril coiling, plant response to wounding and abiotic stress, defense against insects and pathogens, as well as flower development.
In this work we identified a full length of *InOPR3* cDNA (GeneBank acc. no. HM357793) from *Pharbitis nil*, which consists of 1460 bp and its ORF encodes 400 aa. The predicted amino acid sequence of *InOPR3* is 63% homologues to *OPR3*from *Lycopersicon esculentum*.
Expression analysis by the use of RT-PCR technique revealed that *InOPR3* mRNA was present at different levels in various vegetative and generative organs of *Pharbitis nil* seedlings. Transcript of the gene was accumulated in cotyledons, hypocotyls, roots, shoot apexes, leaf, stamens, pistils and petals. The highest expression level of *InOPR3* was found in the hypocotyls and petals, while the lowest – in the apexes and stamens. *InOPR3* transcript accumulation arrangement is partially similar to the expression pattern of *AtOPR3* gene studied in *Arabidopsis thaliana*.
The data obtained in this work clearly indicate that *InOPR3* is involved in the regulation of jasmonates biosynthesis in *P. nil*, which in turn may regulate similar physiological processes as in *Arabidopsis thaliana*.

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5.11. The identification and characteristic of the *InFY* gene of the flowering induction autonomous path with leaves *Ipomoea nil*

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The research done into the model short day plant *Ipomoea nil* (*Pharbitis nil*) enabled to identify several genes directly or indirectly connected with the flowering induction mechanisms. Apart from the photoperiodic flowering induction genes, others connected with the functioning of the autonomous path (AP). The key task of the proteins coded by them is to stop the transcript activity of the *FLC* gene flowering inhibitor. One of the functional structures responsible for this process is a complex of FCA and FY proteins. Earlier research made it possible to identify *InFCA* occurring with *I. nil.* The second protein of the complex, *InFY* was described with the use of the RACE technique. The analysis of the nucleotide sequence and the anticipated amino acid sequence showed considerable similarity of cDNA *InFY* to the homologues defined with other plant species. Transcript activity was shown with the gene tested with both leaves of the plants undergoing the photoperiodic flowering induction and the non-induced ones. FY proteins and similar ones are described as factors responsible for the poliadenylation process and partially controlling the synthesis of the proper form of transcripts. On the basis of the results obtained, one may assume that with *Ipomoea nil* InFY functions in such a way independently of the mechanisms connected with the photoperiodic flowering induction.

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5.12. *LEAFY COTYLEDON2* overexpression and embryogenic capacity of Arabidopsis explants cultured *in vitro*

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The *Arabidopsis LEAFY COTYLEDON*2 (*LEC2*) encodes a transcription factor, a central regulator of zygotic and somatic embryogenesis (SE). *LEC2* overexpression results in *planta* in spontaneous formation of somatic embryos by transgenic seedlings and *in vitro* impaired embryogenic response is observed in transgenic immature zygotic embryo (IZE) explants cultured on auxin medium. Considering that among *LEC2* targets the genes involved in auxin biosynthesis were found, it was postulated that *LEC2* modulates embryogenic responses of somatic tissue via its influence on auxin level. Thus, the goal of the present study was to evaluate embryogenic capacity of *in vitro* cultured transgenic IZEs in relation to exogenous auxin treatment.
Different auxin types and concentrations were tested in SE induction media and the results indicated that the explants with *LEC2* overexpression displayed a lower capacity for SE on media with increased auxin concentrations. This observation suggested that endogenous auxin level can be elevated in 35S::LEC2-GR transgenic tissue. In support with this postulate, a distinctly increased IAA level in transgenic seedlings overexpressing *LEC2* was found. Moreover, real-time qRT-PCR analysis of an auxin biosynthesis gene controlled by LEC2, the *YUCCA4 (YUC4)* was carried out during SE process. The results confirmed that *YUC4,* encoding flavin monooxygenase enzyme, is up-regulated in culture of transgenic explants. Altogether, the study provides evidence that *LEC2* can influence morphogenic responses of cultured explants via stimulation of auxin level in somatic tissue.

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5.13. Nuclear 5’-3’ exonucleases function in transcription termination and RNA surveillance in Arabidopsis

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Arabidopsis thaliana two nuclear 5’-3’ exonucleases, AtXRN2/3 are homologs of yeast Xrn2/Rat1 which is involved in the degradation and processing of several classes of nuclear RNAs and in transcription termination of RNA polymerases I and II. We have shown recently that AtXRN2 and AtXRN3 contribute to polyadenylation-mediated nuclear quality control for rRNA precursors and excised spacer fragments. Here, we report that in *xrn3* and *xrn2/3* mutants several mRNAs are significantly upregulated, without the effect on their stability. Readthrough transcripts detected upstream of these genes indicate that this may result from defects in polymerase termination. Chromatin immunoprecipitation confirmed increased PolII occupancy downstream of highly expressed genes, pointing to a role of XRN2/3 nucleases in the torpedo mechanism of termination. In contrast, transcriptional readthrough was not observed downstream of the 35S rDNA gene, so this mechanism may not operate for PolI.
Intergenic transcripts detected in *xrn* mutants belong to both classes, polyadenylated or lacking the poly(A) tails, and in some cases they are generated from both strands. It is therefore possible that in plants these aberrant RNAs may activate RNAi via RNA-dependent RNA polymerases, especially considering that plant XRN2/3 act as endogenous silencing suppressors.
Some of mRNAs increased in *xrn* lines are also elevated in plants lacking the major component of the NMD pathway, UPF1. This observation suggests an intriguing possibility that plant UPF1 may possess an additional function, separate from its role in NMD, which is related either to other pathways of RNA surveillance or in one of the RNAi mechanisms.

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Session 6
Organellar triangle - nucleus, mitochondrion, chloroplast

Plenary lectures

Photosystem II – bogenesis and repair

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Photosystem II (PSII) in plants and cyanobacteria performs highly oxidizing photochemistry of water splitting and a concomitant release of hydrogen equivalents. This chemistry exerts photodamaging effects on protein components of PSII resulting in an irreversible damage of the D1 protein, one of the heterodimeric polypeptides in the PSII reaction center complex. Damaged D1 protein is replaced by a newly synthesised D1 copy, which is co-translationally inserted into the PSII complex. This occurs by subjecting the entire PSII complex to a repair cycle in order to keep the photosynthetic light reactions functional. Such repair of PSII has several features common to the biogenesis of new PSII centers and requires a number of assembly factors and molecular chaperones functioning in the chloroplast stroma, thylakoid membrane and the thylakoid lumen compartments. Moreover, in plant chloroplasts there is a spatial segregation of the photodamage and repair of PSII, the actual photodamage takes place in highly appressed grana thylakoids whereas the PSII repair and assembly are taking place on stroma-exposed thylakoid domains where the ribosomes have an access. The relatively well characterized repair cycle of PS II involves an increasing number of various proteins assisting in the degradation of damaged proteins, in the insertion and folding of the nascent protein chains into the thylakoid membranes, in the assembly of the newly synthesized proteins and in reassembly of released proteins and different cofactors into functional PSII supercomplexes. Moreover, the phosphorylation of PSII core proteins has an important role in the repair process by facilitating the migration of damaged PSII complexes from grana to the stroma lamellae for repair.

Pentatricopeptide repeat proteins localized to both organelles and the nucleus

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Pentatricopeptide repeat (PPR) proteins make a novel class of RNA binding proteins universally found in eukaryotes (Schmitz-Linneweber and Small 2008). At the level of entire organisms, the functions of PPR proteins have extremely wide implications, going from human health to crop yield. At the cellular level, their function is mostly related to the biogenesis of organelles through the fulfilment of pivotal and essential gene expression processes. In plants, PPR proteins are particularly prevalent. There is e.g. 80 times more PPR genes in plants than in animals, thus making them one of the largest gene families. The explosion of PPR gene numbers appears to be related to the evolution of plant specific gene expression processes. Our research contributes to the characterisation of the functional diversity that PPR proteins have acquired during the evolution of eukaryotes. In particular, we investigate a new crosstalk pathway between mitochondria and the nucleus that involves a dual-targeted PPR protein bound to mitochondrial polysomes but also involved in expression regulation in the nucleus of genes encoding mitochondrial proteins (Hammani et al. 2011). We also investigate another subgroup of PPR proteins that we find to have RNase P activity in the three compartments where gene expression takes place in plants, i.e. mitochondria, chloroplasts and the nucleus (Gobert et al. 2010). With this work, we intend to understand better how the functions of multi-localised proteins affect the biogenesis of organelles in plants.

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Structural basis of short-term photoacclimation

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Excitation of photosystems I and II is regulated by a process known as "State transitions" that ensures the highest efficiency of photosynthetic electron transport. A mobile pool of light-harvesting complex II (LHCII) is involved in this process in response to N-terminal phosphorylation/dephosphorylation. This pool includes the minor C*ab* subunits CP26 and CP29, and a subset of major LHCII proteins. In a green alga *Chlamydomonas reinhardtii*, State transitions often occur via a switch between the linear (LEF) and cyclic photosynthetic electron flow (CEF). Using electron microscopy and single particle analysis of negatively stained State 1 and State 2 LHCI-PSI supercomplexes, we identified changes in the LHCI-PSI ultrastructure during state transitions in *C. reinhardtii (*Kargul *et al.* 2003, 2005). We showed that redox-dependent multiple phosphorylation of the minor C*ab* subunit CP29 and its docking in the vicinity of the PsaH core subunit of PSI form two essential steps of State transitions, whereby the structural changes of CP29, induced by reversible phosphorylation, determine affinity of the mobile LHCII for either of the two photosystems (Kargul *et al.* 2005, Turkina *et al.* 2006). Recently Minagawa and colleagues reported isolation of the elusive CEF megacomplex that forms in the State 2-induced *C. reinhardtii* cells (Iwai *et al.* 2010). We will present the first structure of this complex derived from single particle analysis and electron microscopy of negatively stained particles.

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Reactive Oxygen Species (ROS) and retrograde stress signalling from mitochondria and chloroplasts

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The production of reactive oxygen species (ROS) increases in plants under stress. ROS can damage cellular components, but they can also act in signal transduction to help the cell counteract the oxidative damage in the stressed compartment. H_2O_2 may induce a general stress response, but it does not have the required specificity to selectively regulate nuclear genes required for dealing with localized stress, e.g., in chloroplasts or mitochondria. We here argue that peptides deriving from proteolytic breakdown of oxidatively damaged proteins have the requisite specificity to act as secondary ROS messengers and regulate source-specific genes and in this way contribute to retrograde ROS signalling during oxidative stress.

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Oral presentations

Temporal changes of the mitochondrial and nuclear genome expression in response to a defect in mitoribosome assembly

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The nuclear *Rps10* gene of *Arabidopsis thaliana* encodes the S10 protein, which is a part of the small subunit of mitochondrial ribosome. Using RNAi strategy we obtained transgenic lines with silenced expression of this gene. Triggering of the *Rps10* silencing results in a mixture of fully assembled and *assembly-defective* mitoribosomes. The goal of this work was to determine alterations at different expression levels of genes coding subunits of OXPHOS complexes in response to impaired assembly of mitoribosomes in *rps10*.
Two phenotypes of hemizygous *rps10* transformants, P2 and P3, differing by the onset of silencing, were studied. We have showed that in both P2 and P3 plants the copy number and transcript abundance of mitochondrial-encoded OXPHOS genes are increased while the level of nuclear-encoded transcripts is decreased. A strong correlation between abundance of mitochondrial genes and their transcripts suggests that a main reason of the transcript increase is higher level of mitochondrial DNA. We have also found, that this increase is more pronounced with the age of plants. Moreover, the abundance of nuclear-encoded transcripts was raised with time. In consequence, transcript level of some nuclear genes returned to the level of wild type plants in old rosettes. Abundance of mitochondrial and nuclear transcripts in polysomal fractions showed the same divergent trend like was observed for the steady-state level of transcripts. However, studies at the protein level have shown that both mitochondrial and nuclear-encoded subunits of OXPHOS complexes in *rps10* were at lower level compared to wild type plants. It was noticeable, that nuclear-encoded subunits were reduced to a lesser degree than mitochondrial-encoded ones. The abundance of complex-bound OXPHOS subunits was also reduced, but the relative stoichiometry of nuclear and mitochondrial subunits within OXPHOS complexes was proper. Comparison of different levels of genes expression strongly argues that in response to a defect in assembly of mitoribosome, expression of OXPHOS genes is adjusted already at mitochondrial DNA and steady-state transcript levels but the proper stoichiometry between nuclear- and mitochondrial- encoded subunits is achieved exclusively at the level of the complex assembly.

Correlation between structure and function of bean chloroplasts during biogenesis

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Chloroplast biogenesis during greening of etiolated bean seedlings was analyzed. This development models the natural initial growth of seedlings under the ground and later growth above the earth surface leading to fully differentiated and functionally mature plastids. This process consists of prothylakoids (PT) and prolamellar bodies (PLB) transformation into grana and stroma thylakoids (Mostowska 1986). These structural changes were correlated with the levels of the key photosynthetic proteins and the function of the photosynthetic apparatus (measured by modulated fluorescence of chlorophyll *a* with a DUAL-PAM-fluorometer and fluorescence at 77 K) during the main stages of bean chloroplast biogenesis.
We found that the formation of grana does not require the presence of all the main photosynthetic proteins at the level comparable with the one observed in fully developed leaves (Garstka et al. 2007). The correlation between structural and functional changes at given times of chloroplast biogenesis provides a way to elucidate the role of particular proteins in the stabilization and transformation of PLB and PT into thylakoid membranes.

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Diverse responses of cauliflower mitochondrial proteome to cold and heat stress conditions

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The aim of our study was to study the response of cauliflower mitochondrial proteome in relation to cold and heat stress and after post-stress plant adaptation. We identified 27 spots showing major variations from 2D (IEF/SDS-PAGE) gels. They represented mostly some carbohydrate metabolism enzymes, HSPs, translation factors, processing peptidase and ATP synthase subunits. Variations in the abundance of ATP synthase subunit 1 were additionally verified by 2D Western immunoassays. Using BN-PAGE, 2D DIGE and protein crosslinking with DSP, the destabilisation of SC I+III_2 which lasted during heat adaptation was found; however, we noticed no significant variations in the transient interactions between respirasome components and respirasome/ ATP synthase. Some alterations in the subunit content of CI, CII and ATP synthase during heat adaptation were also reported. For instance, few heat-stress responsive proteins, including ATP synthase and CII subunits, were identified by LC-MS/MS from DIGE gels. Using *in gel* assays, we showed significant decrease of CI, CII, CIV and ATP synthase activity in all stress conditions. Additionally, the profile of mitochondrial translation was affected especially by heat treatment and heat adaptation. We conclude that biogenesis of cauliflower mitochondria on various levels is significantly, but not equally affected by investigated thermal stress conditions, which considerably extends results obtained from cell culture models.

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Expression of phototropins in Arabidopsis leaves: developmental and light regulation

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Phototropins are blue light receptors involved in reactions responsible for plant adaptation to changes in light conditions. *Arabidopsis* has 2 genes coding for phototropins. Phot1 and phot2 have different light sensitivity but strongly overlapping functions (Christie 2007). In seedlings, both phototropins mediate phototropism, whereas in leaves they are responsible for leaf expansion and flattening, stomatal opening and chloroplast accumulation. Phot2 alone mediates chloroplast avoidance response to strong light. Phototropin expression in seedlings is reported to be regulated by light in a different manner: the expression of *PHOT1*decreases after light treatment (Kang et al. 2008), whereas *PHOT2* is induced by light (JArillo et al. 2001). We examined whether this expression pattern is conserved during plant development. We found that levels of both phototropins are higher in adult leaves than in seedlings, and that white light downregulates *PHOT1* expression and upregulates *PHOT2* in adult leaves. However, in our experimental conditions white light did not influence *PHOT1* mRNA levels in seedlings. Phototropin mRNA level decreased during darkening-induced leaf senescence, but the influence of light was still visible. DCMU treatment resulted in no change in phototropin expression patterns, leading to a conclusion that the light regulation is photosynthesis-independent. In order to identify components involved in light perception we tested expression of phototropins using red and blue light and photoreceptor mutants (*cry1, cry2, cry1/cry2, phyA, phyB, phyA/phyB, phot1, phot2*). We found that both red and blue light affect phototropin expression to a similar extent, and that cryptochromes and phytochromes act together in regulating the mRNA level of phototropins.

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Poster presentations

6.1. Role of phospholipase C in calcium flux and cytoskeleton organization in Arabidopsis during chloroplast movements

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Phototropin-mediated movements of chloroplasts play an essential role in optimizing the photosynthetic activity in higher plants. The actin cytoskeleton and calcium ions play important roles in the mechanism of movements (Anielska Mazur and Gabryś 2009). However, the regulators of actin dynamics and the exact role of cytosolic calcium gradient, remain poorly defined. Transgenic Arabidopsis lines expressing aequorin have shown a transient increases in cytosolic calcium under blue light irradiation when tested in luminometer. Here we show that, PtdInsP2 (PIP2), are involved in shaping calcium waves during chloroplast movements. Thus, PIP2 may have a role in maintaining the integrity of actin cytoskeleton in *Arabidopsis* mesophyll cells. A GFP fusion to the PIP2 binding domain of mammalian PLCδ 1, points to a role of PIP2 during chloroplast movements, by showing stronger GFP fluorescence in regions of cytosol irradiated with blue light in *Nicotiana benthamiana* mesophyll cells. We used pharmacological approach to investigate inhibition of PLC pathway (via neomycin) on chloroplast movements, calcium response and actin network arrangement. Using transgenic aequorin lines we show suppression of calcium transients on blue light irradiation, in the presence of neomycin. Moreover, neomycin shows a dose-dependent inhibition of chloroplast movements. In the same way, alexa fluor staining of actin shows actin disruption in the presence of neomycin. Therefore, we suggest that PIP2 causes internal stores calcium release during chloroplasts movements and it interact with the actin cytoskeleton.

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6.2. The effects of auxin and cytokinin on cell proliferation and induction of endoreplicaction DNA in cell suspension culture of *Nicotiana tobacum* (BY-2)

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Plant organogenesis requires a strict balance between cell proliferation and differentiation. This is subject to a complex regulatory network which, in some cases, depends on the action of a variety of plant hormones, including auxins and cytokinins. Their role in cell cycle control is well documented, but effects on transition from mitotic cycles to endocycles which frequently accompany cell differentiation remains largely unknown.
The effects of auxin and cytokinin deprivation on nuclear DNA content in cell suspension culture of tobacco (BY-2) was investigated in the present study. These cells require auxin (2,4-D) addition to the culture medium for proliferation but are cytokinin-independent. Effects of auxin and cytokinin deprivation can be observed on cultures cultivated on medium without 2,4-D or with simvastatin (a cytokinin synthesis inhibitor). The number of cells and nuclear DNA content increasing during culture growth cycle for four variants medium were measured in the following variant: i) Linsmaer and Skoog (LS) medium (control), ii) LS medium without 2,4-D, iii) MS medium with simwastatin, and iv) LS medium without 2,4-D and with simvastatin. The obtained data suggest that in the cell of BY-2 suspension culture auxin or cytokinin deprivation leads to significant reductions of mitotic activity and can induce transition from mitotic cycles to endoreplication cycles. This second effect indicates inhibition of mitototic activity by auxin or cytokinin deprivation and DNA contents multiplication by endoreplication. Majority of cells in cultures cultivated without auxin showed 4, 8, 16, 32, and sporadically 64C DNA content. Similar effect was observed in the cultures with cytokinin deprivation (simvastatin addition). Increase in the nuclear DNA levels was connected with increase in cell sizes.

6.3. Functional importance of Deg5 protease under non-stressing conditions as studied by a new *Arabidopsis thaliana deg5* mutant

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Deg5 is a serine-type protease peripherally attached to luminal side of thylakoid membrane. There are indications that this enzyme is engaged in degradation of a few photosystem II proteins in response to various stresses. Much less is known concerning Deg5 role in plant growth and development under comfortable conditions.
To study the functional importance of this protease under non-stressing conditions a new Deg5 insertion line SAIL_645_D07 was obtained from Nottingham Arabidopsis Stock Center in which T-DNA is inserted in the 4th intron of At4g18370 gene (*DEG5*). Germination of the seeds of this line resulted in segregating population of heterozygous plants. Based on PCR analysis mutants homozygous for T-DNA insertion were identified among F2 progeny. By using a series of polyclonal antibodies raised against Deg5 and other chloroplast-targeted Deg proteases (Deg1, Deg2, Deg8) it was demonstrated that the mutant plant was totally lacking Deg5 whereas Deg1 and Deg8 content remained unchanged and Deg2 seemed to be moderately, though significantly repressed with regard to value typical for wild type plants protein.
For up to two weeks after germination the growth rates of the wild type and mutant leaves were identical but a steep increase in the growth rate of mutants' leaves of all whorls occurred after that time point so that eventually expanded, 4-weeks-old leaves became markedly larger than wild type ones with an exception of the ones belonging to fourth whorl. This can be contributed to changes in length and width of leaf blade since both these values were significantly increased in the mutant leaves in relation to wild type ones. To have a better insight into the importance of Deg5 protease for various aspects of chloroplast metabolism starch accumulation during diurnal cycle and Lhcb1-6 apoproteins abundance have been analysed in the mutant leaves vs the ones of wild type plants.

6.4. Formation of shade–type chloroplasts may be expression of antagonistic interaction between herbicides MCPA and chloridazon to duckweed *Lemna minor*

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MCPA (auxin-like growth inhibitor) and chloridazon (CHD) (PSII-inhibitor) are the most commonly used herbicides for weed control in Polish agriculture. The extensive use of these chemicals poses a threat to non-target aquatic organisms, due to their leaching and runoff from fields. Moreover, herbicides can co-occur and interact, potentially increasing their toxicity to aquatic biota. Previously, toxicity of MCPA and CHD was determined in growth inhibition tests with *Lemna minor*. The EC_{10} and EC_{50} values based on the fronds number reduction observed after 7 days were calculated. They were: 0.8 and 5.4 mg/dm^3 for MCPA and 0.7 and 10.4 mg/dm^3 for CHD. Here, herbicide mixtures at concentrations corresponding to the EC values were used to assess their interactive effects. The two-way ANOVA and Abott's formula used for this assessment revealed an antagonistic interaction between MCPA and CHD. Analysis of pigments content, measurements of chlorophyll fluorescence *in vivo* and ultrastructure observations were performed to probe this interaction. Intensified chlorophyll pigmentation accompanied by formation of chloroplasts rich in lamellar system as well as improved fluorescence parameters suggests the so-called "shade adaptation". Therefore we propose that the formation of shade-type chloroplast may be assumed as an expression of antagonistic effect of MCPA and CHD.

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6.5. Photoassimilate translocation in nitrogen-fixing root nodules of *Medicago truncatula*

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In higher plants, sucrose, major transport form from photoassimilated carbon, is transported from source organs to sink organs via the phloem. Root nodules induced by nitrogen-fixing bacteria represent strong facultative sinks. The process of biological nitrogen fixation demands a high level of sucrose supply. Phloem unloading and post-phloem transport of sugars can proceed symplastically via plasmodesmata or apoplastically via plasma membrane sugar transporters. It is not clear which kind of route – symplastic or apoplastic in the cells of indeterminate root nodules is in favour. It is already known that during nodule organogenesis the symplastic connection between plant phloem and nodule initials is created. To understand how sucrose is transported and incorporated into metabolism in nodules of MT, 6(5)carboxyfluorescein diacetate solution was introduced to the abraded surface of the basal leaflet.
6(5)carboxyfluorescein diacetate (6(5)CFDA) is a phloem-mobile tracer and it is widely using to study symplastic continuity in leaves and stems. It presumes to monitor sap translocation in real time, during short- or long-term experiments. 30 minutes or 2 hours after CFDA application, freehand sections were prepared and immediately observed with a microscope. Firstly CF-associated fluorescence appeared in uninfected cells (after 30 min.). After 2hr fluorescence in infected cells was detected.The results indicate that pathway flow of assimilates between noninfected and infected cells in nodules of *Medicago truncatula* is considered to be symplastic. Uninfected cells may mediate as a pathway to the infected cells.

6.6. Exploring the mechanisms of nucleolar dominance – epigenetic studies in *Brachypodium* allopolyploids

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Nucleolar dominance was initially described by Navashin in interspecific hybrids of *Crepis* (Navashin 1934). This phenomenon takes place in some plant and animal hybrids, and consists of selective suppression of activity of the rRNA gene set inherited from one ancestral species. During the last decade, considerable attention has been paid to discover the molecular mechanisms which are responsible for a particular state of ribosomal DNA-linked chromatin. Several studies using chromatin immunoprecipitation, bisulphite-mediated methylcytosine mapping (Lawrence et al. 2004), DNA hypomethylating agents and immunolocalisation of modified histons (Earley et al. 2006) clearly reveal that nucleolar dominance has an epigenetic origin. This phenomenon has also been described in the *Brachypodium* genus (Idziak and Hasterok 2008). Several *B. distachyon* cytotypes are natural allotetraploids (2n=30) with putative parental genomes originating from diploid *B. distachyon* (2n=10) and the cytotype known as ABR114 (2n=20). Silencing of ABR114 ribosomal genes was confirmed in these hybrids.
Here we demonstrate preliminary results of an epigenetic study based on immunolocalisation of DNA methylation and histone modifications in several *B.distachyon* allopolyploid cytotypes, originating from distinct geographical locations, such as South Africa (ABR101), Portugal (ABR113) and Australia (ABR137).

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6.7. Chromosome arrangement in interphase nuclei of the model grass *Brachypodium distachyon*

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Plants are considerably more difficult to investigate at the cytogenetic level than animals because of large amounts of repetitive DNA in their nuclear genomes. For a long time this hampered the painting of individual chromosomes using fluorescence in situ hybridization which is considered to be the most precise method for the analysis of the distribution of chromosome territories during interphase. Chromosome painting in plants is enabled by a special and very rare combination of several features, such as a small and compact nuclear genome of the plant, completion of its genome sequencing, availability of large genomic DNA fragments in the form of ordered BAC libraries as well as advanced molecular cytogenetic methodology. So far this technique has been successfully used only in the case of a dicot model organism, *Arabidopsis thaliana* and its close relatives (Pecinka et al. 2004). Recently (IBI 2010, Idziak et al. 2011), for the first time in the monocots, chromosome painting has also been successful in *Brachypodium distachyon*, a model species for temperate cereals and forage grasses.
Here, we demonstrate the results of a preliminary study of chromosome distribution and associations in 3-D interphase nuclei of *B. distachyon*. The primary aim was to determine if there is any specific arrangement of homologous or heterologous chromosomes in somatic cells, and to define the associations between arms of the homologous chromosomes.

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6.8. Photochemical characterization of mesophyll and bundle sheath chloroplasts of C4 plants of NADP-ME subtype.

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Leaves of C4 plants contain the two distinct types of photosynthetic cells, mesophyll (M) and bundle sheath (BS). NADP-ME species show quantitative differences in grana development in BS chloroplasts and thus variation in ATP and NADPH production. We examined three grasses: *Echinochloa crus-galli*, *Digitaria sanguinalis*, and maize (all NADP-ME), where BS chloroplasts differed in granal index.BS thylakoids in maize exhibit only PSI activity and little is known about the amount, composition and the function of PSII centers. We have investigated activities of PSI and PSII, amounts of thylakoid proteins, composition of Chl-P complexes in M and BS chloroplasts in these plants under the same light conditions during growth. M and BS chloroplasts were obtained mechanically. Chl-P complexes were analyzed by polyacrylamide native gel electrophoresis, fluorescence excitation. The electron transport activity was determined by oxygen electrode and DCPIP assay. Proteins were identified by immunodetection and chemiluminescence. Fluorescence of dark-adapted leaves was measured by the saturation pulse method and quantum yields of PSII was calculated. It has been found that M and BS chloroplasts of examined species differed in Chl-P complexes, PSII was more active in M than in BS chloroplasts where was higher amount of oligomeric form of LHC. The results indicated that PSII is inactive in maize BS chloroplasts, whereas in *D. sanguinalis* and *E. crus-galli* PSII activity was only twice lower than in M, but PSI activity was 2-3 fold higher in BS. There was correlation between composition of Chl-P complexes and activities of PSI, and PSII in both types of chloroplasts.Higher activity of PSI in BS chloroplasts was accompanied with larger amount of PSI-LHCI. The results show that M and BS chloroplasts in different C4 species, even the same subtype, differ in organization and activities under the same light conditions during growth.

6.9. Changes of chloroplasts, mitochondria and nucleus in Tobacco rattle virus infection

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The objective of our ultrastructural studies was to present the participation of cellular organelle during the process infection of *N. tabaccum cv. Samsun*. Tobraviruses are characterized as having a genome consisting of two species of (+)ssRNA, RNA1 and RNA2, contained in straight rod-shape particles of two lengths. The long (L) ones, infective, 180 to 197 nm long, encode proteins involved in multiplication of virus genome and intercellular transport (MacFarlane 1999). The short (S) ones are non-infective, 43 to 114 nm long, encode capsid proteins (CP) of both particles and two nonstructural proteins involved in transmission by nematodes (Hernandez et al. 1997). Plants with four levels of leaves were inoculated mechanically with the PSG strain of TRV. Ultrastructural analysis revealed that TRV particles of two lengths are presented in encapsidated (multiplying, M) and uncapsidated (nonmultiplying, NM) forms in dispersed and organised inclusion type. Recent investigations have revealed presence of TRV particles only in cytoplasm of mesophyll cells and replication areas are connected with cytoplasm. We observed intense, significant changes in internal structure of chloroplasts, mitochondria and nucleus. It was noticed considerable deformations of mitochondria connected with its enlargement and the presence of internal vesicles. For the first time it was observed TRV particles in mitochondria, chloroplasts and cell nucleus. The disorganized chloroplasts lost a typical lamellas arrangement whereas M and NM TRV particles of two lengths are visible in stroma. Inside cell nucleus was observed electron-transparent areas with fibril material and infective NM TRV particles.

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6.10. Impaired stomata functioning in Arabidopsis *phb2/phb6* mutant

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Prohibitins (PHB) are highly conserved proteins of all eukaryotic cells whose functions have been well recognized in humans and yeasts but their role in plants has not been fully elucidated. PHBs are possibly involved in multiple developmental processes, such as mitochondrial biogenesis and homeostasis, cell cycle, ageing and senescence, and finally in the plant cell response to different stresses. We characterized comparatively morphology and anatomy of *Arabidopsis* double mutant *phb2/phb6* and WT plants to better understand PHB function, specifically prohibitin involvement in the water stress response.
Our analyses showed that the mutant rosettes were smaller and lost water more readily than the WT controls. These differences may result from the impaired regulation of transpiration by the dysfunction of stomata closure. The comparison of *phb2/phb6* and WT revealed statistically significant differences in the stomata size and the degree of their closure. *phb2/phb6* mutant, contrary to WT plants, was not able to close completely the stomata, neither *in vivo* nor in experiments with stomata closure induction by 1 μM ABA or 0.5 M mannitol. Coincident with stomata closure dysfunction, the transpiration tests showed a greater loss of water in mutant than in WT plants. The application of exogenous ABA to the rosettes did not protect mutants against water loss, on the contrary to WT, suggesting that the stomata dysfunction could be related to the signaling impairment rather than to disturbed ABA biosynthesis or transport. We postulate that the functional and developmental problems observed in *phb2/phb6* plants may result from prohibitin involvement in the signaling pathways in response to stress conditions.

6.11. Acclimation to low irradiance in *Arabidopsis thaliana* leaves - kinetics of changes in LS Rubisco and reactive oxygen species abundance

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Rubisco catalyzes photosynthetic CO_2 fixation and photorespiratory carbon oxidation and is the most abundant protein in biosphere. In terrestial plants Rubisco exists as a holoenzyme composed of eight large (LS) and eight small subunits (SS). Rubisco abundance varies during a leaf lifecycle and under various stressful conditions. It is suggested that LS Rubisco abundance may be regulated at the level protease-mediated hydrolysis; however, only limited information is available on the triggering mechanisms that cause LS Rubisco enzymatic degradation. Some data point to reactive oxygen species produced in response to various stresses as a trigger in the degradation of LS Rubisco – they may induce oxidative modifications of LS Rubisco molecule which render it more susceptible to proteases-mediated hydrolysis.
To study catabolism of LS Rubisco *Arabidopsis thaliana* plants were grown under moderate irradiance (250µmol quanta m^{-2} s^{-1}) and then acclimated to low irradiance (25 or 50 µmol quanta m^{-2} s^{-1}) for 5min to 96h. LS Rubisco abundance in lysates of chloroplasts isolated from leaves of moderate irradiance-grown and low irradiance acclimated plants were quantitated by immunoblotting. Acclimation to low irradiance was found to be accompanied by a clear decrease in LS Rubisco abundance, to about 60% of its initial level in leaves of plants acclimated for 24h to 50 µmol quanta m^{-2} s^{-1}. No clear signs of H_2O_2 or O_2^- burst preceding the maximal decrease in LS Rubisco abundance was found yet a prominent rise in malondialdehyde (MDA) was identified after 6h of acclimation to both low light irradiances and this suggests that in fact the acclimation to low irradiance was accompanied by an oxidative stress thus decline in LS Rubisco might be ascribed to oxidative modification of its molecule.

6.12. AFM investigation of topography and elastic properties of chloroplasts

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The Atomic Force Microscope (AFM) allows for high-resolution imaging of the topography and elastic properties of biological samples under physiological conditions. Elastic properties determine the resistance of organelles to the mechanical stress and provide insights into their internal structure. The main challenge in the AFM investigations of biological samples is their immobilization. Proper immobilization technique should ensure strong attachment of the sample without affecting its viability.
We devised an efficient procedure for immobilization of chloroplasts, employing the galactose – specific lectin RCA_{120} from castor bean. The lectin strongly agglutines intact chloroplasts, binding preferentially to the chloroplast envelope (Schroder and Petit 1992). Immobilized chloroplasts were successfully imaged both in the contact and tapping modes. The apparent Young's modulus was calculated from force-distance curves. We found that the chloroplasts exhibit strong dependence of apparent Young's modulus on the loading - rate, which points to their viscoelastic nature.

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6.13. Subcellular localization of the family of acyl-activating enzymes of Arabidopsis

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The acyl-activating enzyme (AAE) subfamily of AMP binding proteins comprises 18 members that appear to have a diverse range of functions in Arabidopsis. Determining the subcellular location of the enzymes is will help to predict the function of uncharacterised members or to confirm the metabolic function of others. Of the 18 members of the AAE enzyme subfamily, proteomics and/or GFP targeting studies have identified or putatively identified 6 peroxisomal AAEs and 2 others have been localised to plastids. We have taken a comprehensive approach to gain more information on function by identifying the subcellular location of all AAEs using GFP fusion constructs stably expressed in Arabidopsis. We have confirmed or assigned locations to 8 peroxisomal, 6 plastidic and 3 cytosolic AAEs. One, AAE2, is unique in that it appears to be mitochondrial. Interestingly, masking the PTS-1 of the 8 peroxisomal AAEs resulted in targeting 6 of them to plastids, indicating that they possess viable plastid targeting signals. Since organellar proteomics have not found these AAEs in plastids, it appears that peroxisomes out-compete plastids for importing these AAEs. This raises interesting questions about competition *versus* dual localisation among organelles for importing their protein components.

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6.14. Does the formation of photosystem I trimer in cyanobacteria have a photoprotective function?

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Cyanobacteria conduct photosynthesis in the way that very much resembles plant photosynthesis. The two photosystems and electron carriers are organized in the plant-like system. Polypeptide composition of photosystem I and photosystem II is similar but not identical as in higher plants. Photosystem I of cyanobacteria exhibits a unique capability of oligomerization into trimeric supercomlexes. In wild type cells trimerization level increases with growth temperature increase. There have been several attempts to uncover the significance of this process, but to date the functional differences between the two photosystem I forms remain unclear. Our earlier studies with trimerless psaL$^-$ mutant show that the absence of photosystem I trimers results in elevated carotenoid content in *Synechocystis* sp. PCC6803, what is most pronounced for myxoxanthophyll. Similar response had been previously reported for various stress conditions.

Here, we present oxidation-reduction kinetics and maximum changes of P700 signals in cyanobacterium *Synechocystis sp.* PCC6803 wild type and psaL$^-$ mutant measured by Dual-PAM-100 Measuring System. With the use of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and methyl viologen (MV) we were able to investigate both linear and cyclic electron transfer around photosystem I. Significant differences were found between response of wild type and mutant P700. We postulate that photosystem I trimer formation is important for one of the photoprotection mechanisms that occur in cyanobacterial thylakoids.

6.15. RbcX proteins from *Arabidopsis thaliana* – chaperones involved in Rubisco biogenesis

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Form I of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) is composed of eight large (RbcL) and eight small (RbcS) subunits. Assembly of these subunits into functional holoenzyme needs the assistance of additional assembly factors. One of such factors is RbcX, which has been shown to act as a chaperone in the assembly of most cyanobacterial Rubisco complexes expressed in heterologous system established in *Escherichia coli* cells. Analysis of *Arabidopsis thaliana* genomic sequence revealed the presence of two genes encoding putative homologues of cyanobacterial RbcX protein: *AtRbcX4*(*At4G04330*) and *AtRbcX5* (*At5G19855*). *Arabidopsis thaliana* plants were transformed with RbcX proteins encoding genes fused with C-terminal Strep-tag. Pulldown experiment enabled establishment of stromal protease cleavage sites of investigated peptides and identification of their putative "partners" by successive mass spectrometry analysis. Apart from binding RbcL AtRbcX4 protein seems to interact with β subunit of chloroplast ATP synthase. Using commercially available antibodies raised against Strep-tag sequence we were also able to confirm chloroplast localization of both AtRbcX proteins. Quantitative RT-PCR experiment suggest that under various stress condition *AtRbcX5* in most cases is transcribed at relatively stable level, while transcript level of *AtRbcX4* changes significantly. However further crystallization studies are planed we present our first attempts to elucidate secondary structure of these proteins using CD spectroscopy. Both of RbcX proteins from this plant show assembly chaperone activity during biosynthesis of Rubisco from thermophilic cyanobacterium *Thermosynechococcus elongatus* in *Escherichia coli* cells. Presented results are the first known data concerning to role of RbcX proteins in higher plant Rubisco assembly mechanism.

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6.16. Changes in lipid and pigment content upon a heavy metal action

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A common consequence of most abiotic and biotic stresses is an increased production of reactive oxygen species. Exposure of plants to heavy metals induces oxidative stress developed as lipid peroxidation and an oxidative burst generating free radicals and active oxygen species. As a primary mechanism of toxicity, heavy metals may cause disruption of cellular and organelles membranes, resulting in rapid impairment of membrane function and loss of membrane integrity. Susceptibility to heavy metal-induced membrane permeability and toxicity may be partly dependent on the lipid composition.
In the present studies we have investigated the effect of lead (Pb) and cadmium (Cd) on the membrane lipid composition and photosynthetic pigment content. Moreover, the level of lipid peroxides was monitored. The data show that upon Cd or Pb 6-hours treatment the amount of the major thylakoid lipids – monogalactosyldiacylglycerol (MGDM) and digalactosyldiacylglycerol (DGDG) increased. However, the longer incubation time (24 hours) resulted in decrease of galactolipid level, which could be caused by peroxidation processes. Interestingly, in contrast to DGDG content, which was shown to increase for the longest incubation time (72 hours), SQDG was accumulated only at the early stage of heavy metal treatment.
Photosynthetic pigment analysis revealed an elevated level of antheraxanthin in plants treated with Pb and even higher in those treated with Cd. Violaxanthin content markedly increased in wheat plants treated with Pb and Cd for 6 and 72 hours, respectively, while neoxanthin level was dependent both on concentration and incubation time. The content of lutein and zeaxanthin in stressed plants was not significantly changed.
The results suggest that the phytotoxic effects of Cd and Pb in wheat seedlings is achieved by an enhanced production of ROS and subsequent lipid peroxidation. Changes in wheat membrane lipid composition might have a stabilizing effect on photosynthetic membranes during heavy metal stress.

6.17. Short term NO pre-treatment alters photosynthesis during development of young apple seedlings

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Nitric oxide (NO) is an intracellular signaling molecules playing important role in diverse processes in plants such as: germination, senescence or responses to stresses. Seedlings developed from dormant apple embryos (*Malus domestica* Borkh. cv. Antonówka) are characterised by one of morphological anomalies: asymmetric growth and greening of cotyledons. The aim of this study was to investigate some aspects of NO involvement in light signaling during apple embryo dormancy alleviation and seedling development.
Short term (3h) pre-treatment of dormant apple embryos with NO accelerates growth of embryonic root and stimulates chlorophyll biosynthesis in both cotyledons. Similarly, short term (3h) NO fumigation of 5 days old dormant seedling (with typical anomalies) led to greening of white cotyledon. The stimulatory effect of NO on chlorophyll concentration in young seedlings was associated with development of chloroplasts. As a consequence photosynthetic activity of the seedlings developed from embryos pre-treated with NO or seedlings with abnormalities shortly fumigated with NO was higher than in control. Moreover, chlorophyll *a* fluorescence indicated that NO did not produce a severe effect on photochemical efficiency of PSII. It is also possible, that NO plays a key role in anterograde/retrograde signalling cascade. Synthesis of the RubisCO small subunit was stimulated by NO pre-treatment of both dormant apple embryos and 5-day old control seedlings. This process was correlated with induction of chlorophyll biosynthesis, as well as symmetric development of cotyledons.

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6.18. Cytological maize response with different level of resistance to sugarcane mosaic virus (SCMV) infection

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Sugarcane mosaic virus (*SCMV*) is one of the representant in the genus *Potyvirus*. SCMV has filamentous, flexuous particles +ssRNA. The natural hosts are restricted to some species of the Poaceae: sugarcane, maize and sorghum. Cultivated cereals such as wheat, barley, rye and rice are rarely infected naturally (Trzmiel and Jeżewska, 2006). The aim of our study was to find and compare effects of cytological response of maize plants with different level of resistance to sugarcane mosaic potyvirus (*SCMV*) infection. The three leaves seedlings of maize cultivars *Moncada* (susceptible) and *Cyrcon* (resistant) were mechanically inoculated with virus suspension obtained from susceptible infector plant. Inoculated and non- inoculated leaves were collected 4, 10, 24 hours (hpi) and 1, 3 weeks (wpi) post infection. In compared *Moncada* and *Cyrcon* cvs, first symptoms occured respectively: 6 and 21 days post inoculation. Ultrastructural analysis showed presence of *SCMV* particles and cytoplasmic inclusions in leaves mesophyll and vascular tissues both *Moncada* and *Cyrcon* cultivars. SCMV particles occured one week after infection and two and three weeks post inoculation at non- infected leaves of *Moncada*. In the case of *Cyrcon*, SCMV particles were found three weeks post inoculation only at non- infected leaves. Viral particles were located both in mesophyll and vascular tissues. Actually our ultrastructural investigations concentrate on maize generative organs tissue infected with SCMV.

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6.19. Effect of physicochemical parameters of inverted micelles on violaxanthin and diadinoxanthin de-epoxidation

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Violaxanthin de-epoxidation and diadinoxanthin de-epoxidation are light dependent steps in one of the most important photoprotecting processes called respectively violaxanthin and diadinoxanthin cycle. Violaxanthin cycle which operates in all higher plants, ferns, mosses and several groups of algae, involves interconversion between: violaxanthin (Vx), antheraxanthin (Ax) and zeaxanthin (Zx). These reactions are catalyzed by violaxanthin de-epoxidase (VDE) and zeaxanthin epoxidase. Diadinoxanthin cycle is present in diatoms in which interconversion between diadinoxanthin (Ddx) and diatoxanthin (Dtx) occurs. Enzymes catalyzing these reactions are diadinoxanthin de-epoxidase (DDE) and diatoxanthin epoxidase.
In the present studies influence of lipids on de-epoxidation of Vx and Ddx was investigated. In particular, the dependence between the conversion of Vx into Ax and Zx as well as Ddx to Dtx and the molecular dynamics of hydrophobic fraction of aggregates formed by inverted micelles, which are necessary for de-epoxidation, was studied. Thickness of the hydrophobic fraction of the aggregates, size of the inverted micelles, suggested by mathematical description of these structures and solubility of Vx and Ddx in various kind of lipids were the next tested parameters. Obtained results show that the rate of de-epoxidation is strongly dependent on physical/ chemical properties of lipids. The key role for VDE and DDE activation play non-bilayer lipids and the parameters of inverted micelles created by them, such as thickness, diameter and molecular dynamics of their hydrophobic core. Mutual orientation of enzyme and substrate molecules and dilution of pigments by lipids are postulated as main mechanisms to explain the results.

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6.20. Chloroplast protease AtDeg2 is engaged in stress responses in Arabidopsis

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AtDeg2 is a serine-type chloroplast protease, peripherally attached to the stromal side of thylakoid membrane. To have a better insight into the physiological importance of this protease its possible role in the degradation of Lhcb4-6, i.e. the apoproteins of minor peripheral antennae of photosystem II was investigated. It has been demonstrated that exposure of detached laves of *Arabidopsis thaliana* wild type plants to six short time (3h) stresses led to significant disappearance of Lhcb5, Lhcb6 (high salt, desiccation, wounding, cold, heat, high irradiance) and Lhcb4 (heat). The disappearance observed *in vivo* was continued in the *in vitro*system, in which the thylakoids isolated from previously stressed leaves were incubated for next 6h in darkness but the degradation of Lhcb6 was arrested in thylakoid samples isolated from high salt, wounding, heat and high irradiance-treated leaves in the presence of aprotinin, a typical inhibitor of serine-type proteases. However, the aprotinin did not influence significantly the disappearance rate of Lhcb 4 or Lhcb5 in any *in vitro* assay performed. Two independent T-DNA insertion lines devoid of Deg2 were found to resist Lhcb6 disappearance *in vivo* under high salt, desiccation, wounding, cold, heat, high irradiance therefore our results demonstrate that Deg2 plays an important role in the short-time stress related degradation of Lhcb6. Furthermore, by applying chlorophyll *a* fluorescence measurements it was shown that Deg2 was engaged in protection against photoinhibition.

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6.21. Calcium localization in *Arabidopsis thaliana* mesophyll cells after blue light treatment, determined by X - ray microanalysis

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Calcium ions serve as a second messenger in plant responses. In mesophyll cells, they take part in signal transduction from phototropins, blue light photoreceptors responsible for rapid movement reactions of plant cells and organs. Two phototropins of *Arabidopsis thaliana* show different light sensitivities, but share redundant functions. Although they both mediate chloroplast accumulation, chloroplast avoidance response is controlled solely by phot2 (Christie 2007). Phototropins elevate cytosolic Ca^{2+} after activation by blue light. Ca^{2+} comes from intracellular stores and extracellular spaces. In higher plants both types of chloroplast movements need Ca^{2+} and internal calcium stores are critical for these processes (Harada and Shimazaki 2007). Different sources of calcium mobilized by phototropins probably trigger a specific spatio-temporal response pattern, which decides about the signaling process. Localization of calcium ions after blue light treatment in mesophyll cells of *Arabidopsis*, WT and phototropin mutants, could clarify how light intensity directs chloroplast movement. Free calcium ions were precipitated with potassium hexahydroxyantimonate, forming electron-dense structures visible under TEM, which were analyzed by energy-dispersive X-ray microanalysis system to reveal quantitatively their element composition. Calcium precipitates were observed in the cell wall of dark-adapted wild type *Arabidopsis* leaves, where they formed spherical structures. 3 minutes after strong blue light irradiation, the efflux of calcium to the apoplast plays a prevailing role – precipitates on the cell wall are bigger, mushroom – shaped, with a couple of layers. Moreover, spherical calcium precipitates on chloroplast surface pointing to the vacuole are formed. The precipitates on the cell wall tend to be smaller and less numerous under control red light and in phototropin mutants.

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6.22. Towards understanding the interaction of saponins with biological membranes. A DSC study

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Steroid saponins are glycosides widely distributed in plants. They consists of sugar units covalently bound to one or two sites of a steroid or triterpene framework (the “aglycone”). Apart from being the key ingredients in a host of traditional Chinese, African and Indonesian medicines, they are also known as agents for combating cholesterol, microbes, fungi, viruses and tumors. Although the chemical composition and pharmacological activities of saponins have been extensively studied, the interaction of these components of herb medicines with lipid bilayers have received much attention only recently.
With the aim to better understand the molecular mechanisms of the interaction of saponins of different chemical speciation with biological membranes we have utilized well-established models consisted of multibilayers of dimyristoylphosphatidylcholine (DMPC), dimyristoylpho sphatidylethanolamine (DMPE) and dimyristoylphosphatidylserine (DMPS) representative of phospholipid classes which differs in head group charge. By the use of differential scanning calorimetry (DSC) we have followed the thermotropic behavior of multilamellar vesicles prepared from DMPC, DMPE and DMPS upon incorporation of diosgenin, solanine, solasodine and solamargine. The effectiveness of perturbations exerted by various saponin compounds on thermotropic phase transition was further analysed in terms of thermodynamic parameters: transition temperature, enthalpy and molar heat capacity, determined for lipid/saponine systems on the basis of heating and cooling scans.

6.23. A chloroplast located form of the nuclear SWIB-1 protein functions as a nucleoid architectural protein

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Plastids possess their own DNA (ptDNA), which is packaged with proteins into structures analogous to bacterial chromosomes, termed nucleoids or plastid nuclei. In contrast to nuclear chromatin, there is only limited information on the organization and dynamics of the plastid nucleoids and to date only few of the plastid nucleoid-associated proteins (ptNAPs) have been identified.
Proteome analysis of a highly purified fraction of the transcriptionally active chromosome (TAC) from spinach chloroplasts identified a SWIB domain containing protein called SWIB-1. SWIB domain-containing proteins belong to the family of the nuclear SWI/SNF chromatin remodelling proteins. In fusion with GFP SWIB-1 was shown to be associated with nucleoids in chloroplasts and was furthermore identified in the nucleus. Immunological analyses revealed that the chloroplast located form of SWIB-1 has a lower molecular weight than the nuclear form. The reduction in size occurred by N-terminal processing during import of the protein in the organelle. *Escherichia coli* cells overexpressing the *swib-1* gene showed higher compactness of the nucleoids coinciding with arrested cell growth. In a complementation approach it could be shown that the *swib-1* gene could complement an *E. coli* mutant lacking the H-NS protein which is a very abundant histone-like nucleoid architectural protein in bacteria.
Based on these findings we hypothesize that plastids use eukaryotic proteins for remodelling of their nucleoid architecture.

6.24. Chlorophyll(ide) fluorescence kinetics in the course of early phases of deetiolation

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Angiosperm deetiolation is an important developmental process that occurs when dark-grown etiolated seedlings are exposed to light. During this process seedlings are redirected from skotomorphogenesis to photomorphogenesis. The assembly of the photosynthetic apparatus that occurs during the deetiolation has been investigated for more than 50 years using low temperature fluorescence spectroscopy, and specific changes in fluorescence spectra have been well described with respect to the developmental stage of the photosynthetic apparatus. The deetiolation starts with formation of chlorophyllide from its precursor -protochlorophyllide. This reaction is triggered by light and catalysed by a photoenzyme - protochlorophyllide oxidoreductase.
In the present study, using a FluorCam imaging fluorometer, we have investigated changes in the fluorescence kinetics of chlorophyllide during its conversion to chlorophyll in the course of the first 15-30 min of greening of *Lepidium sativum.* The process was triggered by a short flash of white light. The newly formed chlorophyllide has a fluorescence maximum at about 680 nm (at 77K). The following shifts of the maxima were observed: (1) a red shift of about 3 nm; (2) a blue shift to 676 nm, known as the Shibata shift, during which chlorophyll starts to accumulate and (3) another red shift related to incorporation of chlorophyll to photosynthetic complexes. Using the protocol for recording of the Kautsky effect, we monitored chlorophyll(ide) fluorescence during incorporation of this pigment into photosynthetic pigment-protein complexes. We attempted to find some correlation between the measured chlorophyll(ide) fluorescence kinetics and fluorescence emission spectra measured at 77 K. In addition, measurements of gas exchange were performed in parallel during greening in the same conditions to monitor the physiological state of the photosynthetic apparatus.

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6.25. Stability of photoactive Pchlide:LPOR:NADPH complexes in prolamellar body and reconstituted from GST-LPOR fusion protein

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Light-dependent protochlorophyllide oxidoreductase (E.C. 1.3.1.33; LPOR) catalyses a penultimate reaction of chlorophyll biosynthesis, i.e. a trans-reduction of the D-ring of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). This reaction plays a regulatory role in angiosperm deetiolation. In etiolated seedlings, LPOR accumulates in the form of ternary Pchlide:LPOR:NADPH complexes in prolamellar bodies (PLBs), which are lipid paracrystalline structures. Pchlide in these complexes can be converted to Chlide with a flash of light.
In the present study, the stability of photoactive Pchlide:LPOR:NADPH complexes in PLBs, isolated from etiolated seedlings of *Triticum aestivum* and incubated in different media were investigated. Among others, effects of pH, temperature, ionic strength, glycerol, detergents and sorbitol were studied. Fluorescence emission spectra measured at 77 K for PLBs showed, as a result of the treatments, different relative intensities of fluorescence bands centered at 633 and 655 nm, as well as different position of the fluorescence maximum of the newly formed Chlide due to illumination. The fluorescence band at 655 nm, characteristic for aggregated Pchlide:LPOR:NADPH complexes was the highest, and the most stable in time, in the case of pH of 7.5 and in the presence of glycerol in the medium. Addition of glycerol also protected PLBs against solubilisation with n-octyl glucoside. The glutathione S-transferase fusion protein (GST- LPOR) expressed after induction with IPTG and purified from cell extract by affinity chromatography on glutathione-agarose was used to reconstitute the photoactive Pchlide:LPOR:NADPH complexes. Low temperature fluorescence maximum of the reconstituted complexes was blue-shifted compared to that in PLBs, however, it depended on reaction mixture composition. Pchlide-protein interaction will be discussed based on the presented results.

6.26. Respiratory activity of mitochondria from sulphur-deficient *Arabidopsis thaliana*

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Sulphur (S) is an essential macronutrient for the plant cell being a constituent of the amino acid cysteine, variety of S-rich metabolites and forming iron-sulphur (Fe-S) clusters in proteins. Mitochondrial respiratory chain (mtETC) activity depends on sulphur availability since it contains different Fe-S centres in Complexes I, II and III (Droux 2004). The additional internal and external NAD(P)H dehydrogenases ($ND_{in/ex}$) and alternative oxidase (AOX) provide functional plasticity of mtETC and allow plant to survive under stress conditions (Rasmusson et al. 2008). $ND_{in/ex}$ do not contain Fe-S clusters but AOX dimer activity is modulated by the redox status of sulphydryl groups in cysteine residues.
The purpose of this study was to relate the effects of S deficiency in *Arabidopsis thaliana* to the activity of the mitochondrial respiratory chain complexes, mainly additional dehydrogenases and AOX. Plants grown hydroponically for eight weeks on S-deficient medium have changed respiratory properties as compared to the control. Mitochondria isolated from S-deficient plants always had lower efficiency of ATP production (ADP/O ratios). However, the respiration with malate, glycine and NADH was not affected. Cyanide resistant respiration and AOX capacity were significantly higher in S-deficient than in S-sufficient plants. This is opposite to what was previously observed in bean plants (Juszczuk and Ostaszewska 2011). The results are discussed in terms of flexible function of mitochondrial respiratory chain as an important target during plant adaptations to sulphur deficiency.

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6.27. Absence of type-II prohibitins AtPHB2 and AtPHB6 elicits mitochondrial stress response in Arabidopsis plants.

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Prohibitins (PHBs) are very conservative proteins located in the mitochondrial inner membrane where they form high-molecular-weight complexes of 1-2 MDa. It is generally accepted that prohibitins directly or indirectly influence the assembly or stability of the respiratory chain complexes in animals and yeast. Because the role of prohibitins in OXPHOS maintenance in plants is unknown, we used double *phb2*/*phb6 Arabidopsis* mutant to investigate this issue.
BN-PAGE analysis revealed unchanged steady-state levels and in-gel activities of complexes III, IV and V and only slightly decreased levels and in-gel activities of complex I and supercomplex $I+III_2$ in *phb2*/*phb6* mutant. However, analysis of mitochondrial respiratory chain in the *phb2/phb6* plants using Clark-type electrode indicated significant increase in activities of alterinative oxidase (AOX, 2-fold increase) and external alternative dehydrohenases (NDex, 55% increase) accompanied by c.a. 25% decrease in activities of complex I and internal alternative dehydrogenases (NDin). In the same time we analysed transcript levels of a set of genes encoding mitochondrial proteins, which are commonly overexpressed in various stress conditions, and are defined as “mitochondrial stress response”. We observed significantly increased expression of genes encoding alternative dehydrogenases (NDB3 and NDB4), alternative oxidases (AOX1A, AOX1C, AOX1D) and several other proteins (UPOX1, BSC1, Hsp23.5). Expression pattern of these genes indicates that the absence of prohibitins AtPHB2 and AtPHB6 in *Arabidopsis* mitochondria elicits typical mitochondrial stress response resulting in activation of the alternative respiratory pathways.

6.28. ROS metabolism in nitrate- and ammonium-grown *Arabidopsis thaliana*

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Plants can take up and utilize either nitrate or ammonium as the inorganic nitrogen source. However, many plant species grow poorly and show stress symptoms, when cultured on NH_4^+-ions as the exclusive N source. NO_3^- has a higher energetic requirement for assimilation compared to NH_4^+ and as a consequence an excess of energy and reductants can lead to ROS production. Thus, one of the reasons for the ammonium stress symptoms might be oxidative stress. The purpose of this study was to analyse mechanisms of ROS production and detoxification in NH_4^+-fed *Arabidopsis thaliana* leaves.
Our study has shown that NH_4^+ nutrition results in an increased H_2O_2concentration and enhanced lipid peroxidation in leaf tissues. The cellular distribution of H_2O_2 was pictured by electron microscopy. ROS scavenging enzymes (e.g. ascorbate-glutathione cycle enzymes) were quantified on enzymatic activity, protein and/or transcript level in whole tissues and isolated organelles. Also mitochondrial electron bypass components were analysed (e.g. alternative oxidases, type II NAD(P)H dehydrogenases). Furthermore non-enzymatic antioxidants like ascorbate and glutathione were more reduced in leaves of NH_4^+-supplied plants. Collectively higher ROS content and antioxidant defense in NH_4^+-fed *A. thaliana* indicates a disrupted redox/oxidative homeostasis and might lead to oxidative stress.

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6.29. The role of mitochondrial protease FtsH4 in germination of *Arabidopsis thalina*

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AtFtsH4 is one of the inner-membrane-bound mitochondrial ATP-dependent metalloproteases in *A. thaliana*, whose catalytic site is exposed to the intermembrane space. Like other ATP-dependent proteases this enzyme exhibits two activities: proteolytic and chaperone-like. In the present study, a reverse-genetic approach has been undertaken to study the physiological role of the AtFtsH4 protease during germination.
We found, that a loss of AtFtsH4 protease slightly delays *Arabidopsis* germination compared to wild-type under long day condition at optimal temperature of growth of 22℃. However, further seedlings development starting from seedlings with fully opened cotyledons was achieved at approximately the same time in *ftsh4* and wild-type plants under these conditions. The germination delay of *ftsh4* was significantly increased when seeds were germinated at elevated temperature of 30℃. In contrast, the overexpression of *FtsH4* accelerated a radicle emergence especially in the presence of higher concentration of sugars. The functional importance of proteolytic or chaperone-like activity of FtsH4 during germination was tested by generating transgenic plants that expressed a proteolytic or a chaperone-like inactive version of enzyme. We found that expression of the chaperone-like inactive version of FtsH4, but not the proteolytic inactive version rescued germination. The similar result was obtained when seed coats were mechanically removed suggesting that the mitochondrial protease AtFtsH4 proteolytic activity is essential for embryo vitality.

6.30. Colocalization of protamine-type proteins and calreticulin with the use of immunogold technique in the key at V stage of *Chara vulgaris* spermiogenesis

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Ultrathin sections were prepared from the middle spermiogenesis stadium (spV) antheridiostans from *Chara vulgaris* thallus prepared for electron microscopy. Double immunostaining was performed with the use of an anti-calreticulin antibody (Anti-Calregulin Santa Cruz; called calreticulin) which recognizes human, mouse and rat calreticulin (ca. 55 kDa) and a rabbit polyclonal antibody against protamine-type protein isolated from *C. tomentosa* antheridiostans.
At the V stage of *C. vulgaris* spermiogenesis colloidal gold labeling coming from both antibodies clearly cumulated in a nucleus especially at its periphery where condensed chromatin was present. Also labeling of protamines in this area was 2-fold greater as compared to the central part of the nucleus occupied by loose chromatin (Popłońska et al. 2009). Gold grains from both antibodies were also well visible in extensive system of ER cisternae and vesicles and Golgi apparatus. Moreover in nearly all studied cellular compartments colocalization of protamine with calreticulin was slightly or significantly more common than the presence of protamine only. Only in ER vesicles no such differences were observed. Our previous and present EM observations suggest that the chaperon protein, calreticulin participates in the transport of de novo synthesized protamine-type proteins into *C. vulgaris* spermatid nucleus during the key V spermiogenesis stage.

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6.31. Regulation of physiological status of the chloroplasts of *Zea mays* and *Arabidopsis thaliana* by light quality

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We investigated the influence of light of different wavelengths on the acclimation of mesophyll (M) and bundle sheath (BS) chloroplasts from *Zea mays* (C4 plant) and *Arabidopsis thaliana* (C3 plant) leaves. The effect of short-term responses to red and far red light were studied in the chloroplasts of plants grown under medium light intensity (250 µmol m^{-2} s^{-1}). We analyzed PS I and PS II activity, protein phosphorylation of PSII and chlorophyll fluorescence parameters. Transferring plants from one type of illumination to another induced changes in the electron transport chain and modified the organization of the photosynthetic apparatus. We showed that modulation of light quality caused changes in the pattern of chloroplast protein phosphorylation. Phosphorylation of PSII proteins was correlated with electron transport activity driven by PS I (far-red) or PSII (red) specifically absorbed light. Fluorescence data of dark-acclimated leaves acquired by using saturation pulse analysis confirmed observed *in vitro* effects. Our results suggest that light quality play a regulatory role in all investigated chloroplasts and that is a co-regulation of M and BS metabolism by phosphorylation/ dephosphorylation of PSII proteins and PSI excitation.

6.32. Functional consequences of lead ions (Pb^{2+}) interference with light induced chloroplast avoidance response

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Lead is one of the most important heavy metals polluting the natural environment as a result of human activity. The uptake of this metal can cause many destructive changes in plants, affecting also the photosynthetic machinery. Environmental influences, such as light intensity, may modify the effects of heavy metals. Directional movements of chloroplasts are an acclimation mechanism that allows the photosynthetic machinery to adjust to changing light conditions. Strong light causes an avoidance response of chloroplasts, i.e. their movement to the anticlinal walls. This response protects the photosynthetic apparatus from excess energy (Sztatelman et al. 2010). The aim of this study was to characterise functional effects of lead ions on the light-induced chloroplast avoidance response.
Pigment concentration, chlorophyll fluorescence parameters and accumulation of stable free radicals were determined in *Lemna trisulca* L. exposed to lead ion stress under light intensity which induces chloroplast avoidance. This combination of lead stress and light was previously shown to interfere with the avoidance response of chloroplasts (Samardakiewicz and Gabrys 2004). A 24h exposure of *L. trisulca* to 15 µM lead (Pb^{2+}) resulted in a significant reduction of chlorophyll a and b content. The decrease in Fv/Fm (quantum yield of PSII photochemistry in the dark-adapted state) in Pb treated plants indicated photoinhibition. However, the concentration of free radicals was unchanged by lead treatment. The presence of lead in the nutrient solution resulted in a pronounced accumulation of zeaxanthin under very high light treatment. This result suggested synergistic effects of Pb ions and very high illumination on photoprotective mechanisms based on the xantophyll cycle.

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6.33. Loss of mitochondrial protease FtsH4 results in arrest of *Arabidopsis* inflorescence growth under moderate temperature stress

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AtFtsH4 is one of the inner-membrane-bound mitochondrial ATP-dependent metalloproteases in *A. thaliana*, whose catalytic site is exposed to the intermembrane space. Analyses of *Arabidopsis* mutants lacking AtFtsH4 revealed a pleiotropic phenotype under continual stress temperature of 30°C including the arrest of inflorescence growth. In almost 90% of *ftsh4* mutants no inflorescence was visible while in the remaining plants a premature termination of inflorescence growth was observed. Regardless of the inflorescence length, all *ftsh4* plants are unable to produce seeds under these conditions. The elevated transcript and protein levels of AtFtsH4 during the late vegetative and generative phase at 30°C compared to other developmental phases of *Arabidopsis* growth supports the important role of this peptidase for the inflorescence development.

We have found that the inflorescence arrest at 30°C is associated with oxidative stress. The substantially higher level of superoxide was observed in the adult rosette and cauline leaves of mutants with the short inflorescence compared to wild-type plants. Furthermore, the 2-fold increase in *AOX1a* and *UPOX* transcripts of genes usually up-regulated by oxidative stress was observed in leaves of plants without inflorescence compared to leaves of plants with inflorescence. In agreement, the elevated transcript levels of these genes were observed also in the shorter inflorescence (~1cm) compared to longer (~3cm) ones. Furthermore, accumulation of oxidized proteins, another marker of *oxidative stress*, was slightly higher in leaves of rosettes without inflorescence than in leaves derived from rosettes with inflorescence. In addition, oxidized proteins immunodetected *in situ* show a stronger signal in inflorescence meristems and flower primordia of *ftsh4* than in the comparable structures of wild-type plants, suggesting their higher accumulation in the mutant plants.

Presented results strongly suggest that the arrest of reproductive development in *ftsh4* mutant is associated with the protective role of AtFtsH4 against oxidative stress during transition between vegetative and generative developmental phases.

6.34. Levels of DNA methylation and histone methylation and acetylation change in root meristematic cells of soybean grown under different temperature conditions

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Soybean root meristematic cells strongly responded to temperature of 10°C which was manifested in drastic decrease in transcriptional activity in comparison to the control conditions (25°C). While in the stressed plants recovered at optimal temperature (25°C) the increase in transcriptional activity, even in comparison with the control, occurred. The assumption has been put forward that changes in transcriptional activity could result from alterations of chromatin transcriptional competence under these growth temperature conditions. Hence, the aim of the current work was to check whether the chemical modifications of chromatin and its structural organization change in soybean along with the change of plant growth temperature. Thus, the measurements of fluorescence intensity with the use of antibodies to heterochromatin and euchromatin markers were carried out. Moreover, the analysis of the number and sizes of chromocentres was made. The studies showed that during chilling stress the fluorescence intensity for the markers characteristic of heterochromatin increased while for the markers of euchromatin decreased in comparison to the control. After the recovery the converse situation was observed, i.e. increase in fluorescence intensity for euchromatin markers and decrease in heterochromatin markers. Differences in the sizes of chromocentres were observed – the highest number of big chromocentres and simultaneously the lowest number of small chromocentres were in the nuclei of stressed plants. Conversely – in the nuclei of recovered plants there were the lowest number of big chromocentres and the highest number of small ones. These results suggest that DNA and histones can undergo modifications in soybean nuclei under chilling stress and during recovery. These modifications could lead to chromatin remodeling and potentially influence chromatin transcriptional competence.

6.35. The use of DArT markers for assessment genetic similarity of near isogenic lines in rye

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Near isogenic lines (NIL) are genotypes revealing genetic diversity in a very narrow space of the genome. They are a valuable material for genetic research developed usually as result of recurrent backcrossing. Precise assessment of similarity newly developed NILs is possible by application of numerous molecular markers covering the whole genome of the studied species. This work was aimed at application of Diversity Array Technology (DArT) for evaluation of genetic similarity of two sets of near isogenic lines developed from BC_3 progeny of the cross 711C x DS2 and the BC_5 generation of the 544C x Ot0-20 cross. Parental lines of both crosses deferred with respect to the ability of restoration of male fertility in rye with sterilising cytoplasm CMS-C. Maternal lines 711C and 544C used as recurrent parent were male sterile, parental lines belonged to the restorer genotypes. Analyses were performed on the DNA obtained from four parental lines and 16 isogenic lines developed from the cross 711C x DS2 as well as 40 lines from the 544C x Ot0-20 hybrid. Application of more than 2500 DArT markers allowed for precise assessment of genetic similarity within both groups of near isogenic lines. As analysed lines were genetically similar but they still were different with respect to their male fertility, they can be used for expression analysis of nuclear and mitochondrial genes regulating pollen development in cultivated rye with sterilising cytoplasm.

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6.36. Localisation of the gene controlling male fertility in rye with C cytoplasm on the integrated genetic map of the 4RL chromosome

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Cytoplasmic male sterility (CMS) is a phenomenon affecting production of normal, fertile pollen in male organs of the flower. The CMS was observed in many higher plant species and it is caused by interactions between mitochondrial and nuclear genomes. In breeding of hybrid cultivars it is often applied for seed production. That's why, investigation of the genetic mechanism controlling pollen production in plants with the sterilising cytoplasm is important for commercial purposes. It is also a good model for studying relationships between nuclear and mitochondrial genes. Localisation of genes and identification of molecular markers linked to the loci of interest opens possibilities for map based cloning of genes and their further investigations. In rye with the CMS-C cytoplasm the main nuclear gene restoring male fertility (*Rfc1* gene) is located on the long arm of the 4R chromosome. In order to precise localisation of the *Rfc1* gene, the integrated genetic map of the 4RL chromosome was constructed with the use of the JoinMap 3.0 software. Different molecular markers were applied for map construction. Between them DArT markers (Diversity Array Technology) were the most numerous. Two mapping populations were used in this study: [541x2020LM]RIL F_5 and [544CxOt0-20]BC_5 allowing for development of high density map covering the region of the 4RL chromosome with the Rfc1 gene.

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6.37. Mitochondrial respiration and ROS metabolism of Arabidopsis *frostbite1* mutant

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Frostbite1 (*fro1*) plants carry a point mutation affecting the 18-kDa mitochondrial subunit of Complex I show impaired cold-regulated gene expression (Lee et al. 2002). Loss of Complex I activity in mitochondria isolated from *fro1* leaves is compensated by an increased external and internal dehydrogenases activities and cytochrome *c*oxidase protein level. Consequently energy deficit does not occur in those plants. However, mtETC dysfunction may modify antioxidant systems of cells (Juszczuk et al. 2011). Cellular enzymatic (enzymes capacities, protein and transcript level) and non-enzymatic antioxidant systems together with H_2O_2 concentration were determined in *fro1* leaf tissue. Only minor changes in most enzymatic antioxidants were observed although a significant increase in AOX protein level was detected. The most pronounced changes were found in GSH oxidation state. The role of Complex I as a crucial component of the cellular redox regulatory system in photosynthetic cells is discussed.

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6.38. Molecular analysis and ROS production in interspecific somatic hybrids between potato and wild species *S. villosum*

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In the present study the interspecific somatic hybrids, 4*x S. villosum* (+) 2*x S. tuberosum* (VT, 5 clones), expressing high level of resistance to the oomycete pathogen *Phytophthora infestans*, were obtained and characterized molecularly by RAPD analysis and cytogenetically using GISH method. The hybrid ploidy was estimated on basis of chloroplast count in guard cells and chromosome count in metaphase plates. Hybrid nature of all regenerants was proved by RAPD patterns and GISH analysis confirmed the presence of both parental genomes in the VT hybrids genome and allowed to discriminate the chromosomes of both parents.
In search of the mechanisms involved in resistance of *Solanum* species in the interaction with *P. infestans* we have studied production of reactive oxygen species (ROS) occurring after the elicitor, a culture filtrate (CF) derived from *P. infestans*, treatment. ROS production is considered to be one of the earliest reactions induced by pathogens or their elicitors. The ROS produced are assumed to play a key role in the integrations of diverse strategies leading to disease resistance. The ROS production was examined in the resistant wild species *S.villosum*, susceptible *S. tuberosum* and VT hybrid, resistant to *P. infestans*.
We have observed that the relative increase in ROS production in response to CF treatment was higher in leaves of the susceptible potato clone than in the resistant *S. villosum* and somatic hybrid.

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6.39. Photochemical response to lead low light grown pea plants

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The environmental factors as light intensity and/or heavy metals cause changes in fluorescence parameters, pigments content, efficiency of photosystems and in relative levels of thylakoid components in plants. Seedlings of pea were growing under low (LL, 60 µmol m^{-2} s^{-1}) light intensity. Lead was introduced into detached plants with transpiration stream (24h, 5 mM $Pb(NO_3)_2$). The rate of photosynthesis and respiration, chlorophyll content, activities of photosystem I and II, phosphorylation of the PSII proteins and fluorescence parameters were studied. The data show that photosynthesis was inhibited by lead, but heavy metal ions stimulated CO_2 evolution. Increased respiratory activity correlated with higher adenylate contents. The potential photochemical efficiency of PSII was lowered slightly by Pb^{2+}, whereas effective PS II quantum yield was decreased by 35%. We observed that quantum yield of regulated and non- regulated energy dissipation was higher in plants treated lead by 28% and 52%, respectively. Exposure of seedlings to lead demonstrated that heavy metal did not diminished PSII and PSI activity and chlorophyll content. However, heavy metal did not affect amount of photosynthetic pigments, such chlorophyll and carotenoids. Also in our studies phosphorylation of the D1 protein upon treatment with $Pb^{2+}$was very high, indicating a possible role of lead in regulation of PSII turnover. Our results suggests that Pb ions not affected light harvesting complexes, but can change electron transport rate and regulate degradation of PSII proteins by changing their phosphorylation.

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6.40. Immunogold localization of H3 histone and ubiquitin in *Chara vulgaris* spermatids exposed to the proteasome inhibitor – epoxomicin

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Spermiogenesis in the alga *Chara vulgaris* showes many common features with animal spermiogenesis. During spermatid differentiation remodeling of chromatin involving the exchange of histone proteins into protamine-type proteins is the most important process, conditioned by the presence of the ubiquitin-proteasome 26S system. Previous cytochemical and immunocytochemical studies (Wojtczak and Kwiatkowska 2008) indicated that exchange of these proteins during *C. vulgaris* spermiogenesis took place at spV-VIII. An inhibitor of proteasome proteolytic activity, epoxomicin, disturbed nuclear protein exchange, which resulted in the presence of the histones at the late spermiogenesis stages (spIX-X), while in the control only protamine-type ones were then observed.
Immunogold analyses with the use of H3 histone and ubiquitin revealed an immunoreaction in cytoplasm and nuclei of the control and epoxomicin (100mM, 48h) treated *C. vulgaris* spermatids. The preliminary analysis of micrographs confirmed the earlier immunocytochemical results concerning the presence of H3 histone in spermatids. In the control at the early stages (spII-IV) the number of immunosignals was much higher than later (spVII-VIII) when the process of exchange of nucleohistones into nucleoprotamines took place. Comparative analysis of the of micrographs of both variants and verification whether “aggresomes”- like structures are, as it is supposed, aggregates of histones joined with ubiquitin which could not have been degraded due to inhibition of proteasome by epoxomicin need to be done.

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6.41. Mitochondrial proteome during salinity induced programmed cell death in lupine

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In this work, the effect of salinity on proteomic changes in lupine embryo axes mitochondria was studied. Lupine (*Lupinus luteus* L.) embryo axes were grown on modified Heller medium with or without addition of 0,25 and 0,5M NaCl for 12h. Our results indicate that NaCl treatment lead to programmed cell death confirmed by DNA laddering, chromatin condensation and comet (single-cell electrophoresis) assay. Salinity caused changes in cell ultrastructure such as cytoplasmic vacuolization, occurrence of mitochondria with membrane deformation, swollen ER cisternae, ER compartments involute and vacuoles containing inclusion bodies. The ultrastructural changes may indicate the occurrence of authophagic cell death. Soluble mitochondria proteins were analyzed by two-dimensional electrophoresis 2D-IEF-SDS-PAGE in order to detect salt stress induced changes in protein profile. Approximately 318 protein spots were detected on individual 2D gel from control and salt treatments. A total of 182 protein spots were affected by 0,25M NaCl treatment, expression of 86 being up-regulated and 96 down-regulated by the presence of salt. A total of 231 spots changed significantly with 0,5M NaCl treatment, with 110 spots being up-regulated and 121 spots down-regulated. For protein identification 22 protein spots were subjected to MS/MS analysis. All investigated proteins were of mitochondrial origin. Proteins involved in TCA cycle, mitochondrial ETC, stress responses, protein synthesis and folding were identified. The altered expression of these proteins might provide an insight into the mechanism of mitochondria response to salinity stress.

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