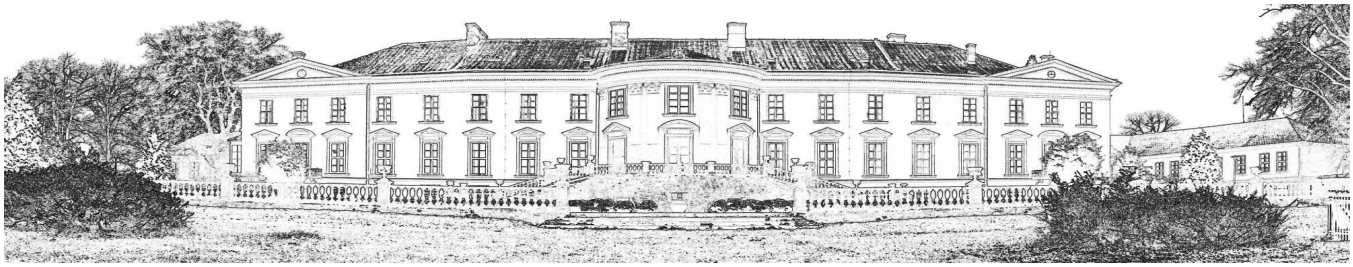
Fresh Insights in Plant Affairs

Nové Hrady 2008

BOOK OF ABSTRACTS

Nové Hrady, 8–10 April 2008



Welcome in Nové Hrady, dear colleagues and friends.

This sentence is something what one would expect to find at opening the first sheet of a conference booklet. I am not going to say more than this but, believe me, it goes from the bottom of my heart.

Science is a multiple ART. Art of clear many-coloured thinking and simplifying, art of intuition and rationality. However also art of “selling”. Many of us do not like it, hate it, but we simply face the reality. "Show! Communicate! Publish! Patent!" Four manager's key-words. And “do not forget to rise the research money! …" Adaptation brings surviving. How to adapt, survive in and still enjoy the Science? Allow me to offer several personal suggestions for your consideration. A mental meal course without any guarantee of gourmet pleasure and completeness; a field guide of (confused) young scientist:

1/ Do not avoid scientific meetings, especially the small and focussed ones, even if you are not a typical party-man/woman.

2/ Enter the "enthusiast's circle": Work hard but do not think you are working hard. Feel as if you are playing a funny game. This and the curiosity feelings lead to passion for science. In turn, the passion allows you to work hard without suffering.

3/ Start with hypothesis and try to finish all what you have started. The topic is finished when it is published. Do not play position warfare in this business.

4/ Find your own way of relaxation and use it often.

By coming to this meeting, you are half way through. You came, worked hard, published (sort of) and relaxed (hopefully).

We wish you good day. Meet your old friends and make new ones, enjoy your stay in the charming Nové Hrady castle!

On behalf of the organisers: Jiří Šantrůček

Program, 8 – 11 April 2008

Tuesday (8 April)

Registration and accommodation 17:00–20:00

Wednesday (9 April)

Additional registration 8:00–9:30
Opening ceremony 9:30–9:45

Presentations 9:45–10:45 (Cell Biology)
Lenka Dvořáková: Study of hybrid proline-rich cell wall proteins: from sequences to the function
Monika Hlavová: Regulation of *Chlamydomonas reinhardtii* cell cycle by A-type specific cyclin dependent-kinase

Coffee break **10:45–11:00**

Presentations 11:00–12:00
Dáša Umysová: Bioaccumulation of selenium in cells of the green alga *Scenedesmus quadricauda*
Petra Vojvodová: Flowering onset in day-neutral tobacco (*Nicotiana tabacum* L. cv. Samsun) is influenced by transformation with cdc25 gene from *Schizosaccharomyces pombe*

Lunch time **12:00–13:30**

Presentations 13:30–15:00 (Phytohormones)
Petra Baťková: Oxidative stress in tobacco caused by an imbalance of phytohormones and biotic stress
Silvia Gajdošová: *Cis*-zeatin type cytokinins are biologically active and their metabolism differ from *trans*-zeatin
Renata Kotková: Effect of phytohormone on growth and bulb formation in garlic at different plant age

Coffee break **15:00–15:30**

Presentations 15:30–16:30
Sibu Simon: Characterization of the auxin efflux machinery in suspension-grown tobacco BY-2 cell line by assessing the specificity towards various auxins and auxin-related compounds
Martin Kubeš: The role of the auxin efflux carrier PGP19 in BY-2 cell suspension

Poster session 16:30–18:00

Dinner **18.30**

Bean-feast 20.00

Thursday (10 April)

Presentations 8:30–10:30 (Green machinery)

Martina Košvancová: Changes in photosynthetic induction of European beech and Norway spruce seedlings grown under elevated CO_2 concentration

Petra Krausová: Phosphorylation of thylakoid membrane proteins in green algae

Viktor Demko: Finding mechanisms controlling the progressive etiolation of initially green larch seedlings

Andrej Pavlovič: Light-independent chloroplast development in four gymnosperm species

Coffee break **10:30–10:45**

Presentations 10:45–12:15 (Eco-physiological point of view)

Alexander Ač: Analysis of energy and matter fluxes within mountain ecosystems on the basis of spectral-optical properties of vegetation

Jana Karbulková: Ecophysiological traits of plant cuticle

Jana Zámečníková: Growth and Development of Yacon (*Smallanthus sonchifolius*) in Field Conditions and *in vitro*

Lunch time **12:15–14:00**

Presentations 14:00–16:00 (Whereof are plants sad?)

Jana Dobrá: Characterization of the drought response of tobacco plants and their subsequent recovery

Katarína Ďurčeková: Oxalate oxidase is strongly activated during cadmium induced premature xylogenesis in barley roots

Klára Kosová: The relationship between development, frost tolerance and accumulation of DHN5 protein in barley (*Hordeum vulgare*)

Zuzana Lhotáková: Detection of changes in mesophyll structure using confocal microscopy and stereology: application on Norway spruce needles treated with elevated CO_2

Closing ceremony, best lecture award **16:00–17:00**

Visitation of Nové Hrady castle

Informal banquet **19:00**

Friday (11 April)

Facultative walking trip to Terčino údolí (for interested persons)

Abstracts are given in alphabetical order to the name of the first author.

Authors of each abstract are responsible for material and linguistic accuracy.

This book did not pass linguistic revision.

Analysis of energy and matter fluxes within mountain ecosystems on the basis of spectral-optical properties of vegetation

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Continuing increase of anthropogenic influence on the surrounding environment could have possibly dangerous consequences. Remote sensing (RS) technology advances could help us to better understand the (global) extent and future development of negative and positive impacts of human actions. Recent RS studies shows an exciting possibility to detect dynamic processes related to carbon sink activity, and even to measure carbon assimilation directly. It has been shown that using RS data, in our case hyperspectral reflectance (amount of reflected radiation, HR), rapid changes in specific spectral regions occur, specifically at 530, 690, 740 and 760 nanometers, due to zeaxanthin pigment and chlorophyll fluorescence (Chl-F) effects. However, most of these studies have been done under laboratory conditions and Chl-F and HR were not measured simultaneously. Further, only very few studies have been done evaluating the influence of leaf temperature on the optical-spectral properties of the leaf.

In the laboratory, HR was measured using spectroradiometer ASD FieldSpec3 (Boulder, USA) for leaf spectra within the range from 400 to 2500 nm and Chl-F was measured simultaneously using open FluorCam (Brno, CR). During the continuous measurements (1 s) leaf temperature oscillated within the range from 15 to 40°C.

For field measurements, HR was measured using Airborne Imaging Spectroradiometer for Applications (AISA, Specim, FI), Chl-F was measured using PAM-2000 (Effeltrich, GER), and CO_2 assimilation rate was measured using infrared gas analyzer LI-6400 (LI-COR, USA).

Laboratory measurements have confirmed that zeaxanthin and Chl-F emission signals can be extracted from HR. Chl-F sensitive vegetation indices tracked the changes in Chl-F emission associated with light adaptation. Temperature had an observable effect on HR and most temperature-sensitive wavelength in the optical spectral region was determined at 700 nm, though the physiological interpretation of this effect remains unclear. Potentially, wavelength at 700 nm could be used for detection of heat-stress induced changes at larger scales.

Field leaf-level measurements have also confirmed that zeaxanthin signal and Chl-F effects on HR can be detected and statistically significant relations between Chl-F and vegetation indices were detected. This supports scientific effort to use satellite RS for a more precise estimation of carbon sink activity of terrestrial ecosystems. At the canopy-level, the correlation between vegetation indices and carbon assimilation is weak, probably complicated by high spatial heterogeneity and non-physiological factors, such as sun angle, viewing geometry and others.

Oxidative stress in tobacco caused by an imbalance of phytohormones and biotic stress

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Potato virus Y (PVY) infects a range of solanaceous crops including potato, capsicum, tomato and tobacco. PVY^{NTN} strain, which has an ability to infect tobacco plants systemically, causes severe necrosis in tobacco. Beside many roles in plant growth and development, plant hormones cytokinins (CK) affect processes caused by virus infection, particularly those associated with the development of symptoms. In various plant-pathogen interactions an increased production of active oxygen species has been observed. Consequently, the changes in the activities of enzymes of antioxidative system might play an important role in plant resistance to pathogens.

In this study we examined the influence of PVY infection on the activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APOD), guaiacol peroxidase (GPOD), syringaldazine peroxidase (SPOD) and another stress connected enzyme NADP-malate dehydrogenase (NADP-ME) in control (C) and P*ssu-ipt* transgenic tobacco overproducing cytokinins (T). Both plant types were grown as rooted as well as grafted on control rootstock (C/C, T/C). In our previous research C plants were found most susceptible and T/C plants most tolerant to PVY infection. Enzyme activities were measured spectrophotometrically in leaf extracts. Isozyme patterns were studied using native polyacrylamide gel electrophoresis. The study was accompanied by histological localization of certain enzyme activities and phenolic compounds using light microscopy.

PVY infection caused significant increase in peroxidase (GPOD, SPOD) activity in all plant types except T/C plants. Activity of NADP-ME increased mainly in PVY infected C and T plants, only slightly increased in C/C plants and was not changed in T/C plants. CAT activity was not changed markedly due to PVY infection in any type of plant. APOD activity increased only in PVY infected C/C plants and did not significantly change in any other plant type. PVY infection increased the activity of GR in all plant types. Changes in isozyme pattern and especially changes in the activity of certain isozymes caused by PVY infection were also recorded.

In this study, changes in the activity of NADP-ME proved to be a good indicator of the extent of stress caused by PVY infection. The only minor changes in the activities of all measured enzymes in T plants confirmed the smaller effect of PVY infection in those plants. Higher tolerance of T plants to PVY infection can be contributed to pre-activation of plant defense mechanisms by the stress caused both by grafting and overproduction of cytokinins.

The impact of drought and/or heat stress on tobacco plants with decreased content of plant hormones cytokinins

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Due to their sessile character, plants have developed many effective mechanisms to cope with a wide range of unfavourable environmental conditions. Important role is played by plant hormones as well as by osmoprotective substances, especially proline. In our work we compared the effect of drought stress, heat shock and their combination on content of proline and plant hormones cytokinins (CKs) in tobacco plants *Nicotiana tabacum* L. (WT) and in transgenic tobacco plants with modulated CK content. This was achieved by over-expression of the gene for the main CK degrading enzyme cytokinin oxidase/dehydrogenase, either constitutively (*35S::CKX1*) or in roots and stressed leaves (*WRKY::CKX1*).

Tobacco plants were grown in soil for 6 weeks. Drought stress was applied by water cessation. Samples were taken from upper and lower leaves and roots during the drought stress progression and 1-day recovery or after heat shock application (40 °C for 2 or 6 hours). Combined stress was done by heat shock (2 h) at the end of the 7-day drought period.

In order to characterize the drought stress in different genotypes, weight of the pots was followed during the water deficit progression. Biggest decrease of soil humidity was determined after one day. No significant differences were detected among the genotypes. The strength of the stress in leaves was estimated using Relative Water Content (RWC). The level of chlorophyll was measured in non-invasive way by SPAD Metre (Minolta, USA). Chlorophyll content was higher in slowly growing constitutive *CKX1* transformants then in WT and *WRKY::CKX1*. The level of proline strongly increased during the drought. After heat stress tendency to proline decrease was monitored. The level of physiologically active CKs decreased substantially during the prolonged drought. Nevertheless it stayed higher in upper leaves than in the lower ones. Heat shock had only minor effect on the level of physiologically active CKs. Precise evaluation of the changes after heat stress is still in progress.

Proteomic analysis of whole leafs of *Hordeum vulgare* under different environmental stress conditions

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In natural field conditions the photosynthetic apparatus of plants is sustainly forced by environmental stresses affecting their photosynthetic efficiency. We isolated proteins from leaves of *Hordeum vulgare* .In the first dimension we applicated proteins in electrofocusing solvent on IPG strips with pH 4-7. After first dimension we applied strips on SDS-PAGE gels and after running time we stained gels with silver. Consequently the stained gels was analysed by PDQuest software for image analysis of qualitative and quantitative distribution of the spots on gels. The differences among the spots were cutted off and digested in gel. The peptides were identified by MALDI/ToF and LC/MS mass spectrometry. High temperature treatment caused strong changes of parameters characterizing photochemistry.In photosystem II we observed decrease in maximal and actual quantum yield of photochemistry and electron transport rate. Heat stress markedly reduced number of active PS II reaction centres. Decrease of quantum yield of PS I was cuased mainly by limitation of donor side of PS I, especially by limited electron transport from PS II. The ratio of PS I and PS II quantum yield indicates that PS II was much more susceptible to heat than PS I. Proteomic analysis confirmed strong changes in photosynthetic apparatus caused by heat treatment.We found 16 different protein spots in heat shock sample (40°C, 1 hour) as compared with control sample. We identified 14-3-3 protein, which is expressed under high temperature treatment.The next spots were identified between quantitative distribution of spots in high temperature and control sample. In heat shock sample we found LHC1 protein is up-regulated and transketolase is down-regulated. Heat stress caused severe impairment of photosynthetic function accompanied with functional changes shown at proteomic level. Here we found heat shock 14-3-3 protein, which can regulate heat shock responses. LHC1 protein was up-regulated in heat shock samples and transketolase is down-regulated in the same sample. The next investigation of identification of protein spots will reveal potential signal pathways as the response to the environmental stress. Transketolase catalyzes reactions in the Calvin cycle and the oxidative pentose phosphate pathway and produces erythrose-4-phosphate, which is a precursor for the shikimate pathway leading to phenylpropanoid metabolism. 20 to 40% reduction of transketolase activity inhibited ribulose-1,5-bisphosphate regeneration and photosynthesis. 20 to 50% decrease of transketolase activity leads to decreased levels of aromatic amino acids and decreased levels of the intermediates (caffeic acid and hydroxycinnamic acids) and products (chlorogenic acid, tocopherol, and lignin) of phenylpropanoid metabolism.We found decreasion of transketolase on 2-D gels under high temperature and heat shock probably influence on regeneration ribulose-1,5-bisphosphate and photosynthesis. The increase of LHC1 protein on 2-D gels under high temperature serves probably for protection of PS1 under high temperature.

Finding mechanisms controlling the progressive etiolation of initially green larch seedlings

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Light-independent chlorophyll biosynthesis is a prerequisite for photosynthetic apparatus formation in the dark. In contrast to norway spruce (*Picea abies*), dark-grown european larch *(Larix decidua*) seedlings synthesize chlorophyll (Chl) only in early developmental stages and the level Chl rapidly declines during their subsequent development. To investigate a processes responsible for progressive etiolation of initially green larch seedlings we used molecular, biochemical and cytological methods like RT-PCR, restriction analysis of cDNA fragments, protein gel blot analysis, spectrophoto/fluoro-metry and transmission electron microscopy. Analysis of the key regulatory steps in Chl biosynthetic pathway revealed that etiolation of fully developed larch cotyledons is connected with decreased abundance of glutamyl-tRNA reductase which correlates with lower 5-aminolevulinic acid synthesizing capacity. The level of immediate chlorophyll precursor protochlorophyllide also declines in gradually etiolating larch. Interestingly, although the genes *chlL*, *chlN* and *chlB*, encoding the light-independent protochlorophyllide oxidoreductase, are expressed constitutively during larch seedlings development the level of ChlB subunite is regulated posttranscriptionaly and decreases in fully developed cotyledons. Our results indicate that efficiency of *chlB* RNA-editing is reduced in mature etiolated larch seedling in comparison with earlier developmental stages. These features were not observed in dark-grown spruce that accumulates chlorophyll throughout the whole seedling development. In addition, we analysed the functional status of plastids in both species during their development in the dark and after illumination. We suggest that progressive etiolation of larch seedlings is not only result of impaired Pchlide reduction but also more complex developmentaly regulated relaxation of metabolic flow into the Chl biosynthetic pathway.

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Characterization of the drought response of tobacco plants and their subsequent recovery

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Drought response was compared in wild-type (WT) tobacco plants and in transgenics with modulated content of plant hormones cytokinins (CKs), over-expressing *trans*-zeatin O-glucosyltransferase (*ZOG1*) gene from *Phaseolus lunatus* either under constitutive (*35S*) or senescence-inducible (*SAG12*) promoters.

Dynamics of the expression profile of four selected genes was followed in the individual leaves and in roots during the drought stress progression (1, 6, 11 days of water stress) and 1-day recovery. Monitored genes were related to the plant response to water stress (dehydrin *NtERD10B*), senescence (activity of *SAG12* promoter determined as *SAG12::ZOG1* expression), degradation of osmoprotectant proline (gene coding for proline dehydrogenase - *cig1*) and regulation of the stability of chloroplast transcriptome (chloroplast endoribonuclease *CSP41*). The content of plants hormones CKs, auxin and abscisic acid (ABA) was followed in upper, middle and lower leaves and roots.

The levels of the expression were quantified by real time RT PCR. Quality of RNA and PCR products were verified by agarose gel elecrophoresis. CK metabolites were quantified by LC/MS, auxin and abscisic acid by 2D-HPLC.

The expression of dehydrin gene quickly increased in the whole plant after water supply cessation and quickly decreased after rehydration. The *cig1* expression exhibited fast decrease at drought, but only gradual elevation after re-watering. *SAG12* promoter activity depended strongly on the leaf position, being stimulated starting from the lower leaves. After rehydration *SAG12* activity fell down very quickly. Opposite profile was observed in case of *CSP41*, expression of which was higher in upper leaves and during the stress it was diminished. ABA strongly increased during drought stress, especially in upper leaves. In *35S::ZOG1* plants delay in the increase of ABA was observed. Later on ABA levels were comparable to WT. After mild stress, gradient of bioactive CKs in favour of upper leaves was found in WT. As drought progressed, content of bioactive CKs decreased, nevertheless, their gradient was found in all genotypes tested. Under stress conditions *SAG12::ZOG1* expression resulted in elevation of total CK content, but not of bioactive CKs. During drought, significant accumulation of CKs occurred in roots. Simultaneously, auxin increased in roots and lower leaves. This indicates that both CKs and auxin play a role in root response to severe drought, which involves stimulation of primary root growth and branching inhibition.

Oxalate oxidase is strongly activated during cadmium induced premature xylogenesis in barley roots

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The effect of heavy metals (Cd, Hg, Ni, Pb, Cu, Co), salt treatment and elevated temperature on oxalate oxidase (OxO) activity and lignin formation was analysed in barley root. OxO activity strongly increased in the root region 2-4 mm behind the root tip and raised toward the root base in heavy metals treated roots. Elevated OxO activity was also observed in NaCl treated roots and roots grown at 31°C and in all experiments it was localised in the root stele – in the central vascular tissues. In early metaxylem vascular bundles also precocious lignification was observed due to heavy metals and salt treatment. We supposed that OxO is activated during abiotic stress and as H_2O_2 generating enzyme probably causes premature root xylogenesis.

Study of hybrid proline-rich cell wall proteins: from sequences to the function

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Hybrid proline-rich proteins (HyPRPs) represent a group of structural plant cell wall proteins. Their expression is strongly upregulated under varied conditions, but their function remains unclear. HyPRPs consist of a signal peptide and two distinct domains: usually a repetitive N-terminal proline-rich domain and a hydrophobic C-terminal domain with 8 cystein residues conserved in specific positions – the 8 CM domain.

Searching for the function of HyPRPs we used different approaches: detailed phylogenetic sequence analysis, expression analysis and direct modulation of expression of selected *HyPRP* genes. The sequence analysis revealed that N-terminal domains of HyPRPs are very variable with respect to the length and the amino acid composition, including proteins with long or short or no N-terminal domains, rich in proline (PR) or glycine (GR). C-terminal domains were much more conserved and therefore suitable for phylogenetic analysis.

The phylogenetic tree constructed from all available sequences of C-terminal domains from seven plant species (incl. monocots, dicots and gymnosperms) indicated existence of distinct subfamily of conserved HyPRPs, which often have long PR domains with high portion of hydrophobic and aliphatic amino acids. In angiosperms, each species have only few relatively well conserved C-type HyPRPs while in gymnosperms (*Pinus taeda*) this group appeared to be highly diversified. Atypical HyPRPs with N-terminal domains (GR or very short or without them) evolved probably independently.

Based on these results, we suggest possible evolutionary scenario that HyPRPs evolved from a LTP-like protein, which acquired a long PR N-terminal domain. This ancestral HyPRP produced the current diversity through ongoing gene duplications accompanied by shortening, modifications, inversions, rearrangements or loss of sequences encoding the PR domains.

Expression profiles of potato and *Arabidopsis HyPRP* genes were very variable, partially overlapping and complementary in different organs, suggesting high level of redundancy. This could also be the reason for missing phenotypic changes in transgenic potato plants with modified (either up or down-regulated) expression of single *HyPRP* genes that we prepared and characterized. For future transformation experiments we have decided to concentrate on C-type genes with “housekeeping” expression pattern. Both potato C-type genes will be knock outed by way of RNA-interference.

Cis-zeatin type cytokinins are biologically active and their metabolism differ from *trans*-zeatin

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Cytokinins (CKs) are important plant intrinsic compounds that affect many developmental processes such as leaf senescence, seed germination, plant-pathogen interactions etc. Generally, they are N^6 – substituted adenine compounds with isoprenoid aromatic side chain occurring frequently in the form of sugar conjugates. Zeatins represent isoprenoid group of CKs in plants. *Trans*-zeatin (*t*Z) is biologically active CK while none or very weak biological effect has been reported for *cis*-zeatin (*c*Z) so far. However, certain plants such as *Triticum aestivum, Avena sativa, Nicotiana tabacum* contain prevalence of *cis*-zeatin and its derivates.

Here we demonstrate that *cis*-zeatin exhibits biological activity in oat chlorophyll retention and tobacco callus bioassays, although in higher concentrations than corresponding *trans* isomer. The presence of less degraded plastids in oat leaves was confirmed microscopically. Similar results were found after *c*Z- and *t*Z-ribosides and *O*-glucosides treatment in oat chlorophyll retention bioassay. No significant biological response was detected only after *c*Z-9-glucoside treatment. Endogenous CKs level was dramatically changed in case of *trans* isomer at the end of oat chlorophyll retention bioassay but only slightly after application of *c*Z. Treatment of oat leaves with radiolabelled *c*Z and *t*Z revealed an uptake of both isomers. Applied *c*Z was rapidly *O*-glucosylated and then degraded by cytokinin oxidase/ dehydrogenase (CKX) activity, unlike *t*Z that was degraded by CKX at first and then *N*-glucosylated.

Overall these results report biological activity and distinct metabolism of *c*Z when comparing to *t*Z thus indicating possible unique physiological role of *cis*-zeatin and its conjugates in plants.

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Regulation of *Chlamydomonas reinhardtii* cell cycle by A-type specific cyclin dependent-kinase

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Eukaryotic cell cycle is regulated by the activity of kinases named cyclin-dependent kinases (CDKs). Two types of cyclin-dependent kinases, A and B type CDKs, were identified to be involved in the cell cycle regulation in higher plants and algae.

In the model organism, green algae *Chlamydomonas reinhardtii* there is one homologue of CDKA. CDKA transcript is present constitutively during the cell cycle. To study the function of CDKA in the regulation of cell cycle in *Chlamydomonas reinhardtii*, we prepared gene cassette in vector pNE537, where gene of interest is cloned under Ni inducible promoter from cytochrome c6 (Cyc6). In consequence, Ni induced RNA interference (RNAi) lead to downregulation of CDKA and transformants were screened for growth phenotype – progress through the cell cycle, growth rate and daughter cell size.

Transformants were cultivated in minimal medium in microtiter plate and microculture growth was measured by optic density. In the presence of $NiCl_2$ transformants with downregulated CDKA grew slower comparing to the transformants without $NiCl_2$. Interestingly, cell sensitivity to nickel ions depends on growth rate. More detailed analysis revealed that growth of cells with downregulated CDKA is similar in the presence and absence of $NiCl_2$. However, in the presence of $NiCl_2$ the cells attained commitment point later, cell division was delay and daughter cells were bigger comparing to control daughter cells.

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Microarrays as a possible technique for GMO screening

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Genetically modified organisms (GMOs) are already parts of our lives in these days. Especially food and feed produced from GMOs has become a part of our food chain. However, GM food and feed is not widely accepted in several countries including EU - there are applied precautionary principles and there is food labeling laws which also include treshold limits for GM content. Due to asynchronous authorisation of various GM namely between USA and EU built up the problem of approved and unapproved GMOs identification in EU enforcement laboratories. GMO analysis are provided mostly via PCR and real-time PCR. However, only approved GMO can be identified in the food using published and validated PCR, real-time PCR methods. PCR analyses could be money- and time-consuming in case of unknown GMO and multiple stacked more advanced genes. Therefore, high troughput cost effective methods are desired. One of the possible is to use a Microarray technology which could be used to analize as many as hudreds of DNA sequences in one experiment.

We have tested the throughput of in house developed DNA arrays based on control amplicons. In this research there was developed in-house GMO chip for identification of four plants species, five genetically modified organisms (GMOs) and three GMO screening elements. As probes there were used PCR amplicons of selected genes.

There were tested three ways of labeling templates using fluorescent Cy3/Alexa3 and Cy5/Alexa 5 colours. There were tested hybridization of PCR products, total genomic DNA and treated DNA on the chip. The sensitivity was determined in the developed chip trough dilution of the hybridization template.

The main goal was to get a signal after hybridization of labeled total genomic DNA on the chip. However, the signal after hybridization of total genomic DNA wasn’t significant. Thus, we propose another procedures for signal improvement of total genomic DNA.

Evaluation of gene expression in winter barley cv. Luxor during acclimation and cold stress

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Besides the defense mechanisms which were developed during the evolution, some plants are capable of a much faster adaptation to current conditions. However, even this process, called acclimation, requires some time and appropriate environmental conditions (mainly temperature and photoperiod) to develop the maximum tolerance possible. For winter barley cold acclimation investigation we have chosen twenty-one days at 3/2°C (day/night) and 12 h photoperiod after 21 days of cultivation at 18/13°C. Samples (the second fully developed leaf and crown) were taken after 0 (control), 24 hours, 3, 7 and 21 days of cold acclimation. After this the plants were exposed to -3°C for 24 hours.

We aim to compare expression profiles of leaf and crown tissues in the course of chilling and freezing as well as confront different cultivars and thus contribute to better understanding how winter cereals cope with low temperature at the RNA level. As a model cultivar we used locally adopted cv. Luxor that had not been studied before. Leaf samples were analyzed first, followed by crowns as a tissue crucial for overwintering.

Affymetrix chips (GeneChip Barley Genome Array, Microarray Core Facility, The Academy of Science) were used to obtain expression profiles, all samples were done in triplicate. It includes RNA isolation, purification (with DNase treatment), and quantity and quality analysis. Obtained data let us to compare expression of nearly 23 000 genes, including 5000 genes with significantly altered expression ($p \leq 0,05$) or 2000 ($p \leq 0,01$). For further evaluation (clustering and functional analysis), the data from ANOVA (level of significance 0,01) were employed. Using Gene Spring software 25 clusters were identified, each cluster being characterized by a typical course. Functional analysis allowed us further to find genes that are regulated in a similar way. Results and discussion will be presented.

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Ecophysiological traits of plant cuticle

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Plant cuticle is the uppermost layer of the leaf surface, forming the interface between epidermal cells and the atmosphere. More than four hundred million years ago, cuticle contributed to the plant colonization of terrestrial habitats. The cuticle forms an effective barrier protecting plants from uncontrolled loss of water and nutrients and it reduces infection by pathogens. During water stress, when stomata are closed, plant survival depends on the amount of water lost through the cuticle. From a whole-plant point of view, the interplay between stomatal regulation and cuticular properties is therefore essential. In this study, I focused on environmental factors affecting cuticular properties, such as water permeability, wax chemical composition and isotopic signature of cuticles and transpired water.

Several methods were used for investigation of hypotheses: Water permeability of cuticular membranes isolated from the adaxial (astomatous) and abaxial (stomatous) leaf sides. The method used allows separation between water diffusion through the remnants of the original stomatal pores and water diffusion through the solid cuticle. Wax chemical composition was analysed by capillary gas chromatography with flame detection. Identification of wax compounds was done by gas chromatography-mass spectrometry. Isotopic signature of transpired water was assessed with IRMS coupled to TC/EA. Leaf $^{13}C/^{12}C$ isotope ratio was determined using an elemental analyser linked to IRMS.

My results indicate: 1) The water permeability of the solid stomatous cuticles was significantly higher than that in the astomatous ones. The water permeability differed according to drought resistance strategy of investigated plants. 2) Environmental changes (humidity, diffusion coefficients) during plant growth did not change the wax chemical composition, whereas the relative abundance of wax components changed. Further, the wax composition was affected by ABA application. 3) Analysis of water isotopic composition reflected the proportion of stomatal and cuticular transpiration; it indicates that plants cannot completely close stomata during drought stress. 4) Finally, carbon isotopic composition of isolated cuticles reflected the environmental factors such as humidity and temperature.

The findings show that plant cuticle is a sensitive element of the plant–environment interaction network. Carbon isotopic composition could be used as a helpful tool to identify environmental growth conditions.

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The relationship between development, frost tolerance and accumulation of DHN5 protein in barley (*Hordeum vulgare*)

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Frost tolerance (FT) is an essential physiological trait for overwintering of plants of temperate climate habitats. In barley, as well as in other cereals, an increased level of FT can be induced under the conditions of cold acclimation (CA). The capacity of the plant to induce FT is strongly dependent on its developmental stage. The transition from the vegetative stage into the reproductive stage is associated with a significant decrease in the ability to induce FT upon cold.

It is known from wheat that the WCS120 proteins from the dehydrin (LEA II) family can be used as markers of acquired level of FT, *i.e.*, their accumulation correlates quantitatively with the acquired FT. We have found out in the literature that in barley, WCS120 orthologue named DHN5 has been described whose expression is specifically induced under CA. We have tested whether DHN5 can be used as a marker of FT in barley. The accumulation of DHN5 protein on immunoblots was detected by a specific anti-dehydrin primary antibody and the level of DHN5 accumulation was evaluated by densitometric analysis (Quantity 1-D software).

Having used a set of 21 differently frost-tolerant barley cultivars, we have found out that after a 3-week CA (a time when maximum FT level is reached), the amount of accumulated DHN5 in the individual cultivars correlated well ($r = 0.9$) with the level of acquired FT.

We have investigated changes in DHN5 accumulation during a long-term CA in Atlas 68, Igri and a set of doubled haploid lines derived from an Atlas 68 × Igri cross. We have found out that in Atlas 68 and also in spring-type DH lines, the amount of accumulated DHN5 increased during the first three weeks of CA, but it fell down rapidly then and remained low until the end of CA. In contrast, in Igri and winter-type DH lines, the amount of DHN5 also increased at the beginning (after 3 weeks of CA, the amount of DHN5 in Igri was only slightly higher than in Atlas 68, since Igri is a relatively low frost-tolerant winter barley), but they were able to maintain the increased level of DHN5 for a relatively long period - ca 12 – 13 weeks of CA. The maximum FT and the maximum level of DHN5 accumulation in Igri was observed after 9 weeks of CA.

Thus, it can be concluded that the main difference between a spring growth habit and a winter growth habit lies in the capacity to maintain an enhanced level of FT under a long-term CA. The winter growth habit can maintain an enhanced FT and enhanced level of DHN5 accumulation under a long-term CA due to the existence of vernalization requirement while the spring growth habit fails in the maintenance of enhanced FT and DHN5 during a long-term CA due to a more rapid development (absence of vernalization requirement).

Changes in photosynthetic induction of European beech and Norway spruce seedlings grown under elevated CO_2 concentration

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Effects of elevated CO_2 concentration (EC) on net photosynthetic rate (*A*) have been the subject of many studies. Most of these studies, based on **steady-state measurements**, show that *A* is increased by an initial (*short-term*) exposure to EC. However, over *long time periods* substantial reductions in EC-enhanced photosynthesis (down-regulation) may occur. Despite the potentially important ecological consequences, very little is known about the synergic response of photosynthesis to EC and **dynamic light environment**.

The main hypotheses why EC should enhance carbon gain in fluctuating light are: (A) EC may potentially influence the ability to rapidly reach light saturation, since EC can decrease the amount of Rubisco or its activity, (B) EC plants may decrease stomatal conductance, (C) even if actual induction rates are not altered by EC, the reduced maximum activity of Rubisco and rate of stomatal conductance should be reached faster in EC plants than AC ones. Since effective utilization of fluctuating light requires fast photosynthetic induction after leaf illumination, our experiments were designed to study photosynthetic induction curves.

Simultaneous measurement of fluorescence and gas-exchange induction curves was determined using PAM-2000 (H.Walz, Germany) directly fixed to the assimilation chamber of Li-6400 (Li-Cor, USA). The experiments were done on five years old seedlings of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) grown (3 months) under ambient (AC; 375 ± 5 µmol(CO_2) mol^{-1}) and elevated (EC; 700 ± 25 µmol(CO_2) mol^{-1}) CO_2 concentrations using the glass domes with adjustable windows.

We confirmed the hypothesis that EC leads to significant decrease in Rubisco amount in the both investigated tree species. Time constant for Rubisco activation increased under EC in deciduous *F. sylvatica*, while it remained unchanged in coniferous *P. abies*. Both *F. sylvatica* and *P. abies* manifested significant decrease in light-saturated stomatal conductance (G_{Smax}) as well as decrease in time required to reach 90% of G_{Smax}. Further, we found the significant increases of light-saturated CO_2 assimilation rates (A_{max}) in both tree species cultivated under EC. The mentioned photosynthetic adjustments consequently resulted in the reduction of time required to reach 90% of A_{max} in *P. abies* grown in EC; however, it was not changed in *F. sylvatica*. Our results thus show that the adjustments of photosynthetic induction in EC trees are species-specific. The initial hypotheses were confirmed only in *P. abies*.

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Effect of phytohormone on growth and bulb formation in garlic at different plant age

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Increasing of grower coefficient of garlic in *in vitro* culture is engaged in the laboratories on the all world. The main aim of this work was monitoring the plants growth and tillering of *Allium* in different age in *in vitro* cultures in the basal MS medium with application growth regulators NAA and BAP and their combinations.

As an experimental plant was chosen the landrace cultivar of *Allium* known like Czech clone 1669. In the MS medium were applied these combinations of growth regulators: NAA (0-0,05-0,1-0,2-0,25-0,5 µM) and BAP (0-0,1-0,2-0,5-1,0-1,5 µM). From the bulbs of garlic were extirpated shoot tips, which were then cultivated in three variants in the MS medium (1 month – 1 week – 1 day). The plant cultivation was realized in the control conditions.

The rate of biomass growth was very similar in all these three variants. The main frequent of shoot tips was in the 1 month old plants variant and it was in the range concentration 0,25-0,5 µM NAA and 0,1-0,5 µM BAP. There were formed the most number of new bulbs of garlic in this variant and these concentrations.

Phosphorylation of thylakoid membrane proteins in green algae

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Protein phosphorylation is a mechanism used by eukaryotes to regulate various cellular activities. In plants, protein phosphorylation is a key response to environmental signals. The light-induced phosphorylation of several proteins in thylakoid photosynthetic membranes of plant chloroplasts was discovered by J. Bennett in 1977. The major phosphorylated proteins have been identified as D1, D2, CP43 and PsbH subunits of photosystem II (PSII) as well as polypeptides belonging to the light harvesting complex (LHCII) of this photosystem.

The synchronized culture of *Scenedesmus quadricauda* was grown under sinusoidal regimes mimicking the circadian light-dark cycles. Samples collected at time were subjected to biochemical analysis (SDS-PAGE, CN-PAGE, BN-PAGE, 2-D electrophoresis, immunoblotting). The obtained results were similar to those previously using a culture of *Scenedesmus* synchronized by discontinuous constant light-dark cycles. In both measurements significant rhythmic changes in photosynthetic activity and photosystem II protein phosphorylation during the cell cycle have been observed.

These measurements will be completed by thermoluminiscence and fluorescence characteristics of the cultures. Comparison with asynchronic cultures grown at different light conditions will be also performed.

The role of the auxin efflux carrier PGP19 in BY-2 cell suspension

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The cell-to-cell polar transport of the phytohormone auxin is a complex process consisting of both passive diffusion of auxin molecules across plasma membrane (PM) and specific activities of auxin influx and efflux carriers. Thus the polar auxin transport machinery is partly energy-dependent. Molecular-biological studies revealed the dominant role of PIN proteins in polar auxin transport and suggested that PIN-mediated auxin efflux involves interactions with other PM regulatory and transport proteins. Plant cells contain ATP-dependent transporters belonging to the multidrug resistance/P-glycoprotein (MDR/PGP) family. PGP proteins are integral PM-proteins related to mammalian PGPs, with relatively narrow specificity. There are 22 members of the PGP family in Arabidopsis. Three of them (PGP1, PGP4 and PGP19) have been characterized as participants in tissue-specific auxin transport. When they interacted with PIN proteins, the specificity of PGP-mediated auxin efflux was enhanced (Blakeslee et al., 2007). Defective *PGP* genes in *Arabidopsis* result in dwarf mutants with altered gravitropism and auxin efflux.

We used dexamethasone-inducible *PGP19* overexpression in tobacco BY-2 cells (line GVG-PGP19:HA) for comparison of the roles of PIN and PGP proteins in auxin efflux from cells. We have proved the localization of PGP19:HA protein at PM and shown that the accumulation of [^{3}H]NAA was decreased similar to GVG-PIN7 line (Petrášek et al., 2006). However, compared to PIN7-mediated auxin efflux, the PGP19-mediated NAA efflux was notably less sensitive to NPA, the inhibitor of polar auxin transport at the level of cellular auxin efflux. Phenotype of BY-2 cells after overexpression of *PGP19* was highly similar to that of *PIN7*, however, in concert with lesser sensitivity to NPA of PGP19-line, NPA was not able to rescue PGP19-related phenotype.

Blakeslee, J. J. et al.: Interactions among PIN-FORMED and P-Glycoprotein Auxin Transporters in Arabidopsis. - Plant Cell 19: 131-147, 2007.

Petrášek J. et al.: PIN proteins perform a rate-limiting function in cellular auxin efflux. - Science 312: 914-918, 2006.

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Short-term responses of photoprotective mechanisms to artificial photoinhibition in *Arabidopsis thaliana*

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Light represents major energy input into photosynthesis and main precondition for plant growth and development. However, excess light may produce reactive oxygen species (ROS) in chloroplast. Since ROS are harmful for plants, several protective mechanisms exist to avoid negative effects of excess light. Energy that is not used in photosynthetic processes might be e.g. dissipated as heat by xanthophyll cycle pigments, zeaxanthin in particular. Oxidation of glutathione is another photoprotective mechanism. In our study, we investigated non-photochemical quenching of absorbed light energy in *Arabidopsis thaliana* in violaxanthin deepoxidase- and zeaxanthin epoxidase-deficient mutants (npq1-2 and npq2-1 mutant, respectively). We studied also mutants that have reduced amount of protein subunits that form the light harvesting complexes of photosystem II (lhc2-1) and lacking practically all of them (lhc2-12).

The main objective of our study was to quantify plant response, primary photosynthetic processes in particular, to high light doses. Quenching mechanisms and antioxidants production were in focus. *Arabidopsis thaliana* plants were cultivated in controlled conditions of 16/8 h d/n photoperiod, 21/19 ^{o}C temperature, 55 % of relative air humidity, and irradiance of 140 µmol m^{-2} s^{-1} for 65 days. Then, they were exposed to photosynthetically active radiation of 600 µmol m^{-2} s^{-1} for 90 min to induce high light stress in photosynthetic apparatus. Inhibition of photosynthetic processes, as well as their consequent recovery in dark, was measured fluorometrically (PAM-2000, Walz, Germany). We evaluated light-induced changes in capacity of primary photosynthetic processes (Fv/Fm), quantum yield of photosynthetic processes in photosystem II (Φ_{II}), non-photochemical quenching (NPQ). Pigments and antioxidants (zeaxanthin, glutathione, tocopherol) were extracted before and after high-light treatment and evaluated using a HPLC. In Npq mutants having blocked zeaxanthin synthesis, amount of zeaxanthin remained unchanged by light treatment while lhc2-12 mutants exhibited an increase of zeaxanthin due to its light-induced synthesis. Amount of lutein was not affected by light in both Npq and Lhc mutans. Lhc2-12 plants had, however, contrastingly lower lutein amout throughout light treatment and recovery. Oxidized glutathione (GSSG) showed similar response. It increased immediately after high light treatment in control plants, while it was constant in Npq and Lhc mutants. The results indicate that reduced amount of light harvesting complexes may alter zeaxantin formation while amount of other photoprotective and antioxidative compounds may be unaffected.

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Detection of changes in mesophyll structure using confocal microscopy and stereology: application on Norway spruce needles treated with elevated CO_2.

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The internal leaf structure is tightly connected with many important physiological processes in leaves. Recent studies show that relationships between leaf anatomy parameters and photosynthesis are important in leaf acclimation to high or low irradiances or elevated CO_2 concentrations. Elucidation of changes in needle anatomy connected with elevated CO_2 may help to understand the potential of conifers in carbon sequestration. In this study I focused on proportion of intercellular spaces and internal surface density in mesophyll of Norway spruce needles.

Initially, technical aspects of using free-hand sections of frozen needles for three-dimensional analysis of mesophyll by stereology and confocal microscopy was tested. No significant differences in measured parameters of mesophyll were revealed between fresh and frozen needle samples. However, several specific constraints of using stereological methods of fakir and point counting method were established.

The effect of elevated CO_2 concentration on Norway spruce needle anatomy was studied on sun-exposed and shaded needle samples collected from a long-term simulation experiment at Experimental Ecological Study Site Bílý Kříž (http://www.usbe.cas.cz/lefr/bily_kriz.htm) in the Moravian-Silesian Beskydy Mts. in August 2004. Experimental trees were grown for 8 years in glass domes with adjustable windows with following CO_2 concentrations: 350 μmol CO_2 mol^{-1} and 700 μmol CO_2 mol^{-1}; control trees were grown in adjacent open-air stand.

As far, no effect of elevated CO_2 concentration on needle volume and proportion of individual needle tissues was detected, however, anatomical alterations are axpected at more subtle level, which is under investigation: measurements of internal surface density of mesophyll are now in process. Working hypothesis suggests higher internal surface density as response to elevated CO_2 concentration. Moreover, higher morphological plasticity is known for shade spruce foliage, therefore more pronounced changes in internal surface density of shaded needles are expected.

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The impact of drought and heat stress on antioxidant enzyme activities in tobacco plants over-expressing gene for cytokinin oxidase/dehydrogenase

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Plants must often sustain unfavorable conditions in natural environment. Abiotic stress as drought and heat shock affect negatively plant growth and yield. Stress conditions cause accumulation of reactive oxygen species that disrupt cellular redox homeostasis. Plant cell copes with elevated reactive oxygen species concentration by antioxidative system composed of enzymatic and non-enzymatic components. We examined effect of decreased cytokinin concentration on the response of enzymatic antioxidative system in tobacco plants in cosequence of combined stress.

Two transgenic lines and appropriate wild type (WT) were analyzed. Transgenic plants of *Nicotiana tabacum* L. cv. Samsun NN over-expressed a gene for cytokinin oxidase/-dehydrogenase either in roots under promotor WRKY6 (*WRKY::CKX1*) or in whole plant under constitutive promotor 35S (*35S::CKX1*). Fiftyseven-day old plants were treated by 8 days water withdrawal and by heat 40 °C for 2 hours. Activities of antioxidant enzymes were measured spectrophotometrically (superoxide dismutase, ascorbate peroxidase) and polarographicaly using oxygene electrode (catalase). Isoenzymes were visualized on gels after native electrophoresis. Enzymes were analyzed in upper, middle and lower leaves and in roots.

Antioxidative enzymatic system response differed in dependence on the individual parts of plant, stress type as well as cytokinin concentration. Plant transgenosis influenced activity of antioxidative enzymes, e.g. superoxide dismutase activity during drought in WT decreased, while it increased in *WRKY::CKX1*. A distribution of isoforms was also affected, e.g. *35S::CKX1* lacked one superoxide dismutase isoform. The elevated activity of cytokinin oxidase/dehydrogenase influenced both quantity and quality of antioxidative enzymatic system considerably. Plants *WRKY::CKX1* showed usually higher activity of antioxidative enzymatic system in stress conditions than other analyzed plant types which correlated well with apparent higher damage in WT and *35S::CKX1*.

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Light independent chloroplast development in four gymnosperms species

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The transformation of protochlorophyllide (pchlide) into chlorophyllide (chlide) is key regulatory step of chlorophyll (chl) biosynthesis. In angiosperms, the reaction is light dependent catalysed by the enzyme NADPH:protochlorophyllide oxidoreductase (LPOR), which is encoded by nuclear genome. In etiolated angiosperms the Pchlide accumulates, what leads to feedback repression of aminolevulinic acid (ALA) synthesis, common precursor of tetrapyrols. In gymnosperms, plastids encoded light independent protochlorophyllide oxidoreductase (DPOR) catalyses transformation of pchlide into chlide also in the dark. We studied four gymnosperms species with different ability to synthesize chlorophyll in the dark (*Larix decidua*, *Picea abies*, *Pinus mugo*, *Gingko biloba*). We determined chl and pchlide content as well as ALA synthesizing capacity in dark and light grown seedlings and after transferring from dark to the light. Chlorophyll fluorescence (FMS-2, Hansatech, Norfolk, UK) and gas exchange (CIRAS-2, PP-Systems, Hitchin, UK) measurements were also performed. Samples for electron microscopy were stained with uranyl acetate and lead citrate and observed using Joel FX electron microscope. The highest chl, pchlide content and ALA synthesizing capacity was in dark grown spruce (*P. abies*), following by pine (*P. mugo*), larch (*L. decidua*) and gingko (*G. biloba*), which has not the ability to synthesize chl in the dark. After transferring of seedlings from dark to the light Pchlide concentration dropped to zero due to the activity of LPOR and synthesis of ALA and chl rapidly increased in *L. decidua*, whereas in *P. abies* the response to light was weak. Except chl synthesis in the dark, there appears to be a marked reduction in the requirement for light also in the initiation of chloroplast development in these plants. The plastids in dark grown *P. abies* have numerous thylakoids that are organized into grana composed from 2 to 10 membranes. In *L. decidua* grana are minute and consist of only two or three thylakoids. The prolamelar bodies are present in plastids of both species. The effective (ΦPSII) and maximum quantum efficiency (Fv/Fm) in *P. abies* and *P. mugo* achieved the value comparable with seedlings growing in circadian illumination within 6 hours, larch within 48 hours and *G. biloba* need more than 72 hours after transferring to the light. In all studied species the photochemical quenching. (qP) is comparable with light grown seedlings earlier what indicates that PSI becomes fully active before PSII. After 12 hours of illumination the rate of photosynthesis (P_N) was comparable between species, but later P_N was much higher in larch than in the spruce and pine, despite much lower chlorophyll concentration. We hypothesized that higher chlorophyll efficiency and P_N in larch and gingko should lead to the relaxation of functional constraint on DPOR what result in lost of function (gingko) or decreased ability synthesize chlorophyll in the dark (larch). This work was supported by the grants VEGA 1/3288/06 and APVV-20-020805.

Characterization of the auxin efflux machinery in suspension-grown tobacco BY-2 cell line by assessing the specificity towards various auxins and auxin-related compounds

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The plant hormone auxin has been shown to be involved in the regulation of plant growth and development. Auxin is transported from regions of its biosynthesis to regions of its action through unique polar transport pathway. Polar auxin transport depends on directional efflux of auxin molecules from cells and it provides both spatial and temporal signals for various physiological events in plants. The experimental evidence confirms that the polarity of auxin transport through cells and tissues is regulated by the asymmetrical distribution of auxin efflux carriers in the plasma membrane.

In our study we were concentrating on the kinetic characterization of auxin transport in suspension-grown tobacco BY-2 cell line. We have selected various auxins and their structurally-related analogues and assessed their ability to induce the auxin-responsive reporter DR5::GFP. Specificity of the auxin transport pathways has been determined using the assay based on accumulation of radio-labelled synthetic auxin naphthalene-1-acetic acid (1-NAA) which is considered as a good substrate for auxin efflux carriers. The kinetic parameters of BY-2 efflux carriers were evaluated by a simple displacement of radio-labelled 1-NAA by non-labelled auxin analogues. It was found that auxin transport machinery is specific to those compounds which were able to induce DR5::GFP reporter.

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Bioaccumulation of selenium in cells of the green alga *Scenedesmus quadricauda*

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Selenium is an essential micronutrient in the diet of many organisms, including humans. The mechanisms of selenium functions are still unknown. Selenium was shown to have a role in the prevention and slowing down the development of breast, prostate and colorectal cancers, in immune function, and in reproduction. Although it has important biological functions at higher concentrations selenium can be genotoxic and possibly carcinogenic. The toxicity of selenium compounds is caused by the generation of reactive oxygen species (ROS) which can induce DNA oxidation.

While other metals are incorporated into protein structure post-translationally, Se is inserted during translation as a rare amino acid, selenocysteine. Selenoproteins include enzymes involved in maintaining the cell redox potential (glutathione peroxidases, thioredoxin reductases), proteins implicated in the selenium transport (selenoprotein P) and proteins with unknown function (selenoprotein W or R).

Selenium enters the food chain through plants that take it up from the soil. It has been shown that both the dose and chemical form of selenium are the critical factors in the cellular response. Supplementation by inorganic selenite salts is less efficient than usage of organically bound selenium. Therefore the algal cells enriched by selenium could be used as an excellent source of organically bound selenium-compounds.

Green chlorococcal alga *Scenedesmus quadricauda* is able to produce biomass with a high content of organically bound selenium. There is narrow border between deficiency and toxicity of selenium, which can complicate both a dosage of selenium to target organisms and production of selenium enriched source biomass. Therefore, we selected three independent mutant strains differentially resistant to the presence of high concentrations of inorganic selenium in the form of selenite, selenate or a combination of both. The SeIV strain was resistant to high doses of selenite but its sensitivity to selenate was the same as that of wild type. The SeVI strain was resistant to selenate but sensitive to selenite. The strain Se(IV+VI) was resistant to the combination of both selenite and selenate. However, its growth rate was lower than the wild type one. Also, the strain's resistance to both compounds was lower than that of respective resistant strains. Our findings imply that the effect of selenite and selenate on the cells is based on distinct mechanisms.

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Subcellular and organ responses of plants to occurrence of organic pollutant

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The environment is continuously polluted by a large array of hazardous chemicals. An important group is polycyclic aromatic hydrocarbons (PAHs), which are found in all environmental compartments and are mainly the products of incomplete combustion. The plant's ability of PAHs uptake, translocation, transformation and accumulation is a limiting factor for phytotoxicity of these compounds and can affect quantitatively and qualitatively several biochemical and physiological processes. PAHs are lipophilic substances, which influence the membrane integrity. The primary processes of photosynthesis, localized at thylakoid membranes of chloroplasts, are known to be sensitive to these organic contaminants.

The monitoring of stress by chlorophyll (Chl) fluorescence of whole leaves or plants permits very early stress detection in plants at a stage in which countermeasures can still be taken to overcome the stress-induced changes in order to avoid severe damage to the plants. The photosynthetic parameters can be used as reliable indicators of many stressors. Chl fluorescence has allowed better understanding of photochemical and nonphotochemical processes, which could be related to the changes in the structure and might alter the permeability of thylakoid membranes of chloroplasts.

The aim of this study was to evaluate the effect of concentration (0.1 and 1 $mg.l^{-1}$) of important organic pollutant fluoranthene (FLT) on primary processes of photosynthesis on subcellular (chloroplast suspension) and organ levels. Chl fluorescence measurements were done on plants cultivated *in vitro* on whole plants and on the leaves of plants cultivated hydroponically. Pea plants (*Pisum sativum* L. cv. Garde) were cultivated in Reid-York nutrient solution. Murashige-Skoog cultivation medium with IAA (0.1 $mg\ l^{-1}$) or combination of IAA and BA (0.1 $mg\ l^{-1}$) was used for *in vitro* cultivation of plants. Chl fluorescence parameters (F_0, F_V/F_M and Φ_{II}) of chloroplast suspension and soilless-cultivated plants were determined from an analysis of slow (Kautsky) kinetics supplemented with saturation pulses, recorded by PAM 2000 portable fluorometer (Walz, Germany). The spatial distribution of these parameters in plants cultivated *in vitro* was investigated by imaging of chlorophyll fluorescence (Handy FluorCam, Photon System Instrument, Czech Republic).

The obtained results demonstrated that applied concentration of FLT (0.1 and 1 $mg\ l^{-1}$) caused increase of F_0 and decrease of F_V/F_M and Φ_{II} values. FLT (1 $mg\ l^{-1}$) caused the significant changes of Chl fluorescence parameters. The response of primary processes of photosynthesis was more intensive on the chloroplast suspension level. The method of Chl fluorescence can be used as an early indicator of contamination of the environment by organic pollutants.

Flowering onset in day-neutral tobacco (*Nicotiana tabacum* L. cv. Samsun) is influenced by transformation with *cdc25* gene from *Schizosaccharomyces pombe*

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Flowering is the process when the shoot apical meristem (SAM) acquires a new developmental fate characterized by a switch from creating leaves (vegetative phase) to creating flowers (reproductive phase). The transition to flowering is one of the most important developmental decisions made by plants. Early classical studies that focused on the contribution of daylenght to control of flowering time demonstrated that plants have different requirements for floral induction, and can be classified as either short day, long day or daylength neutral with respect to flowering.

The plants of day-neutral tobacco (*Nicotiana tabacum* L.) cv. Samsun were transformed with fission yeast mitotic activator coded by cdc25 (Spcdc25) and besides others precocious flowering was observed (Bell et al. 1993). The aim of the presented study was to determine the component of the multifactorial system controlling flowering onset which was most affected by the Spcdc25 transformation and based on the results to contribute to elucidation of flowering regulation in day-neutral plants.

The transformation led to dramatic change in flowering of tobacco plants cultivated under *in vivo* conditions. Transformed plants of two lines flowered earlier (in 90 days) after reaching lower number of leaves / nodes (about 12 leaves / nodes) compared to the control plants flowering in 120 days and after reaching about 42 leaves / nodes. The grafting experiments enabling combination of parts of transformed and control plants indicated, that the speeding up of flowering was preferentially caused by the effect of the transformation on the SAM. This finding was supported by anatomical analysis of SAM which revealed substantial differences between the SAM structure of transformed compared to wild type plants. Interestingly, the phyllotaxy of transformed plants does not appeared to be shifted.

If SAM of transformed plants is changed there could be also a change in the structure of axillary bud meristems. This idea is supported by the observed disturbance of flowering capacity gradient along the axis of transformants. In addition, in the SAM and in axillary buds of transformed plants shifts in expressions of important genes, like TFL1 or FD, involved in the flowering onset can be expected. At present we try to find out the answer to these questions.

Bell MH, Halford NG, Ormrod JC, Francis D. 1993. Tobacco Plants Transformed with Cdc25, a Mitotic Inducer Gene from Fission Yeast. Plant Molecular Biology 23(3): 445-451.

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Growth and Development of Yacon (*Smallanthus sonchifolius*) in Field Conditions and *in vitro*

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Yacon (*Smallanthus sonchifolius), Asteraceae* family, is a plant which is native to the Andes and is cultivated mainly for its tuberous roots which are rich in fructooligosaccharides and leaves with high antioxidant content. Newly established Peruvian landraces in the Czech Republic were studied. First, they were introduced *in vitro* condition, by newly optimized pre-treatment resulting in average 78.3 ± 4.4% of *in vitro* growing plants without contamination. Theoretical number of i*n vitro* propagation was 52 million plants / year when optimal concentration of 0.43 mg/l KIN and 0.093 mg/l NAA was used. Micro-tuberization did not occur with any treatment of different concentrations and combination of sucrose, salts, BAP, JA. However, the best medium for root thickening (0.6cm in diameter after 90 days cultivation) was 2 mg/l BAP and 90 g/l sucrose. Chromosome number 58 (2n) for landraces L15/20, L40, L50 was determined by caryological analysis. Secondly, acclimatization of micropropagated plants to *ex vitro* conditions was successful for 87 ± 3.8 % of plants when commercially available soil substrate was used and plants were cultivated at the temperature 23 °C. The average yield of Peruvian landraces at experimental fields at the Czech University of Life Sciences in Prague was 0.62 kg/plant of tubers, 0.67 kg/plant of rhizomes and the average sugar content was 9.17 °Brix. When compared with non-Peruvian landraces, for Peruvian landraces yield of tuberous roots was 0.31 kg/plant lower. Highest value of sugar content was measured for Peruvian landrace L65 (11°Brix) which is the same value as for the highest non-Peruvian landrace obtained from New Zealand. Negative correlation was found in Peruvian landraces contrary to non-Peruvian in relation of tubers and rhizomes yield. The newly established Peruvian landraces in the Czech Republic can be recommended as a new genetic source for commercial utilization according to their easy *in vitro* propagation, formation of sufficient tuber yield and high sugar content.

Společensko kulturní program konference

Milí mladí kolegové, vážení hosté!

Konference, kterou by tvořila jen věda, by byla nudná a vyčerpávající. Proto jsme pro Vás – jak bývá zvykem - přichystali přivítací večer (9.4.), kterého se můžete, ale také nemusíte zúčastnit. Stejně tak i trocha hradebního historického chleba obohatí náš čtvrteční jídelníček.

Více akcí se na tak krátkou konferenci snad ani pořádat nedá. Přesto jistě mnozí pocítí ve volných chvílích nutkání někam vyrazit. Proto jsme se v další části tohoto programu pokusili stručně vyzdvihnout co se v Nových Hradech (a okolí) a času konání konference děje zajímavého a co by mohlo stát za vidění…

Pokud se Vám kulturní program (ne)bude líbit, vězte, že za to může Jiří Kubásek.

Krásné zážitky Vám přejí organizátoři.

A/„Kultůra“ organizovaná - Společenský večer 9.4. od 20 hod

Kromě přivítání organizátorů, „nenucené družby účastníků“ a občerstvení se můžete těšit na koncert skupiny **Mons Pubis**. Tato formace složená z doktorandů Přírodovědecké a Zemědělské fakulty naší univerzity ještě není moc známá, ale tipoval bych, že v popularitě půjde strmě nahoru. Jak sami prohlásili, hrají něco mezi jazzem a ničím, ale to něco je podle mého docela původní a neokoukané (teda neoposlouchané). Pěkně to šlape a navíc to není úplně triviální ve snaze zavděčit se hlavnímu proudu. Písně staví hodně na textech, které proto možná stojí za to poslouchat.

Kluci mají za sebou několik desítek koncertů v různých hudebních klubech, na naší univerzitě a jako doprovodný program společenských akcí. Právě vydávají své první demo. Připojit se k jejich fanklubu a poslechnout si několik hudebních ukázek (v ne moc dobré technické kvalitě) můžete na serveru Bandzone na adrese: http://bandzone.cz/monspubis. V Nových Hradech budou hrát s hostujícím kytaristou Tomášem Beštou alias Hruškou (mj. členem skupiny Asmodeus).

Prohlídka hradu Nové Hrady – 10.4. v 17 hod

Přeneseme se do dob minulých, do míst, „kde Vitorazská stezka překračovala zemskou hranici, byl kolem poloviny 13. století založen hrad, který v té době sloužil především jako strážné místo. Za husitských válek byl vypálen, roku 1467 vyhořel a obnoven byl až v 1. polovině 16. století za Viléma z Rožmberka. Roku 1573 hrad silně poškodil výbuch prachárny a roku 1605 zemětřesení. Za Švamberků byl hrad silně opevněn, takže roku 1619 odolal prvnímu útoku císařských vojsk, avšak ještě téhož roku byl dobyt generálem Buquoyem“ (zdroj: http://www.mysmas.cz/ceske-budejovice/?text=50-mesto-nove-hrady).

Terčino údolí – 11.4.

Zájemce provede divukrásnou jarní krajinou Jirka Kubásek.

B/„Kultůra“ neorganizovaná

1/Příroda

Podle Nových Hradů se jmenují **Novohradské hory**. Přestože po mnoha letech dohadů nemají stále statut CHKO, jejich půvab a hodnota jsou obecně uznávané. Proti Šumavě – se kterou jsou někdy srovnávány – jsou mnohem menší (asi 90 km^2, NP + CHKO Šumava mají 1670 km^2). Díky tomu jsou i druhově (zejména floristicky) chudší. Reliéf je velmi členitý (ne že by na Šumavě taková místa nebyla, ale celkově je zarovnanější). Nejvyšší vrchol **Viehberg** leží v Rakousku (1 112 m). Na našem území doporučuji k vylezení **Kraví Horu** (953 m) s rozhlednou na vrcholu. Vzdušnou čarou od našeho zámku je přesně 8 km. **Poutní kostel v Dobré Vodě**, který můžete vidět cestou je dle mého fenomenálně zasazen v krajině. Dalšími známými atrakcemi hor jsou nejstarší přírodní pralesovité rezervacemi v České republice i střední Evropě – **Žofínský a Hojnovodský prales**, které byly vyhlášeny již v roce 1838. Novohradské hory sloužily po mnohá desetiletí jako honitba šlechty. Připomeňme rod Buquoyů, který se velmi osvíceně angažoval i v ochraně přírody a založil zmíněné pralesovité rezervace.

Když jsme u Buquoiů, nemůžeme nezmínit **Národní přírodní památku Tereziino (také Terčino) údolí,** kterou máte za humny (1.2 km od zámku). Cituji: (http://www.infocesko.cz/Czechia/Content/clanek.aspx?clanekid=5923&lid=1) “Údolí přiléhající od jihozápadu k Novým Hradům bylo budováno v průběhu tří století hraběcí rodinou Buquoyů. Původně sloužilo jako bažantnice, roku 1756 tu vznikl rozsáhlý anglický park zvaný "Vallancherie". Hraběnka Terezie Buquoyová, podle níž dostalo údolí svůj název, zde v letech 1788-1797 nechala vybudovat malé soukromé lázně a postupně zde vznikly další zajímavé budovy, které měly dokreslovat romantický vzhled parku. Ojedinělým dílem bylo zbudováním umělého vodopádu, v jehož okolí byly do skal vytesány verše romantických básníků.” Na vyvýšenině nad přírodním parkem stojí tvrz **Cuknštejn**, ale to už vlastně volně přecházíme do další kapitolky.

2/Památky

První jistě třeba představit zámek. Ranně klasicistní (empírový) **zámek v Nových Hradech** není zdaleka tak slavný jako jeho rokokový jmenovec ve východních Čechách. Postavili si jej (myšleno ten jihočeský) na přelomu 18. a 19. století Buquoiové jako „upgrade“ svého sídelního barokního domu na náměstí. Zajímavé je, že jeho první majitel Jiří František August von Buquoy byl matematik, přírodovědec, technik a filozof. Už to snad zámek předurčilo k jeho osudu. Po zestátnění v roce 1945 byla na zámku nejdříve dětská ubytovna a později střední zemědělská škola. Nyní zde sídlí **Ústav fyzikální biologie** a **Ústav systémové biologie a ekologie AVČR.** Město se však může pochlubit i **hradem,** který navštívíme ve čtvrtek**.** V Nových Hradech najdeme dále **klášter Servitů** z let 1679-1685. Dnes se nazývá “Božího milosrdenství” a mimo jiné plní funkci jídelny pro akademické osazenstvo zámku.

Východní stranu **náměstí** v Nových Hradech tvoří původní barokní residence rodu (hádejte kterého ☺), dále na náměstí najdeme kamennou kašnu a **radnici,** v jejíž zadním traktu byl pivovar. Pokud si vyjdete na místní hřbitov, najdete tam novogotickou **hrobku Buquoyů** (1890–1892) s místem pro 28 rakví.

V blízkém okruhu města mohu za nejpozoruhodnější jistě označit již zmíněný **poutní kostel Panny Marie dobré rady v Dobré Vodě.** Hodnotná, vrcholně barokní stavba vystavěná nad mírně radioaktivním pramenem byla postavena v letech 1706 - 1718 podle plánů neznámého autora (autorství bývá nejčastěji připisováno K. I. Dientzenhoferovi, ale to asi proto, že podobně jako J.B. Santini-Aichel toho u nás během baroku postavil spoustu). Podle mého, tato instalace v krajině působí nejimpozantněji za soumraku.

A když už jste na jihu Čech, určitě stojí za to podívat se do „**Budějic**" nebo **Českého Krumlova**. Jen heslovitě něco o Českých Budějovicích (zaujme-li Vás něco, snadno si najdete na internetu): hektarové **náměstí Přemysla Otakara II.** je prý největší čtvercové náměstí v Česku (srovnatelné je ještě ve Vysokém Mýtě). **Černá věž** (1549–1577) má asi 72 metrů, několik zvonů (nejtěžší Bumerin 3.5 tuny) a ochoz, kam si můžete vylézt. **Radnice** (renesanční, barokně přestavěná) je modrá (takže dobrá). Samsonova kašna je uprostřed náměstí (jak kašny bývají) a ani Samson, ani 4 „ksichty" plivající vodu nejsou originály. Ty najdete v přízemí radnice. Přímo na náměstí je také **Česká spořitelna**, **Raiffeisen banka** a jejich **bankomaty**. Poblíž náměstí je ještě c**hrám sv. Mikuláše** a **kaple Smrtelných úzkostí Páně.** Historií dýchá komplex **kostela** a **kláštera dominikánů** + **Solnice** kolem **Piaristického náměstí.** Když už budete tam, nezapomeňte se podívat na **žábu** (jestli ještě nespadla) a strčit si prsty do očnic **lebky** (tajné přání se Vám pak jistojistě splní). Nejcennější a nejpůvodnější městskou architekturu najdete (co se centra týče) asi v uličkách **Hradební**, **Mlýnská** a **Panská.** Pokud máte rádi **secesi**, krásná **SPŠ stavební** a mnoho secesních domů je v **Resslově ulici** a okolí. Na jídlo jsou v ČB asi nejlepší restaurace **Alchymista** a **U Tří sedláků**. Velmi stylové je zajít také do **Budvarky** a staronových **Masných krámů.** Svůj svérázný kolorit má mnoho desítek dalších **restaurací, hospod, non-stopáčů, pajzlů, vývařoven, mléčných barů** a dalších institucí. To je však už nad rámec našeho stručného přehledu.

3/Akce

a/Kina

Nové Hrady – Kino Nové Hrady	9.4. 20:00 „**Na Vlastní nebezpečí"**, 55 Kč
České Budějovice – Kino Kotva	8.4. 18:00 **„Občan Havel"**, 90, s ISIC 80 Kč
	8.4. 20:15 **„Pokání"**, 90, s ISIC 80 Kč
	9.4. 18:00 **„Kladivo na čarodějnice"**, 90, ISIC 80Kč
	9.4. 20:00 **„Občan Havel"**, 90, s ISIC 80 Kč

České Budějovice – Multikino Cinestar (spousta filmů, viz program na letáčku, který jsme Vám obstarali)

b/Divadla

Nové Hrady – divadlo nemají

České Budějovice – Jihočeské divadlo

8. 4. 19:00 **„Síla osudu"** (**La forza del destino**), G. Verdi, opera, v DK Metropol

9. 4. 19:00 „**Sudostbayerisches Stadtetheater Passau Giulio Cesare in Egitto“**, G. F. Händel, opera, v Jihočeském divadle (v době psaní programu vyprodáno)

9. 4. 19:30 **„Mrzák inishmaanský“**, M. McDonagh, hodně drsná irská komedie, v Malém divadle (v době psaní programu vyprodáno)

České Budějovice – k.c.Bazilika

8.4. 19:00 Festival Shakespeare: Divadlo Na zábradlí (Praha) – „**Troilus a Kressida“**

České Budějovice – c.k. Solnice

9.4. 20:00 Festival Shakespeare: Divadlo Vizita – **„Ó, tělo a děs démona“**

České Budějovice – Kabaret u Váňů

9.4. 19:06 Jiří Suchý: **„Dobrodružství pana Půlpána aneb Jak pan Půlpán z Pyšel do Prahy si vyšel a o co tam přišel“**

České Budějovice – Divadelní soubor J. K. Tyl

9.4. 19:00 Vratislav Blažek, Zdeněk Podskalský, Jiří Zajíc: **„Světáci“** (organizátor Kubásek doporučuje).

c/Koncerty

České Budějovice – konzervatoř

8.4. 18:00 Koncert posluchačů – Koncertní síň Otakara Jeremiáše

9.4. 9. a 10.4. 19:30 A. Dvořák: Karneval, E. Gregson: Koncert pro tubu a orchestr, A. Dvořák: Rusalka (orchestrální svita ve zpracování Jiřího Temla, sólista: Roman Hoch – tuba, dirigent: Stanislav Vavřínek)

d/Výstavy a přednášky

V Jihočeském muzeu v Českých Budějovicích můžete shlédnout expozice **„Kréta“**, Thomas Hirsch (Mainz, Německo): **„Světlo a stín“**, Miroslav Koupil – „**Kaligrafy**“ a další.

K.c. Bazilika a Wortnerův dům vystavují **fotografie Jindřicha Štreita**.

Zajímavé je navštívit **Jihočeské motocyklové muzeum** v „malé“ Solnici.

8.4. v 17:00 si můžete zajít do Jihočeského muzea poslechnout přednášku Zdeňka Troupa **„Šumava a já“**. Pokud byste 8.4. směřovali na Kleť, můžete se od 21:00 v místním planetáriu zúčastnit **„Večerního astronomického pozorování“**.

e/Sport

Nové Hrady mají **tenisový kurt** (kontakt: p. Jelínek tel.: 728736798), **Fitness** (p. Jelínek tel.: 728736798) a **Tereziiny lázničky** (sauna, ruční masáž, rašelinné termoobklady).

V Českých Budějovicích si můžete zaplavat v **krytém bazénu** nebo zalézt na několika desítkách různě obtížných cest ve velkém „**lezeckém centru Lanovka“**

Organisers:

- Faculty of Science, University of South Bohemia, České Budějovice

Tomáš Hájek, Marie Hronková, Jana Karbulková, Jiří Kubásek, Jiří Květoň, Anna Ruprechtová, Jiří Šantrůček, Marie Šimková, Martina Vašková, Daniel Vrábl

- Czech Society of Experimental Plant Biology
- Institute of Microbiology, AS CR, Třeboň
- Institute of Plant Molecular Biology, AS CR, České Budějovice
- Institute of Systems Biology and Ecology AS CR

Fresh Insights in Plant Affairs, Nové Hrady 2008

Book of Abstracts

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