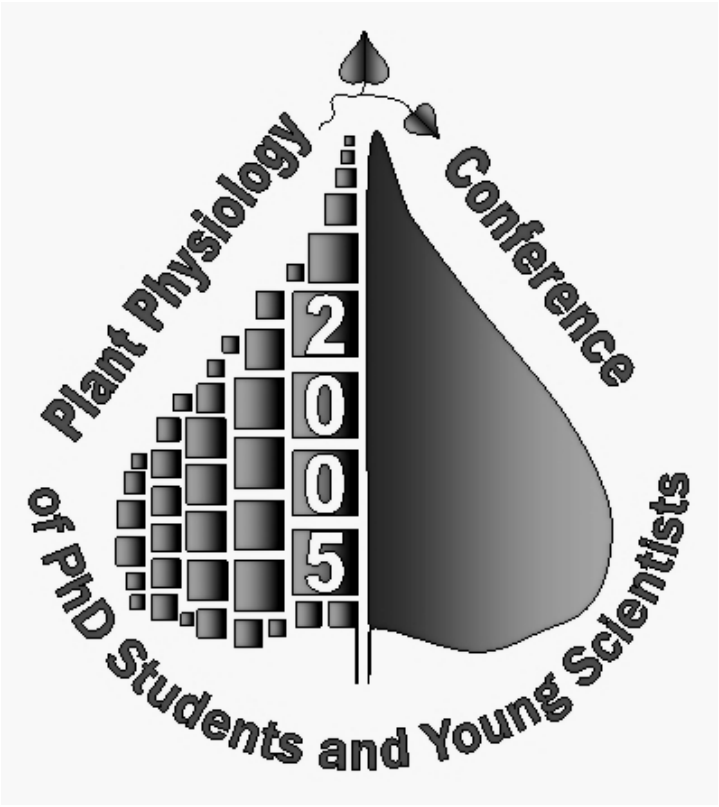
Plant Physiology Conference of PhD Students and Young Scientists



Book of Abstracts

June, 6-8th, 2005, Modra, Slovakia

Plant Physiology Conference of PhD Students and Young Scientists

Organized by:

Department of Plant Physiology
Institute of Botany
Slovak Academy of Sciences, Bratislava

Department of Plant Physiology
Faculty of Natural Sciences
Comenius University, Bratislava

June, 6-8th, 2005, Modra, Slovakia

SCIENTIFIC PROGRAM

SCHEDULE OF LECTURES

TUESDAY, June 7, 2005

09.00-09.05 **Opening Ceremony**

09.05-09.25 M. Nadubinská
09.25-09.45 M. Kubeš
09.45-10.05 D. Reňák
10.05-10.25 P. Illéš

10.25-10.40 C o f f e e

10.40-11.00 Š. Zezulka
11.00-11.20 J. Štepigová
11.20-11.40 A. Pavlovič
11.40-12.00 A. Machlicová

12.00-13.20 L u n c h

13.20-13.40 J. Kubásek
13.40-14.00 P. Ferus
14.00-14.20 V. Demko
14.20-14.40 P. Valentovič
14.40-15.00 L. Kolarovič

15.00-15.30 C o f f e e

15.30-17.30 Poster Session

19.00 **Welcome Party**

WEDNESDAY, June 8, 2005

09.00-09.20 M. Martinka
09.20-09.40 P. Vítámvás
09.40-10.00 M. Ollé
10.00-10.20 P. Paľove-Balang

10.20-11.00 C o f f e e

11.00-12.00 Assoc. Prof. M. Kutáček, and Dr. M. Luxová Awards

12.00-13.00 L u n c h
13.30 D e p a r t u r e t o B r a t i s l a v a

NUMBERS OF POSTERS

Section 2 - Plant Growth and Development

1. Dupľáková N.
2. Kákošová A.
3. Kutarňová Z.
4. Mýtinová Z.
5. Sedláková K.
6. Suchomelová P.

Section 3 - Plant Metabolism

7. Franková L.
8. Simerský R.

Section 4 - Photosynthesis

9. Šalgovičová I.

Section 5 - Stress Physiology

10. Czibula K.
11. Grúz J.
12. Hradecká V.
13. Jindřichová B.
14. Kováčik J.
15. Krulová J.
16. Morovič M.
17. Šimonová E.
18. Veselková Š.
19. Zelko I.
20. Živčák M.

Section 6 – Ecophysiology

21. Repková J.

CONTENT

Cena Doc. Milana Kutáčka pro mladé rostlinné biology

Česká společnost experimentální biologie rostlin v Praze a Fyziologická sekce Slovenské botanické společnosti v Bratislavě ve snaze podpořit rozvoj talentovaných mladých vědeckých pracovníků ve vědním oboru biologie rostlin se rozhodla (usnesením dne 23. února 2004) udělovat Cenu Doc. Milana Kutáčka mladým rostlinným biologům za nejlepší prezentaci svých vědeckých výsledků formou přednášky**.**

Statut

1. Cena Doc. Milana Kutáčka (dále jen Cena) se uděluje na počest významného českého rostlinného biochemika a fyziologa Doc. RNDr. PhMr. Milana Kutáčka, DrSc. (1923 –1989), který se výrazným způsobem zasloužil o rozvoj biochemie a fyziologie rostlin v Československu. Svojí úspěšnou vědeckou prací v oblasti rostlinných hormonů, zejména auxinů, se stal výraznou osobností evropského formátu.

2. Cena se uděluje za nejlepší prezentaci vědeckých výsledků formou přednášky na konferencích rostlinných fyziologů ”Dny rostlinné fyziologie” a **“**Dny mladých rostlinných fyziologů” řádným nebo mimořádným členům ČSEBR nebo SBS, jejichž věk v hodnoceném období nepřesáhl 35 roků (podmínkou je, aby oceněný autor byl prvním autorem příspěvku).

3. Vyhodnocení vykoná 4–5 členná komise (předseda poroty a porotci), v níž jsou zastoupeni členové obou pořádajících organizací, jmenovaná Organizačním výborem konference.

4. Cena se skládá z pamětního diplomu a finanční odměny. Součástí diplomu je list s CV Doc. Milana Kutáčka. Cenu předává představitel Výboru ČSEBR nebo SBS (podle toho, kde se konference koná).

5. Výše finanční odměny se stanoví na základě rozpočtových možnosti společností.

6. Cenu je možné udělit jedné osobě pouze jedenkrát.

7. Evidenci o udělení Ceny vedou vědečtí tajemníci obou společností (ČSEBR a SBS).

Doc. RNDr. PhMr. Milan Kutáček, DrSc. (1925-1989)

Mezi českými rostlinnými fyziology koluje jeden krátký příběh: Na mezinárodním symposiu kdesi v Evropě právě probíhá jednání v sekci. Pár minut po začátku se otevřou dveře a do přednáškového sálu vchází vyšší statný muž s bohatými zvlněnými prošedivělými vlasy. Široce se usmívá a pomalu si hledá místo v předních řadách. Současně kyne mnoha přítomným v sále a ti mu s úsměvem odpovídají. Mezi posluchači se jeden účastník naklání k sousedovi a udiveně se ptá: „Kdo je to?“ Druhý s lehkým úsměvem odpovídá: „To je přece Dr. Kutáček, Střední Evropa“.

Ověřit pravdivost této příhody již asi nebude možné, příliš však na tom nezáleží; ať je příhoda zcela autentická, či již nabyla ústním podáním pozměněné podoby, v každém případě velmi hezky ilustruje osobnost Dr. Kutáčka a jeho společenský šarm. Dr. Kutáček byl skutečně – a navzdory minulému režimu – velmi výraznou a mezinárodně známou a uznávanou osobností české/československé fyziologie a biochemie rostlin. Ač původním vzděláním biochemik-farmaceut, byl to právě on, kdo velkou měrou ovlivnil a obohatil fyziologii rostlin v této zemi. Vždy se podílel na zavádění moderních, zejména biochemických a později i molekulárně biologických, metod do základního výzkumu v rostlinné fyziologii a byl to také on, kdo vždy chápal zdejší vědu jako součást celosvětové kultury a vzdělanosti. Sám byl člověkem velmi vzdělaným, osobností až renesanční. Velmi rád a cíleně se obklopoval mladými lidmi, bez ohledu na jejich národnost či státní příslušnost. Postupem doby vybudoval nejen svou laboratoř na Ústavu experimentální botaniky v Praze, ale též se podílel na zavedení oboru biochemie rostlin na nynější Mendelově zemědělské a lesnické universitě v Brně, kde se v r. 1965 pro tento obor habilitoval. Bez nadsázky se dá říci, že „česká fytohormonální škola“ měla v Dr. Kutáčkovi jednu ze svých zakládajících a poté vůdčích osobností.

Dr. Kutáček byl laskavý, i když někdy trochu netrpělivý školitel, s obrovským zájmem o vědecké bádání v nejširším slova smyslu, hrdý na výsledky svých studentů a spolupracovníků, a otevřený jakékoli nové nebo netradiční myšlence. Jeho myšlenky a přístup k vědecké práci žijí dál – pevně zakódovány v myslích jeho studentů a pokračovatelů. On sám zůstává osobností nezapomenutelnou.

Eva Zažímalová
Praha, květen 2003

Cena Dr. Márie Luxovej pre mladých rastlinných biológov

Fyziologická sekcia Slovenskej botanickej spoločnosti v Bratislave a Česká spoločnosť experimentálnej biológie rastlín v Prahe v snahe podporiť rozvoj talentovaných mladých vedeckých pracovníkov vo vednom odbore biológia rastlín sa rozhodla (uznesením dňa 23. februára 2004.) udeľovať Cenu Dr. Márie Luxovej mladým rastlinným biológom za najlepšiu prezentáciu svojich vedeckých výsledkov formou plagátového posteru.

Štatút

1. Cena Dr. Márie Luxovej (ďalej len Cena) sa udeľuje na počesť významnej slovenskej rastlinnej anatómky RNDr. Márie Luxovej, DrSc. (1924 –2000), ktorá sa výrazným spôsobom zaslúžila o vznik a rozvoj anatómie rastlín na Slovensku. Svojou úspešnou vedeckou prácou v oblasti anatómie koreňa sa stala výraznou osobnosťou európskeho formátu.

2. Cena sa udeľuje za najlepšiu prezentáciu vedeckých výsledkov formou posteru na konferenciách rastlinných fyziológov "Dni rastlinnej fyziológie" a **"**Dňoch mladých rastlinných fyziológov" riadnym alebo mimoriadnym členom SBS alebo ČSEBR ktorých vek v hodnotenom období nepresiahol 35 rokov (podmienkou je aby ocenený autor bol prvým autorom príspevku).

3. Vyhodnotenie vykoná 4–5-členná komisia (predseda poroty a porotcovia) v ktorej sú zastúpení členovia oboch organizácií, vymenovaná Organizačným výborom konferencie.

4. Cena sa skladá z pamätného diplomu a finančnej odmeny. Súčasťou diplomu je list s CV Dr. Marie Luxové. Cenu odovzdáva predstaviteľ Hlavného výboru SBS alebo ČSEBR (podľa toho kde sa koná konferencia).

5. Výška finančnej odmeny sa stanoví na základe rozpočtových možnosti jednotlivých spoločností.

6. Cenu možno udeliť tej istej osobe iba raz.

7. Evidenciu o udelení Ceny vedú vedeckí tajomníci oboch spoločností (SBS a ČSEBR).

RNDr. Mária Luxová, DrSc. (1924 – 2000)

Pri mene Dr. Márie Luxovej mnohých iste napadne ďalšie meno – profesor Bohumil Němec. Veľký československý rastlinný cytológ a anatóm a jeho obľúbená žiačka. O profesorovi Němcovi kolujú v obci rastlinných fyziológov mnohé historky. Jedna z nich hovorí, že pán profesor každý deň obišiel svojich študentov a pýtal sa ich čo majú nové. Niekedy bola odpoveď ťažká. Dá sa však povedať, že Dr. Luxová žila celý svoj život tak, aby ani jeden deň nezostal nevyužitý a aby v duchu mala na otázku pána profesora vždy pripravenú odpoveď. Naprostá väčšina tých dní bola pritom vyplnená mikroskopovaním, štúdiom literatúry, alebo písaním. Pre generácie rastlinných fyziológov a vlastne všetkých študentov biológie sa stala pojmom „Luxová – Zemědělská botanika“, v mnohom u nás dodnes neprekonaná učebnica anatómie a morfológie rastlín.

S mikroskopovaním a mikrotechnikou začala Dr. Luxová už ako študentka, keď sa popri štúdiu zamestnala u docenta Dotha na Drevárskom výskumnom ústave. Stála pri zrode Botanického ústavu Slovenskej akadémie vied, kde prežila naprostú väčšinu svojho profesionálneho života. Veľmi dobrá bola aj spolupráca Dr. Luxovej s mnohými výskumnými a školskými pracoviskami, najmä s Katedrou fyziológie rastlín Prírodovedeckej fakulty Univerzity Komenského v Bratislave. V Bratislave tak vznikla cytologicko-anatomická škola, známa naozaj v celej Európe. A nielen v nej, veď i v Japonsku spomínajú dnešní vysokoškolskí učitelia a profesori ako sa učili na seminároch texty Dr. Luxovej pri štúdiu anatómie koreňa. Koreň, jeho štruktúra a funkcia zostali objektom hlavného záujmu Dr. Luxovej, dá sa povedať, že naozaj do posledných dní jej plodného života.

Alexander Lux
Bratislava máj 2004

ABSTRACT SECTION

IN VITRO CULTIVATION OF *THLASPI* SPP

K. Czibula[1] and I. Zelko[1]
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Phytoremediation is a technology that exploits the plant's ability to remove toxic compounds from the environment. Plants used in phytoremediation might have established cultivation method for microporpagation, high tolerance to a specific contaminant, ability of root uptake, translocation and compartmentation of contaminants into the above-ground organs. Hyperaccumulator species are intensively investigated for their unique uptake, translocation and tolerance to toxic metals. A large number of hyperaccumulating species belong to the Brassicaceae family, in particular to the *Thlaspi* genus. *Thlaspi caerulescens* and *Thlaspi arvense* having different characteristics of cadmium tolerance and accumulation were selected to study these abilities in conditions *in vitro*. A protocol for *Thlaspi caerulescens* and *Thlaspi arvense* culture, regeneration and multiplication has been developed.

Cultures *in vitro* were derived from aseptically germinated plants on MS medium without vitamins and growth regulators (Murashige-Skoog 1962). Stem segments, as explants, for callus and/or organ cultures were grown on Nagy and Maliga (1976) (K3) media supplemented with different combinations of growth hormones in 16 h photoperiod, 45-60 $\mu mol.m^{-2}.s^{-1}$ light intensity, and 23± 1 ºC. The best multiplication and development of shoots has been achieved on K3 medium. The most successful callus induction on media enriched with kinetin and 2,4-D has been achieved.

Supported by the COST 859 and VEGA 1/0100/03 UK/222/2004.

References

Murashige T, Skoog F, 1962. Physiol. Plantarum 15: 473-497.
Nagy JI, Maliga P, 1976. Z. Pflanzenphysiol. 78: 453-455.

WHY ARE SEEDLINGS OF *LARIX DECIDUA* ETIOLATED IN THE DARK?

V. Demko[1], D. Valková[2], G. Minárik[2], J. Turňa[2] and J. Hudák[1]
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In angiosperms protochlorophyllide reduction (and chlorophyll biosynthesis) runs in light-dependent manner. In contrast, most of the lower plants and gymnosperms are able to synthesize chlorophyll in the dark using light-independent or dark-active protochlorophyllide oxidoreductase (DPOR), that is encoded by three plastid genes – *chlL*, *chlN* and *chlB*. However, among gymnosperms there are some exceptions with no or restricted ability of dark chlorophyll synthesis (*Ginkgo biloba*, *Larix decidua*).

We tried to elucidate, which factors contribute to etiolation of *L. decidua* seedlings in the dark. At first we studied wheather *chlL*, *chlN* and *chlB* genes are present in larch cpDNA or not. Than we focused our attention on posttranscriptional processing of *chlB* transcripts. Using PCR we have detected and amplified homologs of *chlL*, *chlN*, *chlB* genes in *L. decidua* cpDNA and we cloned, subcloned and sequenced them. Obtained sequences showed high identity in homologous genes in comparison with lower plants, *Picea abies* and different *Pinus* species. We have not found any stop codons or frame shifts.

Asi t was previously described, two codons of *chlB* transcripts in *Pinus sylvestris* and *Picea abies* are RNA-edited in organ-specific manner. We isolated total RNA from *L. decidua* and *P. abies* cotyledons, prepared cDNA and amplified part of *chlB* gene bearing both potentially RNA-edited sites. After the analysis of these DNA fragments we have found that significantly lower amount of *chlB* transcripts in *L. decidua* (when compared with *P. abies*) is edited in CGG codon. Although *L. decidua* possesses all three genes encoding DPOR required for dark chlorophyll synthesis, proper function of ChlB subunits may be dcreased by inefficient RNA-editing. It will be interesting to determine expression level of *chlL*, *chlN*, *chlB* genes ander various experimental conditions using some agents modifying biochemical status of plastids and also to resolve the other factors and mechanisms involved in limitation of dark chlorophyll synthesis in *L. decidua* seedlings.

Supported by grant agency VEGA No.1/0003/03

ARABIDOPSIS GENE FAMILY PROFILE (ARABIDOPSIS GFP) – A NEW FAMILY-ORIENTED GENE EXPRESSION DATABASE

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The rapidly increasing volume of publicly available gene expression datasets for Arabidopsis now demands an environment suitable for easy orientation and that enables genome-targeted questions about expression patterns to be answered. We present a currated gene family-oriented gene expression database with a user-friendly graphic interface. Arabidopsis Gene Family Profiler (GFP) gives the user access to normalised Affymetrix ATH1 microarray data collected from NASC within the scope of the AffyWatch Service (*Craigon et al. 2004*). The database contains transcriptomic data for number of tissues at various developmental stages from wild type plants gathered from nearly 350 gene chips.

The Arabidopsis GFP database has been designed as an easy-to-use tool for users needing an easily accessible resource for expression data of either single genes, pre-defined gene families or custom user-defined gene sets, with the further possibility of keyword search. The environment enables users to access individual chip experiments and mean data for all appropriate microarrays. Arabidopsis Gene Family Profiler presents a user-friendly web interface using both graphic and text output. Data are stored at the MySQL server and individual queries are created in PHP script. The distinguishing features of Arabidopsis Gene Family Profiler database are 1) presentation of normalised datasets (Affymetrix MAS5 algorithm and calculation of model-based gene-expression values based on the Perfect Match-only model); 2) an intuitive interface; 3) an interactive "virtual plant" visualising the spatial and developmental expression profiles of both gene families and individual genes. Altogether Arabidopsis GFP gives users the possibility to start with simple global questions that can be further refined as highly targeted ones.

We gratefully acknowledge support from the GA ASCR (Grant B6038409).

References

Craigon DJ, James N, Okyere J, Higgins J, Jotham J, May S, 2004. Nucleic Acids Res. 32: D575-D577.

ENHANCEMENT OF RADIATION AND WATER USE EFFICIENCY FOR PHOTOSYNTHESIS AND DRY MATTER PRODUCTION OF SPRING BARLEY IN FLUCTUATING ENVIRONMENTAL CONDITIONS

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Production process of temperate cereals is often impaired by water stress, frequently coinciding with high light and temperature. Formation of a proper traits combination enables plant to enhance water use efficiency (WUE) and bring higher yields. Increased capacity for osmotic adjustment, smaller transpiration area and developmental escape seem to be more advantageous than sensible stomatal behaviour. Reductions in photosynthesis imply a decrease of radiation use efficiency (RUE). This can be alleviated by presence of the same adaptation mechanisms as WUE. Application of a organo-mineral fertilizer Avit – 35 did not improve the plant water- and radiation-economy. His protective effect (through polyamine concentration rise) came too late to compensate injury caused by drought. Finally, selection of genotypes is more recommended than application of Avit – 35 in the water and radiation use efficiency regulation during drought.

METABOLIC ASPECTS OF THE AUTUMNAL DEVELOPMENTAL PHASE OF COLCHICUM AUTUMNALE L.

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Colchicum autumnale L. is a clonal, monocotyledonous plant, which flowers without leaves (hysteranthous geophyte). Autumn crocus has three specific features: i) the unusual life cycle; ii) hysteranthous geophytic life style; and iii) the ability to produce therapeutically active alkaloids called colchicinoids. Although this plant has been used as approved medical plant for more than 3000 years, the basic knowledge at the morphological, structural and biochemical level is still missing. To understand the life strategy of autumn crocus we used all three mentioned methodical approaches.

We found that the annual life cycle of autumn crocus consists of two growth and developmental stages: the first, autumnal one (September-December) and the second, photosynthetically active one (March-May) leading to senescence and summer dormancy. The first developmental phase includes the shoot development, its flowering, root formation and development of all other organs of above-ground part (leaves, stem and capsules with seeds). These processes take place under the soil surface without photosynthesis. Arising of whole future new plant in the soil is the first basic feature of *C. autumnale*. In this report we describe mainly the structural and enzymological aspects behind the autumnal, photosynthetically inactive, developmental phase. We determined that the starch is the main storage component of ripe corm; proteins form 8%, free sugars 5% and lipids 3% of dry mass. The major part of starch is reutilized in the mother corm during the autumnal stage. Decline in starch content is paralleled by increasing in total amylolytic activity. α-Amylase, β-amylase and α-glucosidase are the most active enzymes involved in amylolytic process. The presence of pullulanase and starch phosphorylase was not observed. During autumnal phase the amount of total proteins in the new developing corm increases, but the corresponding decrease of proteinous nitrogen was not observed in the mother corm. The presence of five exo- and two endopeptidases indicated that continual proteolysis takes place in both corms. Very low activity of nitrate reductase was found in roots suggesting the minimal nitrogen assimilation during autumnal phase. Total amino acid analyses of the new corm showed that N-rich amino acids - Asn, Gln, Arg - were present at the highest level. These amino acids could be used as another source of reduced nitrogen. By native PAGE we observed the presence of specific protein complex in both mother and daughter corms. This storage complex consists of at least seven subunits. By structural analyses we identified the sclerenchymatic tissue within the protuberance. This tissue supports the function of protuberance as a kind of hollow diverticulum. On the boundary of the new corm and the shoot a meristematic layer was recognized at early autumnal phase. This layer is later on changed into the abscission zone, which initiates the dying back of the above-ground part regularly at the end of annual life-cycle. The results of biochemical analyses showed, that all developmental processes accompanying the autumnal phase are realised only at the expense of storage reserves of mother corm, mainly starch, storage proteins and N-rich amino acids. The rate their utilisation and reallocation determine the plant continuity and perenniality. Finally our findings underline the question how the environmental or endogenous factors can initiate and schedule the sequence of developmental processes of the hysteranthous geophytic life forms. *Supported by grant VEGA No. 1/1275/04*

CHANGES IN THE PROFILE OF PHENOLIC ACIDS DURING PATHOGEN ATTACK

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Phenolic acids (PHAs) are widespread plant secondary metabolites, virtually derived from benzoic and cinnamic acids. They are synthesized from carbohydrates via the shikimate pathway – the natural source of aromatic amino acids. Commonly, they occur as esters, glycosides and bound complexes. The considerable interest in the study of PHAs is associated predominantly with their health-promoting and disease-preventing properties but they also play a primary role in plant-pathogen interactions. Salicylic acid (SA) especially is closely related to systemic acquired resistance (SAR), hypersensitive response (HR), signal transduction and pathogenesis-related (PR) genes expression. However, the physiological function of other PHAs remains an open question.

The purpose of this work was to describe changes in the profile of phenolic acids under pathogen-attack conditions. The following interaction was investigated: *Lactuca* spp.-*Bremia lactucae* Regel. Infected and control leaves were collected from 8 weeks old plants and stored in –80 °C until extraction. The extraction/hydrolytic procedure resulted into fractions of free PHAs, ester-bounded PHAs and PHAs glycosides. All fractions were analysed by high-performance liquid chromatography coupled with on-line mass spectrometry (HPLC-MS) and the quantitation was performed using deuterium labelled internal standards of *p*-hydroxybenzoic and salicylic acid.

A relevant boost in PHAs content was observed suggesting the increase in phenylalanine ammonia-lyase (PAL) activity during pathogenesis. The observed changes in endogenous PHA levels will be discussed in comparison to biochemical and histological aspects of monitored plant-pathogen interactions.

Supported by grant GACR No.522/02/D011.

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PREPARATION OF POLYCLONAL ANTIBODIES AGAINST ABSCISIC ACID (ABA) AND DEVELOPMENT OF IMMUNOAFFINITY CHROMATOGRAPHY (IAC)

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Abscisic acid (ABA) is a key factor regulating transpiration, stress responses, germination of seeds and embryogenesis.

We have developed a polyclonal antibody (pAb) against (+)-*cis, trans*-abscisic acid conjugated to bovine serum albumin (BSA) through its carboxyl group (C1) according to Weiler (1980). This pAb had a low cross-reactivity with coumpound such as (+)-*cis, trans* – 9'OH ABA, (+)-*cis,trans*-7'OH ABA, farnesoll, *trans,trans*-farnesol, phaseic and dihydrophaseic acid, but relatively hight cross-reactivity with (+)-*cis,trans*-ABA methylester, (+)-*cis,trans*-ABA-alcohol, (+)-*cis,trans*-ABA-aldehyde, (+/-)-ABA-glucosylester and with optical enantiomer such as (+/-)-*cis,trans*-ABA methylester, (+)-*cis,trans*-ABA, (+/-)-*cis,trans*-ABA.

Antibodies (IgG) were subsequently isolated and purified on a column with protein A. Pure antibodies were used to prepare an immunoaffinity gel and immunoaffinity columns. The use of prepared immunocolumns for purification of the extract of normal and water-stressed *Nicotiana tabacum* L. leaves is demonstrated in these studies.

The LC-ESI-MS have been used for quantification of endogenous ABA levels in immunopurified extract.

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RESPONSE OF *ARABIDOPSIS* ROOTS TO ALUMINIUM STRESS

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Although the aluminium toxicity is an important growth-limiting factor in acid soils, the mechanism of its phytotoxicity is still not fully understood. Previous experiments showed that root apex represents primary and the most sensitive site of aluminium-induced injury in plants. The main symptom of aluminium effect is the decrease of root growth as a consequence of the inhibition of root cell elongation and root cell division, and the first physiological responses are observed shortly after aluminium application. In our experiments we focused on immediate responses of *Arabidopsis* root cells to aluminium, its uptake and accumulation.

Root growth was observed in plants cultivated for 7 days on medium with increasing aluminium concentrations (10-300 μM). For investigation of aluminium uptake and its accumulation we selected the concentration of 50 μM. We used fluorescence dyes morin for detection of aluminium and FM 4-64 (selective styryl dye) for labelling the plasma membrane and endocytosed membrane compartments. The roots were examined in living state and at real time by confocal microscope. Changes of membrane potentials were measured by standard electrophysiological microelectrode method.

Aluminium inhibited root growth in concentration-dependent manner. Concentrations up to 50 μM slightly inhibited root cell elongation. With higher concentrations growth inhibition was even stronger and radial expansion of root cells was promoted. Measurement of electrophysiological properties of cortical root cells revealed the difference between certain developmental zones in the root. While the application of 50 μM aluminium rapidly depolarised membrane potential in the cells of transition zone, the depolarisation of mature cells was not so massive. Fluorescence labelling with morin and FM4-64 showed, that aluminium was localised in the apoplast of elongated cells. In the cells of meristematic and transition zone, aluminium was internalised from the apoplast into the cytoplasm within 1,5 hours and concentrated into provacuolar compartments and vacuoles 4 hours after treatment.

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DEFENCE RESPONSE OF *BRASSICA NAPUS* AGAINST FUNGAL PATHOGEN *LEPTOSPHAERIA MACULANS*

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The subject of this research is a study of the defence response of *Brassica napus* against fungal pathogen *Leptosphaeria maculans* using microscopy and spectrophotometry methods.
Leptosphaeria maculans causes blackleg, the most serious disease of oilseed Brassicas, particularly canola (*Brassica napus* and *B. rapa*) worldwide. Host responses to infection of *B. napus* by *L. maculans* include hypersensitive responses such as necrosis of guard cells adjacent to arrested hyphae, phytoalexin, callose and lignin production, accumulation of pectin in the lumen of xylem vessels, production of reactive oxygen species, and induction of pathogenesis-related proteins (PR-proteins) including 1,3-□-glucanase and chitinase.
Callose was detected by spectrofluorometry and microscopy methods using aniline blue and calcofluor. Level of callose is increased during defence response *B. napus* against *L. maculans* and differs in compatible and incompatible interaction. Determination of hydrogen peroxide was done using xylenol orange (spectrophotometry method). Proteins extracted from extracellular spaces were analyzed using native PAGE and *in gel* chitinase activity detection. Both isolates of *L. maculans* under study induced accumulation of PR-proteins including a number of chitinase isozymes.

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LIVING TES ARE PRESENT IN *ZINNIA* XYLOGENIC CELL CULTURE IN TWO PERIODS

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Auxin and cytokinin have been considered essential for vascular tissue differentiation. This is supported by recent molecular and genetic analysis (Ye, 2002). Tissue and cell cultures have long been used to study the effect of hormones on xylem differentiation. The most remarkable *in vitro* system developed so far is the *in vitro* tracheary elements (TEs) model system of *Zinnia elegans*. Mechanically isolated *Zinnia* mesophyll cells differentiate into tracheary elements when cultured in medium supplemented with auxin and cytokinin (Fukuda and Komamine, 1980). Many of the major biochemical, molecular and cytological hallmarks of TEs formation typically observed *in planta* is highly conserved during *in vitro* TEs formation (Pesquet et al., 2003).

Our aim was to visualise the earliest possible formation of TEs in the *Zinnia* xylogenic cell culture, to determine the proportion of living and dead TEs during the experimet, and to determine the TEs types formation and the time.

Cells of *Zinnia* xylogenic culture prepared as described by Fukuda and Komamine (1980), were stained with Calcofluor White to detect simultaneously cellulose and lignin in secondary cell walls of developing TEs. For viability staining, cell cultures were stained with 0.01% Calcofluor White and 0.5% fluorescein diacetate (FDA), with 0.001% DAPI, or with 0.2% Evans Blue. Cells were observed using an inverted microscope (DMIRBE, Leica) with bright field optics or epifluorescence illumination. Image acquisition was performed using a CCD camera (Color Coolview).

Xylogenic cell culture of *Zinnia* was observed 10 days. Tracheary elements with typical patterns of secondary cell wall thickenings were visualized in the UV light with the assistance of special FDA and Calcofluor White staining already after 60 h of culture. High viability of TEs (almost 100 %) rapidly decreased by 60 h until 5th day of culture, and it subsequently slowly increased till the end of the experiment. It seems that TEs are formed in two periods. While all types of TEs are formed in the first period, the majority of newly formed TEs are metaxylem-like tracheary elements in the second period. We identified living nuclei in TEs with DAPI staining on the 8th-10th day of culture and also TEs releasing protoplasts.

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RESPIRATION, GROWTH AND SUGAR CONTENT IN THE MAIZE SEEDLINGS AFFECTED BY OSMOTIC STRESS

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Higher plant respiration can proceed via two pathways of mitochondrial electron transport, the cytochrome and the alternative pathway. Cytochrome *c* oxidase is sensitive to cyanide, in contrast to the alternative pathway sensitive to salicylhydroxamic acid (SHAM). The cytochrome pathway is multiple enzymatic complex, alternative pathway consists of only one protein, the quinol-oxidizing alternative oxidase (AOX).

In our study two maize cultivars (*Zea mays* L.) were used. Cultivar NOVA is more drought-tolerant and cv. ANKORA more drought-sensitive. The seeds were germinated in rolls of moistured filter paper at 24 °C in the dark for 3 days. The seedlings were then cultivated 24 and 48 h in Hoagland nutrient solution (pH 5.0) with or without osmotic stress induced by 0.4 M sorbitol (-2.06 MPa water potential). For recovery experiments, seedlings were grown for another 48 h in the same medium without osmoticum. 2 cm apical root segments were used for measurement of respiration rates. Respiration was measured polarographically on 5 mm root segments which were immersed in 3 ml Na-phosphate buffer (pH 6,8), at 25 ºC using oxygen, Clark-type electrode (YSI 5300, Yellow Springs Instrument, USA). Activities and capacities of both cytochrome (v_{cyt}, V_{cyt}) and alternative pathway (v_{alt}, V_{alt}) were determinated using specific inhibitors (SHAM, KCN) and uncoupler (CCCP). Length of primary seminal root and shoot (hypocotyl and coleoptile) was measured before and after application of the osmotic stress and determinations of dry weights were performed. Root leakage was measured using conductometer (OK102/1, Radelkis, Budapest). Content of soluble sugars was performed using 3 cm apical root segments and determined spectrophotometrically at 710 nm.

The water deficiency stopped elongation growth of both shoots and roots. Osmotic stress decreased the ability of maize seedlings to accumulate soluble sugars. More drought tolerant cultivar NOVA showed greater capacity of the sugar accumulation. Electrolyte leakage, a cell injury index, was increased after osmoticum influence. Total respiration (v_T) decreased after drought treatment. There were no significant differences in the v_T among the analysed cultivars. The decrease of v_T was caused by a decline in the activities and capacities of both cytochrome (v_{cyt}, V_{cyt}) and alternative pathway (v_{alt}, V_{alt}) of the respiration. The result of uncoupler use clearly indicated, that coupling was maintained after 48 h of stress influence. The recovery of the respiration rate was comparable to the nonstressed control rates. According to these observations no possible mitochondrial damages are expected. Osmotic stress didn't induce a stimulation of the alternative oxidase so we assume that the stimulation of SHAM-sensitive pathway is not related to drought stress resistance, rather the function of alternative pathway is to balance carbon metabolism and electron transport in a response to changing environment.

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THE INFLUENCE OF CADMIUM ON SOME ASPECTS OF *MATRICARIA CHAMOMILLA* L. METABOLISM

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Matricaria chamomilla L. was found to be able to accumulate a high amount of cadmium in the shoots and roots. Therefore we studied the influence of low (3 μM l^{-1}) and high (60 and 120 μM l^{-1}) cadmium concentrations on some aspects of chamomile metabolism – nitrogen and chlorophylls content, dry mass production and coumarins accumulation (herniarin, (*Z*)- and (*E*)-2-*β*-D-glucopyranosyloxy-4-methoxy cinnamic acids (= GMCA), umbelliferone) as well as the amounts of cadmium accumulated in the leaves and roots. The experiment was carried out in Hoagland solution and plants were exposed to the cadmium during ten days. The nitrogen content was evaluated according to Kjeldahl method, the content of total chlorophylls according to Wellburn, coumarins content by gradient HPLC and cadmium content in plant parts by FAAS.

The only visible effect of cadmium presence was the brownish colour of the roots in the solutions with high cadmium doses. Among plants cultivated at 60 μM Cd l^{-1} the decrease in the leaf rosettes dry mass by 8.55% and among plants cultivated at 120 μM Cd l^{-1} the decrease by 16.24% has been recorded. Dry mass of the roots was not significantly influenced. The content of water in the leaves after ten days of exposure decreased significantly as cadmium concentrations in the culture medium increased: 91.51% (control), 91.04% (3 μM treatment), 89.79% (60 μM treatment) and 89.21% (120 μM treatment). The nitrogen content in the leaf rosettes decreased by 8.22% (3 μM treatment), 10.36% (60 μM treatment) and by 12.06% (120 μM treatment) when compared to the control but this decrease was not significant within tested cadmium doses. Nitrogen content in the roots remained unchanged. The total chlorophylls content after ten days of exposure decreased by 12.63% (3 μM treatment), 15.58% (60 μM treatment) and by 16.56% (120 μM treatment) but the differences were not significant within tested doses of cadmium. Coumarins content in the leaves after ten days of exposure did not display stress accumulation as in the case of short-term abiotic or biotic stress influence: low content of umbelliferone and stagnation of herniarin has been recorded. The content of the sum of GMCA increased approximately double within all tested cadmium concentrations after ten days of exposure. The amount of cadmium accumulated in the roots was higher than that observed in the leaves. The cadmium content increased as follows (leaves/roots, n-multiple of control): 13/19 (3 μM treatment), 95/124 (60 μM treatment), 103/196 (120 μM treatment).

The differences between low and high cadmium concentrations were not significant in the majority of the parameters, the vitality of plants was not visible affected and plants accumulated high amounts of cadmium as its doses increased. Obtained results arguing that chamomile is tolerant to high cadmium concentrations under tested conditions of cultivation and it is able to accumulate high amounts of cadmium primarily in the roots.

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EFFECT OF SUNFLECKS ON PRODUCTION AND PHYSIOLOGICAL PROPERTIES OF C3 AND C4 ANNUAL CROPS

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Transient high or low irradiance of plant leaf lasting from seconds to minutes is called "sunfleck" or "shadefleck". It might be caused by cloud or ambient canopy movement.
"Sunflecks" reduce plant production due to (i) saturation-type dependence of photosynthesis rate on irradiation and, (ii) slow increase of photosynthesis after the sunfleck appear. Many works studied influence of sunflecks on understory seedlings in tropical forest, where most of light is coming in "sunflecks". Contrary to only a few investigations were performed on herbaceous field plants. We wondered sunflecks effect also for (C_3 vs. C_4) crop plants.

Material and Methods: Two C_3 and two C_4 annual crops, grasses and dicots (*Triticum vulgare* – wheat (C_3 grass), *Setaria macrostachia* (C_4 grass), *Celosia argentea* (C_3 dicot) and *Amaranthus caudatus* (C_4 dicot)) were cultivated in growth box in controlled conditions in two identical temperature (26/16 °C day/night) and air humidity (50/70 %) regimes. During the photoperiod (16 h, incandescent lamps), the mean irradiance was 400 $\mu mol.m^{-2}.s^{-1}$ for both "control" and "sunflecks" plants. However, control plants were exposed to invariable irradiance over the whole photoperiod while in "sunflecks" plants the irradiance varied between a "sunfleck" (1000 $\mu mol.m^{-2}.s^{-1}$) and "shadeflecks" (100 $\mu mol.m^{-2}.s^{-1}$) states periodically. Duration of the irradiance states (1-10 min) varied during the photoperiod however the time integral of photon flux was equal for both control and treated plants.

Results: Thirty three days after planting, total dry weight was higher in control plants by factors of 1.45 (*Triticum*), 2.32 (*Setaria*), 2.19 (*Amaranthus*) and 1.99 (*Celosia*) when compared with the dynamic light ("sunfleck") regime. The sunfleck regime reduced total biomass more in C_4 (2.25) than in C_3 (1.72) species. Ratio of root to shoot dry mass (R/S ratio) was (except *Celosia*) higher in controls than in sunfleck plants (due by higher transpiration rate of control plants?). Leaf area per dry mass unit (SLA) was slightly lower at control (thicker leaves in controls).

^{13}C isotope discrimination ($\Delta^{13}C$) revealed only small differences in ratio of leaf internal to atmospheric CO_2 concentration (C_i/C_a ratio) between control and sunfleck regime in C_3 plants. Contrary to in C_4 plants showed significant increase of bundle sheath leakiness in sunfleck than in control plants (control/sunfleck (%): 24/42 (*Setaria*), 27/48 (*Amaranthus*)). It indicates low efficiency of C_4 concentration mechanism under variable irradiance.

Conclusion
We verified high susceptibility of annual crop to sunflecks reduction of biomass in field reliable condition (not so deep shadeflecks as for tropical understore). More suppressed was both C_4 species and we hypothesised, that one possible reason is increasing leakage of CO_2 from bundle sheath in not steady state photosynthetic conditions.

THE INFLUENCE OF EXOGENOUS SACCHARIDE SUPPLY ON THE SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.)

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Conifer somatic embryogenesis represents a promising tool for mass propagation of elite genotypes. Somatic embryogenesis refers to the process in which somatic cells are induced to form bipolar structures called somatic embryos through the series of developmental steps similar to zygotic embryogenesis. The efficiency of the technique depends mainly on the quality of mature embryos, which is very important for further post-maturation phases. Besides structural features also metabolic status of somatic embryos, including accumulation of storage compounds might be important. There are some main regulatory factors, which play principal role during somatic embryogenesis: phytohormones, osmotic stress and carbohydrates. Carbohydrates serve as sources of energy and carbon, osmotica; they have protective roles and recently are often referred as important signal molecules. In order to elucidate the role of carbohydrate metabolism the detailed analysis on carbohydrate status was done in somatic embryos of Norway spruce grown on solidified media (Lipavská and Konrádová, 2004). The present study broadens the image with the help of the cultivation of maturing embryos on rafts floating on liquid media enabling easier manipulation and precisely defined supplementation with exogenous carbohydrates.

Embryogenic culture of *Picea abies*, (genotype AFO 541, AFOCEL France) was grown on Gupta and Durzan´s (1986) media supplemented with 20 μM ABA, either liquid or solidified with agar. When different types of carbohydrates were compared the media were modified to contain 3% (w/v) sucrose or 1,57% (w/v) glucose and 1,57%(w/v) fructose. After six weeks of maturation, three weeks desiccation phase followed and then germination of somatic embryos was examined.

The development of somatic embryos on the sucrose supplemented liquid media was followed and compared with their solid media supported counterparts. The total carbohydrate content in somatic embryos was higher on liquid media than on solid ones, but the carbohydrate dynamics in somatic embryos cultivated on liquid and solid media were similar. The following features were characteristic for liquid as well as solid media supported embryos: 1) the decrease of total saccharide content with proceeding maturation; 2) the fall of hexoses content accompanied by simultaneous increase of sucrose content which resulted in higher sucrose: hexoses ratio; 3) somatic embryos development deteriorated by replacement of sucrose with corresponding concentrations of glucose and fructose; 4) prevailing sucrose detected in matured embryos supported only with exogenous hexoses indicating sucrose resynthesis by developing embryos; 5) accumulation of raffinose family oligosaccharides (RFO) during desiccation; 6) RFO and sucrose content decrease concerted with gradual increase of hexoses content during embryo germination.

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INFLUENCE OF GROWTH REGULATORS ON PROTEIN CONTENT DURING EARLY PHASES OF *DROSERA ROTUNDIFOLIA* L. SOMATIC EMBRYOGENESIS

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Somatic embryogenesis is one way of the plant regeneration which involves changes on molecular, biochemical and morphological level. A large amount of genes is implicated in the process of the gene expression reprogramming.

Somatic embryogenesis we induced on the pre-trated of *Drosera rotundifolia* L. leaf explants on MS medium with 1.10^{-8} M 2,4-D during 6 hours. Explants were subsequently cultivated on two type of MS media (solid and liquid) containing growth regulators (1.10^{-8} M NAA and 1.10^{-8} M BAP) at different temperature T=25±2°C and 30±2°C in *in vitro* conditions.

Morphological changes were observed by SEM, TEM and light microscopy after 7, 14, 21 days. Proteins were isolated from samples and separated by 1D-A-PAGE and SDS-PAGE.

Histological analyses of transversal section of leaf showed that after 7-th days of cultivation appeared dedifferentiated mesophyll and epidermal cells giving bases to the globular structure of somatic embryo. After 14-th days we observed globular somatic embryos with well-developed fibrilar network of extracellular matrix containing glycin-rich proteins (GRPs), hydroxyproline-rich (HPRs) and arabinogalactan proteins (AGPs).

Protein analyses of leaves extracts containing somatic embryo-like structures showed quantitative and qualitative differences between low molecular proteins belonging to the auxin-binding proteins (Mr ~ 11; 22; 24 and 25 kDa). Quantitative content of proteins with low molecular masses decreased after 21 days of cultivation compared to proteins with high molecular masses. Amount of β-1,3 glucanases and chitinases decreased following the somatic embryo development. Glycoproteins and hydrolytic enzymes (glucanases, chitinases, lipoxygenases) play important role during formation of globular state of embryo-like structures. Gene expression of some of them shows to be strongly influenced by presence of exogenous auxin added to the culture medium.

High temperature (T=30±2°C) induced apoptosis after long term (t≥ 3 weeks) cultivation.

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THE INFLUENCE OF ERYTHROMYCIN AND KINETIN ON THE PROTEIN CONTENT IN MUSTARD COTYLEDONS

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Xenobiotics generally evoke many structural, metabolic and physiological changes in plant organism. Some antibiotics generally inhibit the proteosynthesis at chloroplasts showing the loss of photosynthetic pigments in leaves and consequently end in the faster senescence. Affecting the plastid ribosomes antibiotics influence peptide bonds formatting. Thus proteins essential to photosynthesis are not synthesized and the chlorophyll loss occurs. Using antibiotics there can be caused some changes on chloroplast membranes, too (Hudák *et al*. 1990). On the other side cytokinins make the senescence lower by stimulating the synthesis of photosynthetic pigments (Fletcher and McCullagh, 1971).The aim of our study was to evoke the senescence of mustard cotyledons (*Sinapis alba.* L) by exogenous application of erythromycin and observe the effect of kinetin on the protein content of cotyledons.

Mustard (*Sinapis alba* L) seedling were used in our experiments. They were cultivated in medium with various concentrations of erythromycin as well in combination with various concentrations of kinetin for 7-days. The half Knopp´s solution was used as a control medium. After 7-days of cultivation the quantitative content of proteins in mustard cotyledons was determined (Bradford, 1976). There were prepared cotyledons homogenates (Maniatis *et al*., 1989) and subjected to SDS-PAGE for protein analyzes (Laemmli, 1970).

Our results show that erythromycin caused early senescence of mustard cotyledons. Kinetin increased the amount of proteins comparing to erythromycine variant that could indicate that make the erythromycine influence lower.

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LIGNIFICATION AND SUBERINIZATION OF ENDODERMAL CELL WALL AS FACTORS AFFECTING CADMIUM CONTENT IN PLANT

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This study was conducted to examine the response of red campion (*Silene dioica* L.) to cadmium treatment. Red campion is a herbaceous perennial used in geology as bioindicator of high concentration of heavy metals, particularly copper, in soils. The aim of this paper was to investigate whether and how are differences in cadmium uptake to the roots and its following translocation related to lignification and suberinization of roots. These are two main processes involved in development of root apoplastic barriers (Casparian bands, suberin lamellae). The response to different cadmium concentrations in medium was studied in three various populations of red campion from Slovakia (seeds were collected from Špania dolina, Richňava – the areas contaminated by heavy metals, and Javorina – the non-contaminated area).

After cadmium treatment the plant samples were collected, processed and investigated. The samples were estimated by light and fluorescent microscopy, the anatomical structures were compared with image analyze software Lucia, v. 4.80 (2002) and the cadmium content was measured using FAAS.

The results show that the increase of lignification and suberinization of endodermal cell wall affects cadmium uptake and decreases its translocation in the plant. The processes of lignin and suberine deposition to the endodermal cell wall increases with increasing cadmium content in the medium. Differences in this process were found among studied populations.

The present study shows importance of selecting suitable plant genotypes with high lignification and suberinization potential; those affect cadmium uptake and its following translocation in the plant.

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TOBACCO WITH YEAST MITOTIC ACTIVATOR – A USEFUL MODEL TO STUDY THE ROLE OF CELL DIVISION IN PLANT MORPHOGENESIS AND DEVELOPMENT

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Flowering onset of autonomous plants is governed by internal signals. In day-neutral tobacco, the regulation of floral initiation has been proposed to be a function of the interplay among signals from leaves and roots. It is assumed that leaves provide a floral stimulus while a floral inhibitor is produced by root system. Besides phytohormones (mainly cytokinins) sugars, are often quoted among the signals involved in the floral transition. Sucrose especially is supposed to be a part of the multicomponent floral system. Moreover, experiments in tobacco have established that the flowering signals are graft transmissible.

In most eucaryots, regulation of CDK activity is, beside other mechanisms, provided by activatory dephosphorylation through CDC25 phosphatase. During last decade, the effort to find plant activatory G_2/M cdc25 phosphatase failed, nevertheless, only very recently a small isoform of cdc25 phosphatase catalytic subunit has been identified in *Arabidopsis* that indicates the existence of functional plant homologue to the yeast protein.

In glasshouse experiments with day-neutral *Nicotiana tabacum* L. cv. Samsun with fission yeast *cdc25* gene under 35S CaMV promotor (1) showed, besides other changed characteristics, an earlier onset and enhanced intensity of flowering albeit without any detail investigation. Later, Suchomelová et al. (2) reported a cytokinin-like effect of fission yeast *cdc25* transformation on *de novo* organ formation on tobacco stem segments of transformed plants manifested by earlier and more abundant shoot formation and restricted root induction. C*dc25* expression strongly influences cell shape and organisation in cell cultures in the same way as cytokinin application (3). The above-mentioned transformants were used for the study.

In transgenic lines achieved reproductive phase earlier producing lower number of leaves compared to wild type both under *in vivo* and *in vitro* conditions. The levels of *cdc25* transcripts vary between variants; nevertheless there exists positive correlation between the transcript levels and strength of phenotypic manifestations. Under *in vitro* conditions, on 3% sucrose (w/v) media solely the transformants flowered. Increased sucrose supply shortened the time to flowering, lowered the number of leaves till flowering in transformants and led to flowering in controls as well. The increased flowering capacity could at least partly come out from endogenous saccharide levels that are higher in the leaves of transformed plants compared to control. In addition, the floral acropetal gradient along the stem is disturbed in transformants. Under *in vivo* conditions, the grafting experiments proved that the acceleration of flowering onset in transformants is influenced exclusively by scion (shoot apex), while the stock (root system) has no significant effect on the developmental programme.

The results indicate a marked positive effect of sucrose on flowering induction in day-neutral tobacco plants and the remarkable impact of mitotic activator transformation on the flowering gradient, status of apical meristem and overall floral capacity.

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EFFECT OF HEAVY METALS ON SYNTHESIS OF STRESS PROTEINS IN YELLOW LUPINE ROOTS

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It is none that plants are often exposed to various stress factors which can cause different damages. The scale of these stress factors is very wide. Some of them especially act in protein denaturation for example heat shock, heavy metal treatment, oxidative stress and radiation. This kind of the damage can be partially repaired by special „refolding“ mechanism, which the heat shock proteins play a very important role in. The heat shock proteins present a very large protein family divided into subfamilies. Proteins in each subfamily have the specific relative molecular weight and characteristic function in stress-responsing system.

The research is focused on low molecular weight proteins (small heat shock proteins-shsp). These proteins were observed in yellow lupine (*Lupinus luteus)* roots in 1999 by Gwóźdź and Przymusiński as response to heavy metal treatment. The correlation between the expression of these proteins and the tolerance of plants to the heavy metals has not been directly proved.

In our research three different heavy metals (Cd, Pb, As) with three different concentrations were applied. Correlation between increasing metal ions concentration and protein synthesis activity has been studied using 16% (w/v) denaturing polyacrylamide gels. In case of the protein extracts from the seeds growing in presence of the heavy metals the enhanced protein signals were detected in 15-16 kDa area in comparison to control (water) variant. In further experiments it will be important to find out their similarity to the pathogenesis related proteins.

ANTIOXIDANT ENZYMATIC PROTECTION DURING AGEING IN TOBACCO LEAVES WITH ENHANCED EXPRESSION OF CYTOKININ OXIDASE/DEHYDROGENASE

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Plant ageing and its final phase senescence are connected with increase level of reactive oxygen species (ROS). These kinds of toxic oxygen impair cell components. Extent of their action is dependent on efficiency of antioxidant mechanisms, which include enzymatic and nonenzymatic protection. Important role in senescence play also phytohormones. We have been interested in cytokinins, which are known as inhibitors of senescence. One mechanism of their degradation in plants is a cleavage of the N^6- unsaturated isoprene side chain catalysed by cytokinin oxidase/dehydrogenase (CKX).

We studied influence of decreased level of cytokinins, caused by enhanced level of *Arabidipsis* CKX in tobacco leaves, on the activities of antioxidative enzymes (ascorbate peroxidase, glutathione reductase, superoxide dismutase and catalase). The seeds of transgenic plants (AtCKX2, *Nicotiana tabacum* L. cv. Samsun NN) were kind gift of Prof. T. Schmülling from Freie Universität Berlin, Germany. We measured slow induction kinetics of chlorophyll fluorescence, chlorophyll and soluble protein content for determination of leaf development stage and onset of senescence. We used PAM Chlorophyll Fluorometer for assignation of fluorescence parameters. Chlorophyll content was measured with HPLC and concentration of soluble proteins was determined spectrophotometrically as well as antioxidant enzymes, except catalase measured polarographically with oxygen electrode as oxygen production.

Control and AtCKX2 plants were of very different phenotypes. We observed that AtCKX2 were lower and had smaller and thicker leaves. Surprisingly, when the lowest leaves in controls became already yellow all leaves in transgenic AtCKX2 tobacco were still green. According to the markers of ageing the comparable leaves of AtCKX2 aged more slowly in comparison with controls. The activities of antioxidant enzymes in both kinds of plants remarkably decreased with age, however, in transgenic plants, the activities were higher as compared to controls. It seems that level of cytokinins is in some connection with antioxidant enzymes. AtCKX2 plants are better protected by antioxidant enzymes. Efficiency of nonenzymatic protection during age in AtCKX2 plants will be investigated in next studies.

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PLANTS FROM HEAVY METAL- POLLUTED HABITATS: HOW DO THEY COPE WITH INCREASED ZINC CONTENT?

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Zinc is an important trace element; nevertheless increased concentrations are toxic for majority of plants. Development of plant communities at ore heaps, mining vaste deposits or artificially contaminated localities is driven by metal tolerance level of respective plant species. These localities serve as natural laboratories for the research of plant´s adaptations to heavy metals.

The aim of this work was to study structural and physiological characteristics of plants from heavy metal- enriched habitats in Slovakia and Austria. We have focused on plants with different strategies of heavy metal handling: excluders (*Silene vulgaris*), tolerant plants (*Arabidopsis arenosa, Acetosella vulgaris*) and hyper-accummulators (*Arabidopsis halleri, Thlaspi caerulescens*). The latter group accummulated large amounts of Zn in leaves and roots, when grown on Zn-rich soil. Substantially lower zinc amounts were detected in adult leaves or roots of tolerant or excluder plants originating from the same locality.

In order to localize zinc at subcellular level, we used fluorescent labelling in living cells. When leaf cuttings of hyper-accumulating plants were exposed to increased $ZnSO_4$ concentration *in vitro*, Zn was taken mainly to the vacuoles of epidermis. In the mesophyll, Zn was detected only in a few cells. For confirmation of these results at tissue level, we combined fluorescence method with EDX (energy- dispersive X-rays analyse) scanning electron microscopy.

No damage of root or leaf tissues was caused by heavy metals. However, there was variability in anatomy of the leaves of *Silene vulgaris* collected from localities with different heavy metal contamination.

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ASCORBATE PEROXIDASE AND ASCORBATE OXIDASE ACTIVITIES IN BARLEY ROOTS EXPOSED TO CADMIUM

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Cadmium as a non-essential element is toxic to many plant species already at very low concentrations. General mechanism of its toxicity is proposed to be the interaction with –SH groups of biomolecules, but also the generation of active oxygen species strongly contributes to Cd toxicity manifestation. Binding of Cd to glutathione can lead to depletion of free glutathione, which can disturb active oxygen species scavenging, which is mediated by the regeneration of glutathione-ascorbate cycle. We focused our attention to the analyses of ascorbate-related enzymes in response to Cd exposure; ascorbate peroxidase as a key H_2O_2 scavenging enzyme in plants, ascorbate free radical reductase as an enzyme contributing to ascorbate regeneration and ascorbate oxidase (AAO), enzyme involved in plant growth including plasma membrane electron transport, cell elongation and cell wall metabolism.

We analyzed the ascorbate-related enzymes activities as well as the change in the AAO isozyme pattern in several fractions (extracellular, soluble, cell wall and membrane bound) of barley root tips grown on filter paper and exposed to 1 mM Cd for 48 and 72 h. The Cd-induced root growth inhibition correlated with the Cd-induced loss of plasma membrane integrity. Ascorbate peroxidase activity was decreased in the extracellular, soluble and cell wall fractions, while it was strongly activated in the membrane-bound fraction in the presence of Cd. Ascorbate free radical reductase activity was not affected by Cd. Ascorbate oxidase activity was strongly inhibited in all analyzed fractions, however the analysis of AAO isozyme pattern revealed that besides the reduced activity of two anionic and two cationic isozyme one cationic AAO isozyme was activated during Cd treatment.

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EFFECT OF CADMIUM AND MANGANESE ON NITRATE UPTAKE AND REDUCTION IN ZEA MAYS SEEDLINGS

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The aim of the work is to evaluate the toxic effect of cadmium (Cd) alone, or in interaction with manganese (Mn) on the key steps of nitrogen metabolism in young maize seedling under controlled conditions. Cd at 10 and 100 μM showed significant decrease of growth of the root cells. Uptake of nitrate (NO_3^-) and activity of nitrate reductase (NR) correlated with the results obtained by measurements of growth parameters. The inhibition of NR was much stronger then the nitrate content in root tissue, therefore is probably caused by Cd itself, and can not by explained by lower availability of substrate.

Manganese supplied as $MnSO_4$ clearly alleviated the toxic effect of cadmium on the root growth of maize seedlings. The magnitude of alleviation was dose dependent and total abolition of 10 μM Cd toxicity on root growth was observed at Mn / Cd ratio of 20:1. The 12 h pre-treatment with 10 μM Cd was generally toxic for nitrate uptake and reduction (both determined in Cd-free media). The beneficial effect of 100 μM Mn on this toxicity was confirmed for the low-affinity nitrate uptake system, on the other hand, Mn alone seems to be slightly toxic for high affinity nitrate uptake system and on the nitrate reductase activity.

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COST / BENEFIT MODEL AND CARNIVOROUS SYNDROME IN ASIAN PITCHER PLANTS *NEPENTHES ALATA* AND *NEPENTHES MIRABILIS*

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Carnivorous plants are restricted to environments with abundant supply of water and light but poor in nutrients. Costs of carnivory include investments in non-photosynthetic structures as glands, hairs, glue and digestive enzymes. From photosynthetic point of view, cost represents extra energy requirement for respiration of carnivorous organs. On the other hand, benefits of carnivory include increased rate of photosynthesis, growth and reproduction through increased supply of nitrogen from captured prey. If factors like light or water are in a short supply, energy devoted to carnivory exceeds the benefits obtained from prey and carnivory does not pay. Many species of carnivorous plants give up carnivory temporarily in shady or dry conditions. Carnivorous plants are restricted to early successional stages and are less competitive against grasses and shrubs. As succession proceeds, the plants become increasingly shaded and the limitation to carbohydrate from photosynthesis increases. This results in disappearance of carnivorous plants from their habitats. App. from 15 sites of Drosera rotundifolia in Záhorská nížina 30 years ago remains only one.

We studied photosynthesis and respiration in carnivorous plants Nepenthes alata (Philippines) and Nepenthes mirabilis (S.E. Asia and Australia). Nepenthes leaf is composed of assimilation leaf and trapping pitcher. This allowed us to make a direct comparison between assimilation and trapping organs and estimate the cost and syndrome of carnivorous plants. This was unable in former studies of other carnivorous plants such as Drosera or Pinguicula in which the entire shoot is photosynthetic and captured organs. We measured the rate of photosynthesis (P_N) and respiration (R_D) (gasometrically), C, N and H concentration (CHN-method) assimilation pigment concentration (spectrophotometrically) as well as semithin and ultrathin section for light and transmission electron microscopy.

Photosynthesis of assimilation leaves ranged 24.4 – 42.3 nmol CO_2 g^{-1} d.w. s^{-1} and pitchers ranged -2.4 – (-) 0.1 nmol CO_2 g^{-1} d.w. s^{-1} (CO_2 is emitted through intensive respiration and low P_N). We found a set of characteristics that are involved in low P_N in pitchers: low assimilation pigment concentration, low nitrogen concentration, compact mesophyll with small proportion of intercellular spaces and low stomata density result in lower mesophyll and stomatal conductance for CO_2, absent of palisade layer and less chloroplasts per cell. Chloroplasts with numerous grana and plastoglobuli are presented in both tissues. But some of them may provide selective advantage for carnivory. For a plant to grow, it must photosynthesise more than it respires: if a plant respires more than it photosynthesises then it will eventually burn up all its available biomass, run out of energy, and die. Rate of photosynthesis at different irradiance revealed, that at low light intensity (60 μmol m^{-2} s^{-1} PAR), the cost of carnivory (or $R_{Dpitcher}$ + negative $P_{Npitcher}$) exceed the P_{Nleaf} and carnivory does not pay. Total cost of carnivory at 100 μmol m^{-2} s^{-1} PAR is 8 % of total carbon budged during 12 h light/ 12 h dark period and increased with day night/ cycle 10 h /14 h up to 20 % of total carbon budged. This study underlines the main features involved in a syndrome of terrestrial carnivorous plants and explains the restriction of carnivorous plants to sunny habitats.

IDENTIFICATION OF TRANSCRIPTION FACTORS AFFECTING EARLY POLLEN DEVELOPMENT OF *ARABIDOPSIS THALIANA*

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The haploid male gametophyte generation of flowering plants consists of two- or three-celled pollen grains and pollen tubes as an evolutionary result of structural and functional reductions but performs a vital role in the plant life cycle. Hence pollen ontogeny provides an attractive model for the study of fundamental developmental processes like cell growth and division, cellular differentiation and intercellular communication. Despite a long-term research on the field of plant sexual reproduction, the developmental changes and regulatory mechanisms during pollen grain formation have not been properly described yet.

Our research aims to characterize the role of transcription factors (TF) in male gametophyte development of *Arabidopsis thaliana* with special focus on early developmental stages such as uninucleate microspores and bicellular pollen grains. Exploiting microarray technologies (*Honys and Twell, 2003, 2004*) followed by careful bioinformatic analyses, we selected about 30 genes encoding putative TFs expressed specifically during the developmental stage considered. To prove the importance of selected TFs in development, we analysed respective Garlic and SAIL T-DNA insertion mutant lines. After PCR verification of all insertions, we performed the phenotype screening for aborted or structurally abnormal pollen grains by both light and UV microscopy. The second experimental approach comprises the search for segregation ratio distortion demonstrating the functional significance of examined gene without visible phenotype. Selected positive mutant lines with relevant impact on developmental changes will be genetically characterised to reveal the transmissibility as well as the recessive or dominant character of the mutation. Moreover, the influence of TF genes on downstream regulation will be examined by comparative transcriptome analysis of both TF mutant lines and wild type plants in order to determine their role in gametophytic gene regulatory networks.

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PHOTOSYNTHETIC APPARATUS OF SPRING BARLEY AND ITS DYNAMICS IN FLUCTUATING ENVIRONMENTAL CONDITIONS

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Global climatic change is one of the most actual problems of the recent period. Plant ecosystems are considerably influenced by environmental factors, which directly or indirectly limiting their production potential by decreasing of photosynthesis. Under natural conditions drought is often combined with other constraint such as strong light and heat. The key role in the responses of the plants to the stress conditions plays photosynthetic apparatus. Since under natural conditions is important consider about simultaneous operation of several factors which can change stress response of plants. Substantial resistance of photosystem II to water deficit can produce interesting interactions with other environmental factors with possible result in increasing tolerance to stress.

In presented work with spring barley we assessed an effect of stress conditions on the primary photochemistry of photosystem II (PSII). Barley plants (*Hordeum vulgare*, L.) were grown in plastic pots with soil in the natural environment. Water stress was applied by withholding water and then water status in leaves was measured as relative water content (RWC). Effect of elevated temperature and light on photosystem II was monitored by chlorophyll fluorescence. The polyphasic rise of fluorescence transient (OJIP) was measured by portable fluorimeter Handy PEA (Hansatech Instruments, Norfolk, England).The transients were induced by red light of 3500 $\mu mol.m^{-2}.s^{-1}$. All samples were dark-adapted for 30 min prior to fluorescence measurements. Fluctuation of microclimatic factors was determined applying by Datalogger 1400 (LiCor, Nebraska, USA).

In our experiment it has been demonstrated that when a dark-adapted leaf is illuminated suddenly with high intensity actinic light, the fluorescence transient shows a polyphasic rise, including phases O, J, I and P. Under various stress conditions such as heat stress an early step can be seen around 300 μs which has been labelled K. The appearance of the K step can be use as a specific indicator of injury to the oxygen evolving complex.

We observed differences in PSII sensitivity to environmental factors. While water deficit has no effect on the primary photochemical reactions, interaction among water deficit, high temperature and strong light demonstrated different responses of PSII to this treatment. When the strong light occurred with elevated temperature, water-stressed leaves exhibited similar or no damage of PSII as compared to non-stressed leaves. Thus, mild water deficit could contribute to the maintenance of the PSII stability under environmental stress conditions.

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NEW ANTIBODIES AGAINST AMINO ACID IAA CONJUGATES RETAIN VERY HIGH SELECTIVITY

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Polyclonal antibodies specific for indole-3-acetic acid (IAA) and related compounds were raised in rabbits immunised by IAA-alanine (IAAla) or IAA-aspartate (IAAsp) conjugated via amino acid carboxyl group to bovine serum albumin. All prepared antibodies were characterised by enzyme linked immunosorbent assay (ELISA) based on alkaline phosphatase labelled IAAla and IAAsp, respectively. The assay showed very high sensitivity in comparison to classical IAA ELISA and remarkable selectivity. The IAAla antibodies were of the most specific antibodies prepared, exhibiting slight cross-reactivity (7.7 %) with free, nonmethylated IAAla conjugates only. The IAAsp antibodies were less specific, cross-reacting strongly with many of the IAA amino acid conjugates. Both of these antibodies are, however, much more specific in comparison to classical C1-IAA antibodies. The prepared anti-IAAla antibodies could be, because of their high selectivity, useful for immunohistochemical localization of IAAla in different plant tissues. On the other band, the C1-IAA and IAAsp antibodies are currently tested in immunoaffinity chromatography for single step purification of IAA metabolites from plant extracts.

PREPARATION OF POLYVALENT ANTIBODIES AGAINST STEROID COMPOUNDS

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The occurrence of steroid compounds in plants is recently quite well documented. There are many classes of phytosteroids which have been isolated from different plant sources such as brassinosteroids, bufadienolides, cardenolides, cucurbitacins, ecdysteroids, steroidal saponins, steroidal alkaloids, withanolides and also vertebrate-type steroids (3). Indirect evidence of vertebrate-type steroids occurrence was given by studies describing metabolization of applied steroids by plants (1, 2). The wide array of steroid compounds was gradually also isolated from different plant species (3, 4, 5). Although it is known, that many of these structures occur in plants naturally, their role is still uncertain (6, 7).

We have developed polyclonal generic antibodies against steroid structures using dehydroepiandrosterone–17-carboxymethyloxime and 4-androstene-3-one-17-carboxymethyloxime conjugated with BSA. Both antigens led to preparation of high affinity antibodies giving very high sensitivity in ELISA. The measurement of cross-reactivity with large portfolio of steroid structures confirmed expected generic character of these antibodies. The antibodies will be used for development of an immunoaffinity chromatography combined with HPLC-MS.

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PHOTOSYNTHETIC CHANGES OF *BRASSICA JUNCEA* INDUCED BY CADMIUM

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Cadmium is a non-essential element in plants. It is recognized as one of the most potentially hazardous of all metal pollutants since it is extremelly toxic to humans and animals (Cieslinski et al., 1996). It is easily taken up and accumulated by the plants. One of the most important reasons for Cd toxicity is an interaction between Cd and Fe. Cd-induced Fe deficiency leads to serious disturbances of the photosynthetic apparatus like inhibition of chlorophyll synthesis, disorganisation of the chloroplast structure and inhibition of the photochemical reactions (Siedlecka and Krupa, 1999). The aim of our study was to determine of Cd effect on assimilation pigments, photochemical activity of isolated chloroplasts and CO_2 exchange (photosynthesis).

Brassica juncea L. cv. Vitasso was used for experiments. The seeds were grown in thermostat for 4 days than the seedlings were transferred to the Hoagland nutrient solution and cultivated hydroponically in growth chamber under controlled conditions. In the stage of two pairs true leaves the seedlings were transferred to the nutrient solution with content Cd of 6 – 120 μM $Cd(NO_3)_2$ x $4H_2O$ concentrations or to solution without Cd (control). The response of seedlings to the Cd effect was evaluated 5 days after metal application. Chlorophylls were extracted with 80 % acetone, and their content was determined spectrophotometrically and calculated according to Lichtenthaler (1987). The photochemical activity of isolated chloroplasts was determined by method of Henselová et al. (2004) and CO_2 exchange gasometrically according to Masarovičová and Kráľová (2005).

The chlorophyll content decreased with the increasing Cd concentration. The leaves of treated plants possessed significantly lower content of total chlorophylls at 60 and 120 □M concentrations in comparison with the control. The presence of cadmium in nutient solutions decreased the photochemical activity of chloroplasts in *in vivo* system with effective dose value ED_{50} 54 □M. However, photosynthetic rate was not influenced in *B. juncea* at 24 □M concentration.

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PHOTOSYNTHETIC APPARATUS IN THE SEEDLINGS OF SCOTS PINE AND MOUNTAIN PINE

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Photosynthetic apparatus is developing and changing during the whole plant ontogeny (HUDÁK 1997). Chloroplast differentiation starts very soon after germination to equip the seedling with the functional system for light energy utilization. This differentiation relies on synthesis of pigments and proteins, formation of chloroplast membrane system and pigment-protein complexes. The most important process is chlorophyll synthesis that is driven by light and regulates all other steps in chloroplast development. Chlorophyll synthesis in some conifers is not entirely dependent on light (ARMSTRONG, 1998). We have studied the development of photosynthetic apparatus in cotyledons of two pine species during germination and following 14-days growth in darkness or in the day/night regime.

The seedlings of *Pinus sylvestris* L. and *Pinus mugo* Turra were cultivated in the moist sand with following two conditions of illumination: in the darkness and in the day/night regime. Manipulations with dark-grown seedlings were performed under a dim green safelight. The cotyledons of 14-days old seedlings were used in experiments. Photosynthetic apparatus has been observed by light microscopy in cotyledons fixed according to KARNOVSKY, (1965), embeded in DURCUPAN ACM and stained with toluidine blue and basic fuchsin (LUX, 1981). The content of chlorophylls and carotenoids has been determined and analysed using spectrophotometry.

Observations have revealed that Scotch pine and Mountain pine produce chlorophyll even in the absence of light, though the production is less intensive. But this quantity is sufficient for beginning of photosynthetic apparatus development.

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DYNAMICS OF XANTHOPHYLL CYCLE PIGMENTS IN HIGH LIGHT-TREATED LICHENS

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Conversion of violaxanthin (V) to zeaxanthin (Z) via an intermediate anteraxanthin (A) is supposed to provide an effective protection against negative effect of high light in photosynthetising organisms (Adams et al, 1993). In our previous studies (Barták et al., 2004, Vráblíková et al., 2005) we investigated the effect of short term exposure of lichen thalli to high light. Here we present the results of our most recent experiments focused on the effect of high light duration and light/dark recovery. During exposure to high light and recovery we evaluated de-epoxidation state of xanthophyll cycle pigments (DEPS) in thalli of *Lasallia pustulata*.

Two different high light treatments were used to induce high light stress: „short term“ and „long term“. Thalli of „short term“ treatment were exposed to the light of 2000 μmol $m^{-2}s^{-1}$ for 30 min. Changes in content of xanthophyll cycle pigments were measured immediatelly after high light treatment and several times during recovery in dark. Thalli of „long term“ treatment were exposed to 700 μmol $m^{-2}s^{-1}$ for six days. Each 24 hours several thalli were collected and used for pigment analysis. Analyses were done according to Pfeifhofer (2002). DEPS was calculated as (Z+A)/(Z+A+V).

Different response of DEPS was observed for „short term“ and „long term“ treatment. In „short term“ treatment an increse in DEPS immediately after the exposure to high light was found. During the first 30 min DEPS decreased and reached original value after 10 hours of recovery. „Long term“ treatment, contrastingly, showed a rapid increase during the first 30 min of exposure followed by a much less pronounced increase during the following five days. Our results indicate that photoprotective mechanisms, Z formation in particular, are activated specifically according to the modus of high light exposure. In this respect high light dose, high light duration and type of recovery play the most important role.

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THE RESPONSE OF TWO MAIZE CULTIVARS TO OSMOTIC STRESS

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One of the major environmental factors limiting the worldwide productivity and distribution of cereal crops is osmotic stress resulting from drought. Drought stress triggers various interacting events including the decrease of relative water content, increase of reactive oxygen species production and osmolytes accumulation. Reactive oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical, can damage proteins, lipids and DNA. Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of plant cell and resulted in essential solutes leak out of organelles and cells. Osmotic adjustment due to the proline accumulation can protect plant cells against water loss. There is also evidence that proline can improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions.

The effect of drought on oxidative injury and water relations in 13-day-old maize (*Zea mays* L. cv. Ankora, drought-sensitive, cv. Nova, less-sensitive) plants was investigated. The plants were cultivated in Hoagland nutrient solution in the growth chamber (Conviron S10/S10H) at 24/18°C (day/night), 70 % air relative humidity and light intensity of 200 μmol . m^{-2}. s^{-1} with a 12 h photoperiod till the stage of the second fully developed leaf. Subsequently, the 24 h osmotic stress induced by 0.3 M sorbitol (-1.4 MPa) was applied

The 24 h osmotic stress resulted in damage of cell membranes. The level of MDA signifficantly rised in all studied organs of cv. Ankora. In the case of drought-tolerant Nova, the increase of MDA level was observed only in mesocotyl. Similarly, proline content in all studied organs of both cultivars increased significantly. The electrolyte leakage was the highest in the roots of both cultivars but in roots of cv. Ankora was apparently higher than in the cv. Nova. Relative water content in leaves of both cultivars decreased after drought treatment. Sensitive cv. Ankora lost much more water during both control and stress conditions than the cv. Nova. Osmotic stress induced by 0.3 M sorbitol had deep influence predominantly on the roots of both studied cultivars. It is apparent that stress impact on the drought-sensitive cultivar Ankora was deeper than on the drought-tolerant cultivar Nova.

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THE USE OF CHLOROPHYLL FLUORESCENCE PARAMETERS FOR INDICATION OF PHYTOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBON

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Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental organic pollutants and act as potential carcinogens, mutagens and teratogens for all living organisms including humans (Lee *et al.* 2000). The abilities of the plant to uptake, translocate, metabolise and accumulate PAHs are determinant factors for phytotoxicity of these compounds, which affect several biochemical and physiological processes. Inhibition of photosynthesis and a modification of photosynthetic activity is often used as a "bioindicator" of contamination effects (Huang *et al.* 1997). The affection of these processes can be evaluated from the activity of photosystem II by means of the chlorophyll fluorescence parameters. The aim of this work was to study the influence of short-period exposure of fluoranthene on primary processes of photosynthesis of pea leaves and isolated chloroplasts.

Pea plants (*Pisum sativum* L., cv. Lantra) were used as a plant material. Fluoranthene (FLT) (*Supelco*, USA) was applied in the concentration of 0.1, 1, 5 and 10 $mg.l^{-1}$ into the nutrient solution or on the surface of separate leaves. Chlorophyll fluorescence parameters (F_0, F_V/F_M, Φ_{II}; for the definition see Roháček *et* Barták 1999) were determined from a slow (Kautsky) induction kinetics of chlorophyll fluorescence, recorded by PAM-2000 portable fluorometer (*Walz*, Germany) before FLT exposure and after 12, 24 and 48 hours of exposure. The response of primary photosynthetic processes of pea plants to the presence of fluoranthene was evaluated on the level of both leaves and isolated chloroplasts. Results were then processed with software STATISTICA 6 (StatSoft, Inc.®).

The short-time (12, 24 and 48 hours) exposure of pea plants to 0.1 and 1 $mg.l^{-1}$ FLT in the nutrient solution did not affect the primary processes of photosynthesis of leaves and of isolated chloroplasts. The short-time (12, 24 and 48 hours) exposure of separate leaves to 0.1 and 1 $mg.l^{-1}$ FLT induced stimulative and also inhibitive changes in values of chlorophyll fluorescence parameters (F_V/F_M decrease and F_0 increase). The changes of chlorophyll fluorescence parameters of isolated chloroplasts were not always in correlation with the changes recorded on the leaves. The short-time (48 hours) exposure of separate leaves to 5 and 10 $mg.l^{-1}$ FLT caused significant increase of F_0 and decrease of F_V/F_M and Φ_{II}.

Polycyclic aromatic hydrocarbon fluoranthene applied on separated leaves affected primary photosynthetic processes more rapidly and notably compared to fluoranthene applied in the nutrient solution. Particularly high treatment caused significant changes of chlorophyll fluorescence parameters already after short-time exposure.

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ACTIVITY AND CHARACTERISATION OF PHOSPHATIDYL-CHOLINE - SPECIFIC PHOSPHOLIPSE C IN PLANT TISSUE

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Phospholipases (A_2, C and D) are key enzymes of broad range of signal transduction pathways in animals as well as in plants. They operate as parts of the phospholipide signaling system that transduces signals from the extracellular space to the cell [1]. Phosphatidylcholine-specific phospholipase C (PC-PLC) is an enzyme,which catalyses hydrolysis of lipid phosphatidycholine into phosphocholine (PCho) and diacylglycerol (DAG), both potential second messengers. The enzyme was described up to now biochemically only in bacteria and animal cells. On the gene level all available sequences of PC-PLC was yet known only from two groups of prokaryotic PC-PLCs. We have detected (independently of [2]) *in silico* PC-PLC homologs in *Arabidopsis thaliana* genome (6 putative PC-PLC genes), comprising a novel gene family in plants [3].

The subject of the presented work was detection of PC-PLC activity in plant material and its biochemical characterization. In our investigation we have used various plant materials as tobacco cell suspension culture, *A. thaliana* leaves, *Brassica napu*s stems and corn roots. PC-PLC activity was determined using radiolabelled PC as substrate. Products of enzymatic reaction were separated by extraction and than checked by HPLC.

The highest specific activity of this enzyme was found in PM tobacco cell culture BY2 and corn roots. We studied biochemical characteristics of the enzyme in PM of BY2 cell culture. The enzyme was activated by milimolar Ca^{2+} concentrations and 0,15% (w/v) triton and its pH optimum was around 7. We also tested inhibitors of PLD, PLA and phosphatases to eliminate possible formation of PCho by another metabolic pathway. There were no significant variations. Now we have focused on specification genes of enzymes in this tissues by RT-PCR and on effect of some abiotic stresses.

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INFLUENCE OF VERNALIZATION ON DYNAMICS OF COLD REGULATED PROTEINS IN WHEAT DURING COLD ACCLIMATION

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Vernalization requirement (VR) is a critical determinant between spring and winter cultivars of wheat species, and also one of the major genetic factors for controlling heading time and ability to induce freezing tolerance (FT). In common wheat, *Triticum aestivum* L., three major homoeologous genes concerning vernalization requirement have been identified, i.e. *Vrn-A1*, *-B1*, and *-D1* on chromosomes 5A, 5B and 5D, respectively. The degree of requirement to complete heading depends on the *Vrn* genotype.

Plants with ability to develop FT are able to acclimate to cold during exposure to low, but above freezing temperatures. The expression of several genes during cold acclimation was found to be positively correlated with the capacity of each genotype to develop FT. Among these, the *wcs120* gene family in wheat is considerable for our research as a marker of capacity to develop FT. This gene family encodes a group of highly abundant proteins ranging in size from 12 – 200 kDa. This protein family is coordinately regulated by low temperature (LT) and accumulates to high levels in freezing-tolerant plants of the *Poacea*. The WCS120 proteins are rich in glycine and threonine, highly hydrophilic, soluble upon boiling and have pI above 6.5. WCS120 protein family shares homology with the D11 dehydrin family.

Original substitution lines with substituted 5D chromosomes were produced after crossing the aneuploid wheat between two winter cultivars Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ). These reciprocal substitution lines and the parental cultivars were exposed to cold up to 16 weeks in order to study changes of cold regulated proteins (mainly WCS120 proteins) in relation to vrn and Fr loci situated on the 5D chromosomes. Both the cultivars and substitutions lines did not differ in the level of FT during the first six weeks of cold acclimation but then their FT gradually decreased in the order: BEZ(MIR5D), BEZ, MIR and MIR(BEZ5D). A similar order was observed in the saturation of VR when BEZ(MIR5D) and BEZ showed much shorter VR (about 6 weeks) than MIR and MIR(BEZ5D) (9 weeks). WCS120 proteins increased during cold-acclimation in both lines. However the gradual decrease of FT after saturation of VR was accompanied by decrease of WCS120 proteins. We detected a different pattern of heat stable proteins in non-acclimated and cold-acclimated wheat by 2D SDS-PAGE and mass-spectrometry. After saturation of VR the winter wheat showed similar accumulation of WCS120 proteins as it was in cold-acclimated spring wheat ‘Leguan’. The results support the hypothesis that genes for VR figure as a master switch regulating the LT induced FT and that substitution of 5D chromosomes influences FT as well as level of WCS120 proteins.

We thank Fathey Sarhan for the polyclonal WCS120 antibody, Gerhard Saalbach for mass spectrometry and members of the Ahmed Jahoor laboratory (Risoe, Denmark) for helping us with genomic research. We thank Kateřina Panková for supplying the substitution lines. This work was supported by grants MZE 0002700602 and GA ČR č. 206/03/H137.

EFFECT OF CD STRESS ON *IN VITRO* CULTIVATED *THLASPI* SPP. SEEDLINGS

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In this study two species of *Thlaspi* with contrasting characteristics of cadmium tolerance and accumulation were compared. *Thlaspi caerulescens* is known as a tolerant plant which can hyperaccumulate cadmium, zinc and nickel. *Thlaspi arvense* is sensitive and non-accumulating species, closely related with *T. caerulescens*.

Seeds of *T. caerulescens* subs. *tatrense* from metal polluted soil and seeds of *T. arvense* from non polluted habitat were sterilised (using detergent and aqueous solutions of NaClO and $HgCl_2$) and sown on solid medium. For control variant Murrashige-Skoog medium without vitamins and growth regulators was used, for Cd-variant $Cd(NO_3)_2 \cdot 4H_2O$ in concentration 10^{-5} M was added. Seedlings were germinated and cultivated in growth chamber over a period of two weeks. After cultivation, segments of root were fixed in glutaraldehyde-osmium tetroxide and embedded in the Spurr embedding medium. Semithin sections of roots (1 μm thick) were prepared with ultramicrotome with glass knives and stained with toluidine blue and basic fuchsine.

In both variants the seeds of *T. caerulescens* started to germinate on 3rd to 4th day and in those of *T. arvense* on 4th to 5th day of cultivation. In both variants germinability of *T. caerulescens* was 71 % and of *T. arvense* 51 %. Growth parameters showed differences between species (growth rate in both variants was significantly higher in *T. arvense*) but there were no significant differences between control and Cd-variants. Root structure in both species is very similar. Differences were observed particularly in development of cell wall modifications (lignification and suberinization) in two innermost cortical layers. Cadmium slightly increased vacuolisation of meristematic cells in *T. arvense*.

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TOXICITY OF PHOTOMODIFIED POLYCYCLIC AROMATIC HYDROCARBON FLUORANTHENE FOR LICHENS

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Terrestrial and aquatic ecosystems are affected by a number of noxious compounds including polycyclic aromatic hydrocarbons (PAHs). These compounds penetrate into the food chain and act as potential carcinogens, mutagens and teratogens. (Lee *et al.* 2000). They are formed during incomplete combustion and pyrolysis of organic matter (coal, oil, wood and plastic).

PAHs as lipophilic organic substances, their metabolites and products of their photo-modification can affect structures and functions at the cellular and subcellular levels. The plasma membrane and inner membranes are the first targets, followed by changes in enzyme activity (Duxbury *et al.* 1997). Inhibition of photosynthesis is often a key mechanism of the toxicity of pollutants in plants and a modification of photosynthetic activity is often used as a "bioindicator" of contamination effects (Greenberg *et al.* 1997). The chemical properties and biological activity of PAHs can be altered both abiotically and biotically. One of the most important abiotic factors is short-wave radiation (UV-A and UV-B). During photomodification, mostly photooxidation, PAHs are structurally altered to a complex mixture of (more than 20) compounds, mainly oxidation products, which may be more toxic than the parent compounds due to the combination of increased solubility, reactivity and bioavailability (Huang *et al.* 1997). Many studies have used vegetation which bioaccumulates air pollutants for monitoring studies. Lichens have been often used as bioindicators or as bioaccumulators of environmental pollution by heavy metals and some organic pollutants.

The aim of our experiments was to evaluate the effect of the intact and photomodified form of fluoranthene on chlorophyll fluorescence parameters in two foliose lichen species *Lasallia pustulata* (L.) Mérat and *Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm.. The intact and photomodified form of fluoranthene (*Supelco*, USA) was applied in the concentration of 0.1, 1 and 5 $mg.l^{-1}$. Chlorophyll fluorescence parameters (F_0, F_V/F_M, Φ_{II}; for the definition see Roháček *et* Barták 1999) were determined from a slow (Kautsky) induction kinetics of chlorophyll fluorescence, recorded by PAM-2000 portable fluorometer (*Walz*, Germany) before FLT exposure and after 24, 48 and 72 hours of exposure. Results were then processed with software STATISTICA 6 (StatSoft, Inc.®). The obtained results demonstrated that the applied concentrations of FLT and especially photomodified FLT (1 and 5 $mg.l^{-1}$) affected primary photochemical processes of photosynthesis of algae in both lichen species (increasing F_0, decreasing F_V/F_M and Φ_{II}). Chlorophyll fluorescence parameters showed different sensitivity to the content of FLT in *Lasallia pustulata* and *Umbilicaria hirsuta*, respectively.

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APPLICATION OF CHLOROPHYLL FLUORESCENCE TECHNIQUE FOR DROUGHT AND HIGH TEMPERATURE TOLERANCE ASSESSMENT IN WHEAT GENOTYPES

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Chlorophyll fluorescence analysis represents useful and non-invasive tool to screen for the effects of many biotic and abiotic parameters on photosynthesis in plants. Main disadvantage of many of such techniques is their slowness. One of exceptions is fast fluorescence measurement analyzed by JIP-test. Therefore we applied this technique for considering its ability to record the differences in sensitivity of genotypes to abiotic stresses.

In our experiments with different genotypes of winter wheat (*Triticum aestivum* L.) we assessed the influence of drought and high temperature on their PS II efficiency. The plants of wheat genotypes of different provenance were cultivated in pots in natural climatic conditions. A part of plants was exposed to slowly incoming water stress induced by restriction of irrigation. Water status in leaves was measured as relative water content (RWC). The heat stress was applied on leave segments exposed to high temperatures (30 – 45 °C) for 1 hour; segments were closed in glass tubes immersed in thermostated water bath. Fluorescence parameters were measured in laboratory conditions before and after temperature treatment. The chlorophyll *a* fluorescence emitted by leaves after excitation with red light was measured using portable fluorimeter (Handy PEA, Hansatech Instruments, Norfolk, England) in dark adapted plants (30 minutes shielding by leaf clips) and the collected data were analysed using JIP-test. The result of each measurement is fluorescence transient plotted on a logarithmic time-scale (OJIP-transient) and plenty of derived parameters calculated by software Biolyzer©.

In time of water stress we measured out some changes in OJIP-transient compared to control plants. We observed also differences among genotypes in sensitivity of PS II to comparable water deficit in leaves. Although classic florescence parameters as Fv/Fm were less sensitive up to the strong water deficit, some other parameters of JIP-test, e.g. Performance Indices, were much more useful. However application of this method in screening for drought tolerance remains problematic.

Heat stress induced by temperatures over 40 °C strongly affected primary photosynthetic processes as showed by substantial decrease of OJIP-transient and appearance of step K of transient, that is a specific indicator of damage to oxygen evolving complex (OEC). Leaves of different genotypes exposed to border temperature (40 °C) showed large variances in sensitivity of PS II, expressed as parameters of fast fluorescence calculated by program Biolyzer©. Therefore this method looks very promisingly for screening of genotypes for better termostability of leaves.

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