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ABSTRACTS

4th Conference
of Polish Society
of Experimental Plant Biology

**Experimental Plant Biology.
Why not?!**

September 21–25, 2009
Cracow, Poland

Polish Academy of Sciences – Cracow Branch
Jagiellonian University

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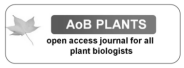

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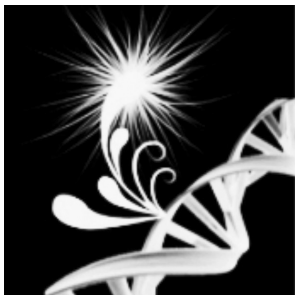

4th Conference of Polish Society of Experimental Plant Biology

Experimental Plant Biology. Why not?!

September 21–25, 2009, Cracow, Poland

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Volume 51, suppl. 2, 2009

Session 1

Plant structure and development

PLENARY LECTURES

1.1.

Development of shape in plants

Enrico Coen

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Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities with in an organism during its development. However, the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify growth of multicellular tissues in 3D space at multiple scales. We have been addressing this problem using a combination of genetic, morphological, computational and imaging approaches in collaboration with Andrew Bangham (Universtiy of East Anglia) and Przemyslaw Prusinkiewicz (Calgary). The results provide new insights into how genes interact with patterns of growth at various scales to modify shape. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

1.2.

Quantitative approaches to plant development

Emmanuelle M. Bayer, Richard S. Smith,
Therese Mandel, Naomi Nakayama,
Przemyslaw Prusinkiewicz, Cris Kuhlemeier

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In the plant shoot apical meristem, gradients of auxin are set up not by diffusion from a localized source or by a reaction-diffusion mechanism, but through a feedback loop between auxin and its transporter, the PIN1 protein. Two distinct molecular mechanisms for the subcellular polarization of PIN1 have been proposed. For leaf positioning (phyllotaxis), an "up-the-gradient" PIN1 polarization mechanism is proposed to direct auxin towards cells with higher auxin concentration, yielding localized auxin maxima that determine the positions of initiating leaves. In contrast, the canalization hypothesis proposes a "with-the-flux" PIN1 polarization that reinforces the direction of auxin flow, leading to the formation of vascular strands. During the initiation of the midvein, these two patterning mechanisms intersect, and thus the question arises as to how two different PIN1 polarization mechanisms may work together. Our detailed analysis of PIN1 polarization during midvein initiation in combination with computer simulations suggests that both mechanisms for PIN1 polarization are operating simultaneously, and that some cells in both the epidermis and in internal meristem tissue appear to switch from one polarization strategy to another.

1.3.

The role of chemical dynamics in morphogenesis

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How plants and other organisms with cell walls achieve their shapes is a very complex problem, which will ultimately require a synthesis across many disciplines, including molecular and cell biology, biophysics and physical chemistry. My work focuses on the interplay between the pattern formation of growth catalysts on plant surfaces and the localized expansion of these surfaces to generate shape. While an enormous amount of information has been gained in recent years on the molecules involved in patterned growth, the broader issues of how these molecules are localized, what maintains localization upon growth, and how growth symmetries are broken remain far less explored. While some simpler types of morphogenesis, such as extending tip growth, may arise from a particular asymmetry in growth which is perpetuated, many morphogenetic sequences involve repeated symmetry-breaking, for example repeated branching from a tip or shoot apex. Selection of different patterns over the course of development requires that not only must the chemistry direct growth, but that the chemistry must respond to growth (and geometry) such that appropriate pattern is generated or maintained. By computing pattern-forming (Turing-type) reaction-diffusion mechanisms on three-dimensional surfaces, I am determining the constraints on reaction kinetics and transport properties necessary for sequences of plant morphogenesis. To focus on the growth-patterning feedback, it is helpful to apply modelling to simpler systems, such as unicellular green algae. Many genera create body architectures as complex as higher plants, but in a single cell with a continuous surface. In these cases, pattern formation is not cell-specific gene expression, but rather spatial concentration patterns in the same plasma membrane. The particular mechanisms of surface expansion are likely to differ from higher plants, but the overall constraints on growth rates and patterning boundaries to generate coherent form are likely to be shared. I will describe my work in modelling the morphogenetic sequences of different species in the genus *Micrasterias*, which display many variations on repeated dichotomous branching; species shapes are distinguished by variation in particular growth and rate parameters. By further variation of these parameters, additional morphologies, such as whorled structures, can be produced. I will describe experimental work on conifer cotyledon number selection to extend the application of the model to higher plant morphogenesis.

ORAL PRESENTATIONS

1.4.

Does a developing lateral root in *Arabidopsis thaliana* obey the growth tensor rules?

Joanna Szymanowska-Pułka[1], Lewis Feldman[2], Jerzy Nakielski[1]

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Lateral root development is a complex and interesting process resulting in the formation of a new meristem. Moreover, because the lateral root initiates in the pericycle of the mother root, between the time of its initiation and maturity it is continuously changing in size and shape. Like all other plant organs the lateral root grows simplastically, which means that walls of neighbouring cells do not slide past each another but rather maintain their mutual cell wall contacts for their lifetimes (Erickson, 1986). Such growth may be described in terms of the growth tensor (GT) and its principal directions, that indicate planes of divisions of cells (Hejnowicz and Romberger, 1984). It was shown that an unsteady (time-dependent) variant of the GT needs to be applied to the case of lateral root formation (Szymanowska-Pułka, 2007).

The objective of this current study was to construct a simulation model for growth including cell divisions for a case of the lateral root formation in *Arabidopsis thaliana*. Empirical data were obtained on the basis of observations of anatomy and morphology of the organ in different stages of its development. The unsteady growth tensor was defined and the algorithm for cellular divisions applied in which cells divided with respect to principal directions of the GT. The input data to the model was a meshwork of polygons representing the cellular pattern of the initiating lateral root. In the simulations the meshwork was deformed in a manner showing the lateral root developing in time in 2D. The computer-made sequence appeared realistic when compared with the empirical data obtained from anatomical and molecular studies (Malamy and Benfey, 1997). Thus the growing lateral root in *Arabidopsis* seems to obey the GT rules.

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POSTERS

1.5.

Response of some Polish wheat genotype to anther culture system

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Double haploid techniques provide plant breeders with pure lines in a single generation, which may save considerable time in the breeding of new cultivars. There are two major techniques for haploid production in cereals – anther/microspore culture and chromosome elimination using wide hybridizations.

The former technique is usually considered as simpler, more efficient, and more cost-effective than the latter.Wheat in particular is known as a recalcitrant species with regard to *in vitro* androgenesis techniques such as anther and microspore culture. Use of anther culture in wheat breeding programs is limited by strong genotype specificity, low frequency of haploids, and a high rate of albinism in regenerants. Despite these problems, the anther culture technique has been successfully applied in some wheat breeding programs, resulting in new cultivars. It is known that anther culture response is highly genotype-specific and typically, it would produce many individuals from only a few selected crosses.

The goal of this study was to investigate the response of 20 Polish wheat cultivars in anther culture system for haploid plantlet regeneration. Significant differences were found between genotypes. The results indicated that both the androgenic response and regeneration ability were greatly genotype depend. It could to be concluded that genotypic response to anther cultivars is vital.

This work was supported by COST ACTION–FA0604.

1.6.

Regulatory function of sucrose in gene expression of lipase and NAD^+- and $NADP^+$-dependent isocitric dehydrogenase in organs of germinating yellow lupine seeds (*Lupinus luteus* L.) grown *in vitro*

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Some mono- and disaccharides control the expression of many genes whose products are involved in processes of glycolysis, respiration, photosynthesis, degradation and synthesis of sucrose and starch, nitrogen metabolism, and mechanisms controlling the cell cycle and defense reactions. There are fewer reports on the control of lipid metabolism especially in leguminous plants. It was shown that in organs of germinating yellow lupine seeds sugar deficiency stimulates lipid degradation. It was reflected in lowering number of lipid bodies and in higher activity of lipase and catalase. However activity of NAD^+- and $NADP^+$-dependent isocitric dehydrogenase

(IDH) which catalyses subsequent steps of storage lipids catabolism in lupine seeds was inhibited in these conditions (Borek et al., 2006). In that paper it was postulated (based on experiments with transcription and translation inhibitors) that changes in enzymes activities were caused by gene expression modifications.

In this work the level of mRNA of lipase, NAD^+- and $NADP^+$-dependent IDH was determined. The expression level of these genes was investigated in isolated embryo axes and excised cotyledons as well as in seedling axes and cotyledons grown *in vitro* for 96 hours in darkness on Heller's medium with 60 mM sucrose or without the sugar. Content of mRNA was determined by real-time PCR.

The highest stimulation of lipase gene expression caused by sugar deficiency was observed in isolated embryo axes and was over 26 times higher than in organs fed with sucrose. In contrast $NADP^+$-dependent IDH gene expression in isolated embryo axes was lower in sugar deficiency conditions and was over 28 times lower than in organs fed with sucrose. Changes in NAD^+-dependent IDH mRNA level were not as significant as lipase and $NADP^+$-dependent IDH and were not unambiguous. In seedling organs and excised cotyledons changes in gene expression were observed as well but in a lesser extent.

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BOREK S, RATAJCZAK W, RATAJCZAK L. 2006. Ultrastructural and enzymatic research on the role of sucrose in mobilization of storage lipids in germinating yellow lupine seeds. *Plant Science*, 170: 441–452.

This work was supported by grant no. 2 P06A 004 29 from science fundings in years 2005–2008.

1.7.

The influence of hydration conditions upon seed germination after long-term storage

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The long-term storage of seeds is a necessary to preserve and protect plant biodiversity. During storage, even under optimal conditions seeds lose their ability to germinate. Lack of seed germination does not always mean seed embryo death. In these cases the potential of seed germination can be increased or even renewed.

The purpose of our work was to determine different indicators of winter wheat seeds' viability and to investigate the ability of these seeds to increase their germination potential by making the water uptake slower with osmotic active compound during seed imbibitions.

In our investigation, we used two different cultivars of winter wheat seeds (*Triticum aestivum*), Albatross from the 2003 and 2006 harvests and Strumok from the 1998 and 2006 harvests. Seed viability was estimated by the tetrazolium method, vigor test, and the standard germination test. Also lipid peroxidation was analyzed in whole seeds and in seed embryos. For decreasing the water uptake by seeds osmotic active compound polyethylene glycol 8000 (PEG 8000) was used. In the control variant, the seeds were germinated in Petri dishes with distillated water for 7 days.

According to our results, the germination test showed 4% for Albatross seeds from the 2003 harvest and 40% for Strumok seeds from the 1998 harvest. At the same time, the viability of these seeds was tested according to tetrazolium method was much higher. According to our data, the influence of PEG 8000 increased germination potential of both cultivars' old seeds in all variants of the experiment. The biggest increase of germination potential, 20% compared to control variant, was gained from old seeds of the Albatross cultivar after germinating them in 5% PEG 8000 for 7 days. The same increase of germination potential was obtained for the old seeds of the Strumok cultivar, after germinating them in 20% PEG for 7 days.

Thus according to the experimental data, slowing the water uptake during seed imbibitions, increases the germination potential of old winter wheat seeds for both cultivars.

1.8.

Studies on pollen viability among interspecific rhododendron hybrids

Malgorzata Czernicka, Maria Klein

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The aim of our study was to evaluate viability and structure of pollen grains in 14 populations of interspecific rhododendron F1 hybrids derived from crosses between: *Rhododendron aureum, R. brachycarpum, R. purdomii*, and cultivars which belong to *R. catawbiense* and *R. yakushimanum* Hybridum groups. Pollen viability was analyzed according to Alexander method (Dafni, 1992) and morphology of pollen grains was studied on the basis of their three-dimensional images, obtained by application of scanning electron microscopy (SEM). Results indicated that pollen viability was reduced and ranged from 12.2% for the 'Old Port' × *R. brachycarpum* hybrids to 55.2% for the *R. brachycarpum* × *R. purdomii* hybrids. There were also identified numerous abnormalities in pollen structure. We found such configurations of pollen grains as: monads, diads or poliads existing among the typical for rhododendrons tetrads. Most of these abnormal structures (i.e. 49.2%) were observed for *R. aureum* × *R. brachycarpum* hybrids whilst none were identified for *R.* 'Koichiro Wada' × 'Catharine van Tol' plants. Furthermore, an average of 39.9% of the pollen grains revealed degeneration and granulosis in cytoplasm for all of studied hybrids. Following on micrographs made with the scanning electron microscope it occurred that tetrads containing fertile pollen grains were significantly bigger and had regular shape. On the contrary, size of sterile pollen grains was smaller and their sporoderm was degenerated and sunken, with cracks and fractures of unknown origin. In order to find a source of pollen structure disorders it is planned to analyze the process of microsporogenesis in interspecific rhododendron F1 hybrids.

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1.9.

Expression of carboxypeptidase III and GAMyb in the triticale seedlings

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Carboxypeptidase III is one of the hydrolytic enzymes which is synthesized and secreted by the aleurone layer in response to gibberellic acid. Gene encoding carboxypeptidase III contain several

conserved sequences upstream to transcription start site: GARE and pyrimidine boxes responsible for binding of transcription factors GAMyb and Dof class proteins respectively. The gibberellin signal cause hydrolysis of proteins related to ABA and expression of GAMyb in the aleurone layer. The latter process is responsible of activation of promoters of genes which participate in degradation of reserves in starchy endosperm. The expression of GAMyb transcription factor was observed in all tissues of triticale seedlings, but the highest level of transcript was found in endosperm of the grains. The expression of the gene increased up to second day after the beginning of imbibition, and decreased to the last day of studied period. It was correlated to expression of carboxypeptidase III which increased up to third day of germination. The transcript level of GAMyb gene was higher than the carboxypeptidase III gene. It may suggest involvement of GAMyb in the control of transcription of carboxypeptidase gene in endosperm of germinating triticale grains. The profiles of expression of the transcription factor and carboxypeptidase III gene in scutellum were also similar. GAMyb is thought not to control the expression of carboxypeptidase III in roots since expression of the enzyme is simultaneous with PCD. Nonetheless the expression of GAMyb in shoots and roots of the triticale seedling was observed at the very low level, but its function is not recognized.

1.10.

Uptake of D-*chiro*-inositol and D-pinitol by developing pea embryos

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Myo-Inositol occurs in all living plants and animals tissues and plays important functions in cellular metabolism. Some plant species synthesize *myo*-inositol isomers or its methylated ethers (like D-*chiro*-inositol and D-pinitol, respectively). Inositols can be transported into maturing legumes embryos, decreasing accumulation of raffinose family oligosaccharides. The mechanism of cyclitols uptake from apoplast by embryo remains still unknown. We investigated the involvement of sucrose, glucose and *myo*-inositol on the uptake of cyclitols by pea embryos, naturally accumulating *myo*-inositol only.

Embryos at 14–20 DAP were incubated in water solutions of D-*chiro*-inositol and D-pinitol (at 10 mM concentration) in the absence or presence of sucrose, glucose and *myo*-inositol (at 10 and 20 mM concentration). Additionally, the effect of 50 μM CCCP and 10 μM antimycin A (both sucrose transporters inhibitors) on the uptake of D-*chiro*-inositol was tested. The levels of cyclitols in embryos after 0, 2, 4, 6 and 24h of incubation were analyzed by gas chromatography method.

Developing pea embryos indicated the ability to uptake of both cyclitols. Embryos fed with D-pinitol converted a small amount of this cyclitol into D-*chiro*-inositol. In the presence of glucose or sucrose the rate of cyclitols uptake was not decreased. Addition of *myo*-inositol to feeding solution drastically decreased absorption of D-*chiro*-inositol and D-pinitol by embryos, suggesting that a common cyclitols transporter can operates in pea embryos. In regard to inhibitory effect of CCCP and antimycin A on the uptake of D-*chiro*-inositol, we suggest that cyclitols transport may be energy dependent.

This work was partially supported by grant No N30312532/4015, obtained from Ministry of Science and Higher Education of Poland.

1.11.

Physiological and molecular analysis of glutamate dehydrogenase in germinating triticale kernels

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An physiological and molecular approach was developed in an attempt to define the role of glutamate dehydrogenase (GDH; EC 1.4.1.2-4) during germination of triticale kernel. *In vitro* GDH catalyses a reversible reaction involving ammonium ion, 2-oxoglutarate, glutamate – and NAD(P)H in the role of coenzyme. Hence the enzyme is known as operating where the paths of C and N metabolism intersect. The dynamics of aminating and deaminating activity of GDH as well as their ratio reported in embryo and endosperm of germinating triticale grain testify to a particular role of the enzyme in the process. Changes in aminating and deaminating activities of GDH as well as their ratio in different parts of the kernel and young plant seem to indicate that its enzymatic function is organ/tissue-specific.

In the molecular approach the aim of the present project was cloning of glutamate dehydrogenase (*GDH*) gene from triticale. First part of sequence GDH based on reverse transcriptase polymerase chain reaction (RT-PCR) was cloned. The cloned cDNA was designated as *TsGDH1*. Complete *TsGDH1* cDNA was obtained by 3' i 5' RACE (Rapid Amplification of cDNA Ends). The *TsGDH1* cDNA is 1781 bp long and contains an open reading frame of 1236 bp encoding 411 amino acids. TsGDH protein contains all conserved regions found i GDH and shows a high level sequence similarity to many other plant glutamate dehydrogenases. The level of *TsGDH* expression in germinating kernels was studied by semi-quantitative RT-PCR analysis. The results showed that *TsGDH* has the highest expression level in the root of the seedling and a somewhat lower one in the embryo after 24h and 48h of germination. A low level of expression was observed in dry embryo and coleoptile.

This work was supported by the Ministry of Scientific Research and Information Technology in Poland, grant No N310 301134.

1.12.

1,3-β-D-glucan in vegetative buds of Norway spruce during dormancy

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The object of the study was Norway spruce, and the analysed material consisted of the embryonic shoots isolated from vegetative buds. Buds were collected from grafts of *Picea abies* clone 04-118, in a clonal archives in the Institute of Dendrology "Zwierzyniec" Experimental Forest near Kórnik. Material was collected from the middle part of the tree crown from November 2008 until April 2009 to analyse changes in content and distribution of 1,3-β-D-glucan.

The location of 1,3-β-D-glucan in the Norway spruce embryonic shoot was determined using immunogold labeling and TEM. Western-blotting analysis of the accumulation of 1,3-β-D-glucan and 1,3-β-D-glucanase was performed too.

Probably, symplastic communication plays the main role in synchronisation development, and is selectively blocked during the dormancy. Specific pattern of 1,3-β-D-glucan distribution was observed, and it was not changing during the dormancy period. 1,3-β-D-glucan was detected in procambium and peripheral meristem. It blocked plasmodesmata and therefore affected symplastic communication.

The work was supported by Institute of Dendrology and Ministry of Science and Higher Education (project no. N N303 068934).

1.13.

The changes in the ultrastructure of root cells in *Pisum sativum* after incubation in inhibitors of protein kinases and phosphatases

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Chromatin is a highly dynamic structure that plays an important role in regulating all nuclear processes (replication DNA, DNA repair, transcription and recombination).The ultrastructure of cells within root tips of *Pisum sativum* was studied after 3-days incubation with roscovitine – the protein kinase inhibitors and NSC – an inhibitor protein phosphatase. Electron microscopic studies demonstrate that depending on the chemical agent, ultrastructure of cells reveals some specific features, comprising both chromatin condensation and the appearance of cytoplasmic structures. As compared with the untreated control plants, ultrastructure of roscovitine treated cells, is distinctly different. The regions of nuclear envelope and central areas of nuclei are characterized by extremely condensed chromatin. Ultrastructure of NSC95397 stimulated cells is similar to roscovitine treated root cells. The electron microscopic observations revealed that, treatment of roots with NSC are characterized by large areas of strong condensed chromatin. Moreover, total surface engaged by this regions is relatively big.

1.14.

AFLP analysis of genetic diversity in natural population and tissue culture plants of *Saxifraga hirculus* L.

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Amplified Fragment Length Polymorphism (AFLP) is a powerful tool for investigation of genetic variation within species, especially in conservation context. *Saxifraga hirculus* (L.) a postglacial relict is protected by international and national law because of significant reduction its habitats. This molecular method was employed to investigate genetic stability the *in vitro* cultured plant of *S. hirculus* and to compare with populations in nature. Although, conventional propagation methods are available, tissue culture techniques reduce risk of losing the valuable material, especially in case of an endangered plants and allow to store limited wild accessions. The effects of pH value and the different regimes of temperature on the shoot proliferation were studied. Application of tissue culture methods improve the rate of plant propagation but may induce somatic variation. Genetical similarity among individuals of both populations is close to one another and, calculated based on Jaccard index, it ranges between 85% and 98% for natural population and 89% to 97% for *in vitro*-cultured plants. Gene diversity values for both populations do not show any differences and are 0.98 for natural and 1.00 for *in vitro*-grown plants. Average gene diversity for both equals 0.1. High genetic similarity was found also in analysis of variance, that showed higher level of intrapopulation (52%) than interpopulation (48%) diversity (statistically significant at $p< 0.001$, $F_{st} = 0.48$). Results of studies, showed no difference in spatial patterns of variability where genetical variability of the plants derived from tissue culture was compared with that of the natural population of *S. hirculus*.

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1.15.

Localization of mRNA in Cajal bodies

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In European larch microsporocytes, spherical structures 0.5 to 6 μm in diameter are present in which poly(A) RNA accumulates. There were one to several bodies per cell and they were often present in the vicinity of the nucleolus. No nascent transcripts were observed within them. Splicing factors of the SR family, including protein SC35, which participates in bringing the 3' and 5' sites closer in the splicing reaction, were also not observed. The absence of the above-mentioned elements within bodies containing poly(A) RNA disqualifies them as sites of synthesis and preliminary stages of primary transcript maturation. However, they contained abundant elements of the splicing machinery commonly occurring in Cajal bodies (CB), i.e. Sm proteins or small nuclear RNA (snRNA). The molecular composition as well as the characteristic ultrastructure of bodies containing poly(A) RNA prove that these were CBs. The question arises whether the observed poly(A) RNA in CBs are mRNA's or other polyadenylated RNAs, i.e. non-coding "mRNA-like" transcripts. There were examined several probes complementary to known mRNAs from *Larix* sp. Double labeling to U2 snRNA (marker of CB) and mRNAs of (1) Sm proteins (SmE, SmD1 and SmD2), (2) Pol II RNA RPB2 subunit and (3) some house keeping gene's mRNAs like catalase, ATPase, α-tubulin or peroxidase, showed that there are present in CBs. This is the first report on the presence of mRNA within Cajal bodies.

The investigations performed here indicate that in the analyzed microsporocytes, the level of poly(A) RNA is high. The discrepancy of the results obtained here with data from the literature could came from lower level of poly(A) in somatic cells than generative cells like larch microsporocytes. Thus, it cannot be excluded that its detection was easier than in somatic cells. It's unknown if mRNA accumulation in CBs represents typical developmental strategy of cells during microsporogenesis. The lack of data on this subject may be due to the fact that the localization of mRNA during microsporogenesis in other plants hasn't been performed to date, and investigations on CBs in these cells are still fragmentary. The role of mRNA-rich CBs in RNA metabolism in larch microsporocyte development will be discussed.

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1.16.

Interactions between auxin, cytokinin and ethylene during *in vitro* rhizogenesis of ice plant

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Hypocotyl explants of *Mesembryanthemum crystallinum* produced roots regularly from basal part when cultured vertically either with apical (AE) or basal end (BE) in media containing indole-3-acetic acid (IAA). The inhibitors of polar auxin transport (PAT), α-naphtylphtalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) inhibited rhizogenesis and changed IAA-induced pattern of root formation only from AE-cultured explants, indicating the role of PAT in root regeneration in this system. Cytokinin (zeatin, kinetin, BAP) added to auxin containing medium caused thickening of the explants and promoted formation of roots with long root hairs. Additionally, all cytokinins strongly reduced rhizogenesis from the explants maintained with BE and AE, and similarly to TIBA and NPA changed IAA-induced pattern of rooting in AE-cultured explants by favoring rooting from apical end and middle part of the hypocotyl with its concomitant reduction from the basal end. The addition of kinetin did not influence the endogenous content of IAA in the explants maintained with AE but it strongly enhanced ethylene production. The cross-talk between ethylene and cytokinin during PAT-dependent rhizogenesis was tested by using an ethylene antagonist ($AgNO_3$), an inhibitor of ethylene synthesis aminoethoxyvinylglycine (AVG), and a precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC). $AgNO_3$ applied together with IAA or IAA and kinetin reduced the production of ethylene, inhibited rhizogenesis and induced non-regenerative callus from BE suggesting the need of ethylene signaling to elicit the rhizogenic action of auxin. A reduction of rhizogenesis and decrease of ethylene biosynthesis was also caused by AVG. Most importantly, AVG at 10 μM reversed the effect of cytokinin on root patterning resulting in roots emerging only from BE on the medium with IAA and kinetin. AVG and $AgNO_3$ also abolished the kinetin-induced effect on root morphology making roots relatively thin and almost devoid of root hairs Conversely, ACC at 200 μM significantly enhanced the production of ethylene and partly mimicked the effect of cytokinin when applied with IAA alone, thus confirming that in cultured hypocotyls of ice plant cytokinin affects IAA-induced rhizogenesis through an ethylene-dependent pathway.

1.17.

Is *Artemisia absinthium* var. *calcigena* a well defined endemic taxon? Morphological, anatomical and cytogenetic characteristics of *Artemisia absinthium* var. *absinthium* and var. *calcigena*.

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Artemisia is one of the biggest genera in the Asteraceae family. Its distribution area covers different habitats mostly in the Northern hemisphere, with landscape-dominant species in dry areas. The large taxa number, geographical distribution range and ecological differentiation have caused morphological, anatomical and cytogenetic adaptations. Due to this, different forms have been considered at subspecific or lower taxonomical levels. A proper classification of these taxa is difficult, particularly considering only morphological features. One doubtful taxon is *A. absinthium* var. *calcigena*, endemic for Pieniny Mountains. The aim of this study was to perform comparative analyses of this taxon and its type variety, *A. absinthium* var. *absinthium*. The analyses included different organization levels: morphological, anatomical and cytogenetic. At the morphological level, no significant differences were observed. Covering and glandular trichomes exist in both forms on the leaves surface. No differences were noted in pollen grains and fruit surface sculpturing. Both taxa have the same ability to produce slime in fruits. Anatomical shoots cross-sections were also very similar. One characteristic feature for *A. absinthium* var. *calcigena* is the ability to form a periderm in very early stages (two month-old seedlings). In older plants of this taxon, the periderm has 2–3 more layers than in individuals of the typical form, and builds an unbroken layer. Karyological and cytogenetic results were also similar in all studied plants. Genome size, chromosome number, karyotype symmetry as well as fluorochrome banding and FISH analyses did not demonstrate any discriminative power. Results from the presumed endemic variety fall in the range of variability found in other populations of *A. absinthium*. Overall, no significant differences between *A. absinthium* var. *absinthium* and var. *calcigena* were found. This suggests that the observed variability does not support distinguishing the Pieniny Mountains endemic as an independent unit, and its particularities may result from adaptations to different habitats rather than implying any systematic independence.

1.18.

The characteristics of circumnutation of *Helianthus annuus* growing on vertical clinostate in different light conditions

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Circumnutations are a helical plant movements inducted by turgor – growth changes. They are universal movements for plants examined so far. They are constitutional, however they are modulated by different external factors. We have studied the characteristics of circimnutation movement in different light directions: when the light source was applied aside and also "moved around" plants growing on vertical clinostate in clockwise or counter clockwise directions. We have examined the frequency of the left-handed and right-handed circumnutation as well as the dependence of the period and length of circumnutation on applied light direction.

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1.19.

Comparative analysis of floral structures attracting insects in selected species of Bulbophyllinae Schltr. and Pleurothallidinae Lindl. (Orchidaceae)

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Pollination is a complex process – flowers have evolved various features to attract the insects' attention. This is the effect of pollinators' demands – they prefer flowers with particular structures and forms of flowers.

Species from Bulbohyllinae and Pleurothallidinae represents one of the most interesting sample of morphological convergence caused by the pollination strategy – myophily (Dressler, 1981). The aims of these studies were: examination of morphological structures connected with pollination by Diptera, and searching answer for the question: does the convergence exist on the micromorphological level as well? Species were observed in stereomicroscope and scanning electron microscope (SEM). Cytochemical and molecular research were also done.

Bulbophyllinae is pantropical taxon, Pleurothallidinae – neotropical. Molecular data show that both subtribes are not closely related. The structure of vegetative parts is different. Nevertheless, there are many similarities in floral structures. The first visual attractant is the floral colour. In both subtribes species have dark flowers: mainly red and brown. The motile elements (appendages, trichomes and hairs on petals and/or on the lip, thickened tips of petals) cause the increase of the floral attractiveness for flies. The function of landing platform for insects belongs to the lip, and also connated sepals. Nectar is observed on superficial grooves on the lip and the abaxial sides of sepals. In both subtribes the mechanism of hinge lip is present.

The floral similarities were also visible on the micromorphological level: an undulating cuticle, conical papillae and stomata in the top-parts of petals, nectar traits beginning from the cavity at the base of lip, the imbricate arrangement of cells and papillae on the lip, trichomes occurring at the bases of petals close to gynostemium, papillae on the column-foot of gynostemium.

In both groups similarities in pollination strategies are noticed. Mimicry to other flowers is observed (i.e. umbellate flowers similar to Asteraceae, occurrence of pseudonectar instead of nectar). Brood-site imitation and mimicry to hidden animals are also present. In Pleurothallidinae pseudocopulation is proved. In Bulbophyllinae some features suggest this pollination strategy, but field works are necessary to demonstrate it.

Species from these two groups are geographical vicariants and occupy ecological niches for myophilous plants, respectively in Old and New World.

1.20.

Unusual plasmodesmata in the embryo-suspensor of *Sedum* (Crassulaceae)

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The development and ultrastructure of the plasmodesmata in the embryo-suspensor of *Sedum acre* L. and *S. hispanicum* L. were investigated using light and electron microscopy. The ultrastructure of plasmodesmata in both the investigated species are very similar, the results will be described together.

Differentiation of the basal cell (BC) begins shortly after the first division of the zygote. Two-celled embryo consist of the large (BC) and the smaller apical cell. The wall surrounding the (BC) is thicker then the apical cell wall. The inner wall of the embryo is thick and separating the apical cell from the (BC) contains a number of a simple plasmodesmata, but there are none in the outer walls of the embryo.

When the embryo proper reaches the early-globular stage of development, the (BC) undergoes stage of following differentiation. The chalazal suspensor, linking the (BC) with the embryo proper, consist of two layers of elongated cells. The part of wall, separating the (BC) from the first layer of the chalazal suspensor cells, is perforated by unusual, compound plasmodesmata. Numerous unusual plasmodesmata are branched. Plasmodesmata, on side of basal cell surrounding the electron-dense, none membranous body in the cupola shape. There are dark network of tubules connecting with RER membranes inside this body.

The mature suspensor of *Sedum* consist of a large pear-shaped basal cell and a few chalazal cells in two layers. The wall surrounding the (BC) is thicker then the chalazal suspensor and the embryo proper cells. There are no plasmodesmata in the outer walls of the whole embryo, but they are numerous in the inner walls of the chalazal suspensor and embryo proper. The wall separating the (BC) from the first layer of the chalazal suspensor cell contains numerous unusual plasmodesmata, too.

The (BC) reaches its maximum size in the torpedo stage of embryo development. At this stage, the suspensor begins to degenerate. The chalazal suspensor cells are arranged in two layers. In transverse wall separating the basal cell from the first layer of the chalazal suspensor cells contains numerous

branched and flattened plasmodesmata. The mechanism and sort of transport through these compound plasmodesmata is discussed.

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1.21.

Expression pattern of *LEAFY COTYLEDON1* and *FUSCA3* genes during somatic embryogenesis in *Arabidopsis*

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In *Arabidopsis thaliana, in vitro* culture of immature zygotic embryos (IZEs) on solid medium supplemented with auxin results in effective production of somatic embryos via somatic embryogenesis, SE (Gaj, 2001). The *LEAFY COTYLEDON* genes (*LEC1, LEC2* and *FUS3*), encoding transcription factors involved in zygotic embryogenesis were suggested to play a key role in SE as knockout mutations in the *LEC* genes dramatically decrease embryogenic ability of *in vitro* cultured explants (Gaj et al., 2005).

The present study was aimed at analysis of *LEC1* and *FUS3* expression pattern during SE process induced in culture of IZE explants. Real-Time PCR was used to monitor the relative level of *LEC1* and *FUS3* expression at various time points of the culture induced on auxin (E5) and auxin free (E0) media. Comparison of *LEC1* RNAs level in E5 and E0-cultured explants revealed their gradual and auxin-stimulated increase between the 3rd and 15th day in E5-treated culture. In contrary to *LEC1*, the *FUS3* expression level was found auxin independent as activity of this gene was similar on E5 and E0 media. The significant difference in the expression levels of *LEC1* and *FUS3* genes was noticed between embryogenic and non-embryogenic cultures. It was indicated that 15-day old Col-0 embryogenic culture displayed up to a 20-fold higher activity of the analysed genes than non-embryogenic one. Similarly, the expression of the *LEC1* and *FUS3* genes was indicated to be significantly lower in cultures derived from the mutants impaired in SE (*axr4-1* and *cbp20*) and characterized by abundant callus formation.

The differential pattern of *LEC1* gene expression observed during the time course of SE culture implicated that the gene, similarly to *LEC2* (Ledwoń, 2008), may be specifically involved in development of somatic embryos. In contrast, a stable, auxin-independent activity of the *FUS3* gene suggests its more general, not SE-specific functions in cultured cells.

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1.22.

Variable shoot morphology of NPA-treated plants

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The experiments on *pin-formed1 (pin1) Arabidopsis* as well as pin-like *Arabidopsis* and tomato shoots obtained with NPA-treatment are fundamental for contemporary models explaining phyllotaxis mechanisms. Pin-like shoot apices are virtually unable to produce a new primordium but the primordium formation can be induced by the local application of IAA. Therefore, it is assumed that polar auxin transport is a crucial process involved in phyllotactic patterns generation.

However, preliminary results indicate that the morphology of pin-like shoot apices of *pin1* is variable and putative early stages of primordium formation are observed at the apex periphery (Kwiatkowska, 2004). To further study this phenomenon we quantify the geometry and growth of shoot apices in NPA-treated plants in three species: *Arabidopsis*, tomato as well as *Anagallis arvensis* (for which morphogenesis at the untreated shoot apex is known in details) using a non-destructive replica method and a 3-D reconstruction algorithm (Dumais, Kwiatkowska, 2002). We show that pin-like shoot apices in NPA-treated tomato and *Arabidopsis* are dynamic and very variable structures. Even after a long-lasting culture on NPA medium tomato apices seem to retain some ability to produce rudimentary primordia and some positional information about primordium distribution. Relatively big protrusions are apparent at the base of apices, in seemingly spiral pattern. Pin-like shoots originating from the same plant exhibit significant morphological heterogeneity, which only to a certain extent can be explained by differences in their developmental phases. We postulate that the observed trials of primordium formation and heterogeneity may at least partly be explained by the presence of endogenous auxin. An extreme case where its presence is manifested is the shoot apex of *Anagallis*. NPA-treated *Anagallis* shoots instead of being pin-like produce "cups", which are formed by fused leaves of the same nodes.

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1.23.

Functional genomic analysis of the proteins involved in interactions between the components of the cell wall, plasma membrane, and cytoskeleton in *Arabidopsis thaliana*

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Structural and functional continuum between cell wall, plasma membrane and cytoskeleton (WMC) is indispensable for proper functioning of plant cells. It is involved in many developmental processes, like maintenance of the cell shape, and also in plant cell reactions to external and internal stimuli. At the present state

of knowledge it is assumed that proteins, and also polysaccharides, fulfill the role of elements connecting various parts of the WMC continuum. However, the true knowledge about such elements is rather scarce.

Based on bioinformatics analyses, four proteins have been selected as potential linkers anchoring cytoskeleton and plasma membrane in the walls. These were analyzed with respect to their structure, presence of a signal peptide, transmembrane helix(-ces; TMH) or in order to confirm the predicted domain architecture. Identification of homologous proteins from other sources was also done as they can indicate possible function(-s).

Two proteins are defined by the presence of EGF motifs, often found in extracellular proteins, suspected to be involved in intermolecular interactions. One of them contains two EGF motifs, and the exostosin domain, responsible for glycosyltransferase activity (GT). Sequence analysis showed that this protein belongs to family 47 of glycosyltransferases. *Arabidopsis* contains 39 genes coding for such GTs but only a few of plant GTs has been characterized so far. All known GTs are thought to act in the lumen of Golgi, and thus it would be extremely interesting to find out if protein with such activity could also be located in the extracellular compartment. The second of those proteins contains an EGF motif, and the metalloproteinase domain. Homology search analysis revealed the highest similarity to gp63 (leishmanolysin) from *Trypanosoma cruzi* which acts as a zinc dependent protease. Detailed sequence analysis and homology modeling showed that it belongs to metazincin family of proteases, very similar to leishmanolysin, and is probably active. Its function is as yet unknown.

Two remaining proteins belong to the formin family (formin 6 and formin 8) and are defined by the presence of formin homology domain 2. In animal cells, they act as actin nucleation and elongation factors. Not much is known about their functioning in plants. Both selected formins belong to class I, defined by the additional TMH and a proline-rich motif which probably extends into extracellular space, anchoring the proteins in cell wall. Analysis of *A. thaliana* insertion mutants revealed a significant decrease in the root length compared to wild type plants. The difference was greater in the case of plants grown on basal medium compared to plants grown under salt and osmotic stress conditions. However, preliminary visualization of the actin cytoskeleton arrangement showed no significant differences between mutants and wild type plants.

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1.24.

Procambium-cambium transition in ontogeny of *Diospyros lotus* L.

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Diospyros lotus L. (Ebenaceae) belongs to the group of tree-species which are characterised by the double-storied structure of cambium. This pattern develops rapidly in the ontogeny of D. lotus stems and is already observed in the terminal parenchyma of the third annual ring. The main aim of the study was to analyse the ontogenetic changes of *D. lotus* vascular meristem with a special attention paid to transition between procambium and cambium. The features regarded as distinguishing both meristems were examined, i.e. intrusive growth and periclinal divisions of initial cells, ray ontogeny, and the primary versus secondary nature of derived vascular tissues. Initially, *D. lotus* procambium is a homogenous tissue developed as a continuous cylinder ('syphonostele'). The procambial cells may have transverse as well as tapered cell wall ends, the latter resulting from the intrusive growth. During further development, two types of meristematic cells, which are primordial fusiform and ray initials, can be recognised in procambium based on two distinct sets of respectively formed derivatives, i.e. vessel elements and rays. Additionally, the first periclinal divisions of procambial cells occur already during protoxylem formation, while distinct radial rows of cells, resulting from this type of divisions, are present in metaxylem. In *D. lotus*, vessels of late metaxylem as well as those of secondary xylem possess pitted secondary cell walls, making it impossible to classify the vascular element as primary or secondary in origin.

The double-storied structure is rapidly formed in cambium ontogeny of *D. lotus* due to anticlinal longitudinal divisions of fusiform initials and ordering of rays into horizontal tiers. The high primary rays are split at the borders of adjacent storeys, and new secondary rays are initiated by segmentation of fusiform initials exactly within the storeys. Data presented here show continuous and quick transition from the early stage of procambium to the double-storied structure of cambium in ontogeny of *D. lotus*. It would be of a special interest to confirm whether such developmental continuity is coincident with the uninterrupted activity of the genes characteristic of procambium and cambium.

1.25.

Fluorescence properties of solubilized Pchlide:LPOR:NADPH complexes and their changes caused by a short light pulse

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The reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) in Angiosperms is a light-triggered reaction, catalyzed by Pchlide oxidoreductase (LPOR). In the absence of light, Pchlide and LPOR accumulate within etioplast inner membranes (EPIM). Some Pchlide molecules form complexes with LPOR and NADPH, whereas the rest stay unbound to the catalytic site of LPOR. Aggregates of different size of Pchlide:LPOR:NADPH complexes were found only within the highly organized lipid structure (prolamellar body, PLB). A short light pulse induces conversion of Pchlide:LPOR:NADPH complexes to Chlide:LPOR:$NADP^+$ ones. Then, Chlide molecules leave the complexes which is accompanied by the disappearance of the PLB structure. Molecular arrangement of pigment:LPOR:NADPH complexes within the PLB structure and their interaction with the environment have not been elucidated yet.

In this study, isolated EPIM or purified PLBs from wheat (*Titicum aestivum*) were solubilised using n-octylglucoside and Triton X-100. Fluorescence spectra at 77 K, and 295 K and fluorescence lifetimes at room temperature were examined for solubilized Pchlide:LPOR:NADPH complexes for different experimental conditions (time of detergent treatment, detergent concentration). In general, a shift of the fluorescence band from 655-657 nm, characteristic for highly aggregated Pchlide:LPOR:NADPH complexes, to 630-635 nm, originating from Pchlide dissociated from the complexes was observed at 77 K. Room temperature spectra were more complicated and were deconvoluted into Gaussian components. Fluorescence lifetime analysis revealed

two components: one was lower than 1 ns and the other was between 2 and 4 ns depending on the experimental conditions. The short light pulse resulted in appearance of the Chlide fluorescence band having maximum between 676 and 688 nm, which depended on solubilization conditions. The appearance of the new fluorescence band was accompanied by the disappearance of the respective Pchlide fluorescence band. Fluorescence lifetimes of the newly formed Chlide were significantly longer than those observed for Pchlide. These experiments allowed us to characterize fluorescence properties of Pchlide (or Chlide):LPOR:NADPH complexes of different size and compare them to the data obtained for isolated PLB (Myśliwa-Kurdziel et al., 1999) and etiolated seedlings (Myśliwa-Kurdziel et al., 2003).

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1.26.

The unique features of rhizophore growth in *Selaginella kraussiana*

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Rhizophores are leafless dichotomizing axial organs found exclusively in the genus *Selaginella* P. Beauv. (Selaginellaceae, Lycophyta). Their mode of growth has not been examined so far. In our research we showed that the new twin apices are formed by endogenous initial cells which divide few times giving rise to hidden primordia. Peculiarly, these primordia are of adventitious roots, what remains in contrast to typical dichotomy where original axis and both twin ones are organs of the same identity. Hidden primordia are temporarily inhibited in development within the original axis while the latter one keeps growing due to activity of the intercalary meristem.

The intercalary meristem is located basally to hidden primordia at the rhizophore apex. In this zone, intensive divisions, which are perpendicular to the long axis of the organ and result in the ladder-like pattern of short cells, occur in epidermis, cortex cells and procambium.

Dichotomy becomes macroscopically visible when hidden primordia emerge. At the base of each primordium new intercalary meristem develops. Together with the intercalary zone of the rhizophore they contribute to the growth of new axes.

Our data show specific aspects of the rhizophore growth. Limited divisions of root initial cells and transient developmental arrest of hidden primordia force the growth to be located subapically and performed mostly by derivatives of the previous axis instead of newly formed apices. Furthermore, the mother axis contributes significantly to the growth of dichotomous twin apices during their emergence and initial elongation. Based on results we show the uniqueness of the rhizophore dichotomous branching that is exceptional comparing to other known dichotomously branched organs.

1.27.

Proteomic approach to analyze dormancy breaking of silver fir (*Abies alba* Mill.) seeds

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Seed dormancy is controlled by the physiological or structural properties of a seed and the external conditions. It is induced as part of the genetic program of seed development and maturation. Seeds with physiological embryo dormancy can be stimulated to germinate by a variety of treatments including cold stratification. Investigation of proteins, product of genes activated during a complex developmental process as is dormancy breaking was the aim of presented research. Proteomics offers the opportunity to examine simultaneous changes and to classify temporal patterns of protein accumulation occurring during seed dormancy breaking and germination.

These studies were carried out on seeds of silver fir (*Abies alba* Mill.), a gymnosperm conifer plant, during their stratification and germination. After imbibition in water, seeds were subjected to cold stratification in 3°C, which breaks dormancy. To accelerate the germination capacity seeds were moved after 6 weeks of stratification to 20°C. The protein extraction was done separately for embryo and megagamethophyte of the seeds covering the period of stratification in 3°C and germination in 20°C. The process of seed germination was analysed at the stages corresponding to the dormant seeds, seeds after 48-hour imbibition, seeds during each week of stratification and germinated seeds with protruded roots.

Regarding the proteomic approach, proteins of the seeds were separated by 2D-gel electrophoresis and were analyzed by mass spectrometry. The influence of stratification was investigated and main protein variations for embryo an megagamethophyte were pointed out. Analysis of the functions of the identified proteins and the related metabolic pathways would expand our knowledge about gymnosperm seeds dormancy breaking.

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1.28.

Structure and evolution of the carnivorous plant genus *Heliamphora*

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The family Sarraceniaceae consists of three genera of carnivorous plants from the New World (*Darlingtonia*, *Heliamphora*, *Sarracenia*). The genus *Heliamphora* currently comprises 15 species and is restricted to the tepuis and plateaus of Venezuela, and adjacent parts of Brazil and Guyana. The aims of our studies were to investigate distribution, morphology and anatomy of *Heliamphora* nectaries in phylogenetic frame. We examined several species including *H. exappendiculata, H. sarracenioides, H. minor, H. ionasii, H. heterodoxa, H. chimantensis, H. folliculata*.

All species of *Heliamphora* studied, with exceptions of *H. exappendiculata* and especially *H. sarracenioides*, form a distinct nectar appendage (nectar spoon) on the pitcher apex. Most of the giant nectaries in *Heliamphora* are located in the nectar appendage, some of them occur also in the external pitcher wall. The giant extrafloral nectaries are a main source of nectar, which contains also scent compounds. Thus these nectaries display a double role, and nectar appendages act as the extra-floral osmophores. The ultrastructure of the nectaries was studied in detail.

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1.29.

Activity of PLA_2 in *Solanum* species treated with elicitor from *Phytophthora infestans*

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Plant phospholipases A (PLAs) are involved in defense strategy since products of PLA activity function in signal transduction and serve as substrates for oxylipin biosynthesis. In studies on lipid peroxidation induced by elicitor from *Phytophthora infestans* in *Solanum* species we have found a decrease in linoleic (LA), linolenic (LnA) and hexadecatrienoic acid in leaves treated with the elicitor. In plant phospholipids, LA and LnA dominate the sn-2 position so they are released by phospholipase A_2 (PLA_2).

In the present study we have examined PLA_2 activity in leaves of *Solanum tuberosum* cv Bzura and clone H-8105 and *Solanum nigrum* var. *gigantea* that exhibited field resistance, susceptibility and non-host resistance, respectively, in response to the P. infestans. The leaves from *in vitro* grown plants were treated with elicitor, culture filtrate (CF) from *P. infestans*. The PLA_2 activity was determined by spectrophotometric method.

The PLA_2 activities in CF treated leaves from all genotypes differed in respect of intensity and kinetics. In non-host *S. nigrum* the PLA_2 activity reached a higher level than in Bzura and H-8105 and remained elevated during the whole time of experiment. In the susceptible H-8105, PLA_2 activity after a transient increase reduced to the level of control.

To study role of PLA_2 in response to CF, we have used inhibitor of Ca^{2+}-independent PLA_2, haloenol lactone suicide substrate (HELSS). The PLA_2 activity was assayed *in vivo* – detached leaves were incubated in HELSS and *in vitro* – HELSS was added into enzymatic extracts from control and HELSS treated leaves. In all genotypes, *in vivo* the inhibitory effect of HELSS was not dose dependent whereas *in vitro*, PLA_2 activity decreased markedly in the highest dose of HELSS, independently of the previous treatment. Then effect of HELSS in chosen dose *in vivo* and *in vitro* on PLA_2 activity in control and CF treated leaves was examined. Preincubation of leaves in HELSS before CF treatment resulted in a marked inhibition of PLA_2 only in *S. nigrum*. *In vitro*, HELSS added to the enzyme extracts from CF treated leaves and those preincubated in HELSS inhibited PLA_2 activity in resistant genotypes.

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1.30.

Environmental engineering with bamboo: options for Europe?

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Most attention in Western Europe goes to the classical bionergy crops poplar, willow and *Miscanthus*. However, several other plants are useful candidates to provide biomass for the production of electricity, bamboo being most prominently among them. Bamboo is one of the fastest growing sources of biomass on earth with an unsurpassed regeneration potential, and several agricultural techniques and technologies have already been adapted to suit large scale bamboo cultivation.

Of course, it can be doubted whether bamboo, which is more known to grow under (sub)tropical conditions, can be grown successfully in a colder European environment. So far, pilot tests of

bamboo growth in Ireland and Flanders (Belgium) show that bamboo produces enough biomass, even under a rather adverse climate, to make the plant economically an equal investment as the classic crops. Moreover, bamboo tends to have a higher capacity for uptake of heavy metals than poplar and willow, offering possibilities for an efficient combination of biomass production and heavy metal phytoremediation. The identification and isolation of several natural endophytic bacteria offers even the possibility, through well-targeted genetic engineering, to turn the combination of plant and bacteria into an instrument to degrade organic pollutants in situ.

This makes bamboo a useful crop to be grown on marginal soils, offering two distinct advantages: the production of bio-energy without having to compete with food and feed production, and the economic revalorisation of polluted and less fertile fields and areas.

1.31.

Molecular cloning and expression analysis of main gliadin-degrading cysteine endopeptidase EP8 from triticale (× *Triticosecale* Wittm.)

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During development and maturation of cereal grains storage proteins are accumulated in the starchy endosperm. These proteins are a source of amino acids indispensable for growth of new plant. The enzymes responsible for mobilization of reserve proteins during seed germination are cysteine endopeptidases and serine carboksypeptidases. In our previous study we purified from germinating triticale kernels the major gliadin-degrading cysteine endopeptidase EP8. We confirmed in an experiment with exogenous GA_3 that this enzyme is synthesized in aleurone during germination. Mass spectrometry analyses of purified EP8 preparation shows that endopeptidase EP-A from barley is the nearest homologue of EP8. We cloned, based on RT-PCR (reverse transcriptase-polymerase chain reaction), part of sequence encoding EP8. The cDNA clone has high identity to endopeptidase EP-A. RACE (rapid amplification of cDNA ends) method let as to clone 3'end of cDNA encoding EP8. The *EP8* transcript level was analysed by semi-quantitative RT-PCR method. Preliminary results indicate the highest expression of *EP8* on the second and third days of germination in the *scutellum* and aleurone layer respectively. It correlates with the activity of EP8 detected after native electrophoresis on gels with copolymerized gliadin as a substrate. Obtained results might suggest that EP8 is synthesized *de novo* during first days of germination.

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1.32.

Principal growth directions in the development of *Arabidopsis thaliana* petals

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The continuous and coordinated (i.e. symplastic) growth of plant organs is known to be of a tensor nature (Hejnowicz and Romberger, 1984). Its unique features are the principal directions of growth (PDGs) that are three mutually orthogonal directions along which growth rates attain extreme values. Numerous studies have shown that PDGs manifest themselves in cellular patterns – they affect the orientation of cell divisions and arrangement of cell packets.

Petals, like other plant organs, grow symplastically. The question arises whether the influence of PDGs is manifested in their structure. We investigated the cellular pattern in the epidermis of *Arabidopsis thaliana* petals in different developmental stages. The adopted staining enabled us to discern the cell packets on the basis of differences in wall thickness. In general, the packets develop symmetrically along the proximo-distal axis producing a fountain-like pattern. For further investigation of petal growth we used the sequential replica method (Dumais and Kwiatkowska, 2002). The comparison of cellular pattern in the same petal in two different stages allowed us to evaluate the directions of maximal and minimal growth, i.e. strain, for individual epidermal cells (protocol by Goodall and Green, 1986). The computed directions correspond to the arrangement of cell packets in petal epidermis. We performed simulations of the growth of *Arabidopsis* petal by means of the tensor method with the assumption that the distribution of PDGs trajectories is symmetrical and resembles both the computed growth directions and fountain-like cellular pattern. The tensor field of growth rates was specified and a model of growth of the whole petal was developed. We used the same field and tensor method to simulate also the expansion and divisions of individual cells, so that we were able to generate cell packets in different parts of the petal. The performed simulations accurately depict changes in shape and size of the whole petal as well as the cell packets. Our results demonstrate the significance of PDGs during petal morphogenesis and growth.

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1.33.

Spatial and temporal distribution of arabinogalactan proteins in female generative organ of Gymnosperms

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Arabinogalactan proteins (AGPs) have been implicated in a variety of plant development processes including sexual plant reproduction. The complex structure of the sugar fraction of AGPs

makes them a potential source of signaling molecules. The ovule is an especially interesting organ to study processes of development and differentiation because it is composed of two totally different tissue types: sporophytic and gametophytic, which represent different ploidy levels and variable functions. In angiosperm plant *Arabidopsis thaliana*, AGPs are molecular markers for certain cell and tissue types in very precise stages of sporogenesis and gametogenesis.

The aim of this work was to analyze the spatial and temporal localization of AGPs in the maturing ovule of *Larix decidua* Mill. through the use of specific monoclonal antibodies (LM2, JIM4, JIM8 and JIM13) that bind to structurally complex carbohydrate epitopes. In the present research the pattern of distribution of different AGPs was investigated in successive stages of the ovule development – from megasporogenesis to the mature gametophyte stage.

AGPs are abundant in the ovule of *L. decidua* and show characteristic spatial pattern of distribution reflected in the presence of variable quantities of these proteins within the cells constituting a different region of the organ. During megasporogenesis the epitopes recognized by mAbs LM2, JIM8 and JIM13 were present throughout the whole ovule. Just after the end of meiosis the signal from JIM4 appeared only in functional and degenerating megaspores. At the stage of free nuclei in female gametophyte, labelling obtained with mAbs JIM8 and JIM13 was present in gametophyte and almost all nucellus cells without tapetal cells. Whereas epitopes recognized by mAb LM2 were detected only in the cytoplasm of free nuclei. JIM4 fluorescence was specific for tapetal cells. In a mature ovule high levels of AGPs were observed especially in the nucellus. Epitopes recognized by mAbs LM2, JIM8 and JIM13 were also detected in the cytoplasm of the egg cell.

The results obtained during the research show that the differentiation of generative and somatic tissues of the gymnosperm plants ovule is reflected in the changes in the composition of AGPs.

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1.34.

Identification of wheat embryo mRNAs expressed in response to abscisic and gibberellic acid

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The study was performed using isolated wheat embryos which were incubated in either 100 μM abscisic acid (ABA) or 10 μM gibberellic acid (GA_3). The embryos collected 6, 12, 24 and 48 hours after hormonal treatment as well as untreated controls were used for RNA isolation and differential display (DDRT-PCR) analysis of gene expression. Using 18 primers combinations (six different oligo[dT]NN primers and four random decamers) 41 ABA-induced and 33 GA_3-induced cDNA fragments were identified. Six of these fragments were cut out from the gel, re-amplified and T/A-cloned. Sequence analysis of two cloned ABA-specific cDNAs revealed homology to the genes coding for trehalose-6-phosphate phosphatase and serine palmitoyltransferase 1 (aminotransferase, class I and II). Three GA_3-induced mRNAs were highly similar to the gene for the putative gag-pol poliprotein and one to the surroundings of the *FL1* gene. Differential expression of the identified sequences will be verified using real-time PCR.

1.35.

Spatial changes in the degree of symplasmic continuity in the cambial region of *Acer pseudoplatanus* and *Populus tremula* × *tremuloides*

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The degree of symplasmic continuity, the functional aspect of cell-to-cell communication via plasmodesmata, is seasonally changing in a cambial region. It is high in a dormant cambium and generally low in an active one, as visualized by either free or limited movement of a symplasmic tracer between ray and fusiform initial cells (Sokołowska and Zagórska-Marek, 2007). Further, more detailed analysis of fluorescent dye distribution in a cambial region has revealed that the degree of symplasmic continuity changes not only in time but also in space. In active cambium the level of symplasmic communication, was either decreasing or increasing longitudinally from the site of dye application. In some cases the direction of the observed tendency altered suggesting that the changes were of a periodic character. It is also possible that for the certain time period the radial transport of fluorescent tracer from xylem rays to the cambium (the tracer had been loaded through the xylem) is more extensive in some areas of a cambial cylinder than in others. Do these spatial changes show any dynamics? Are they related to the other periodic patterns known to exist in cambium, such as domain pattern or auxin waves? Answering this questions will be possible soon, as the research is still in progress.

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1.36.

Production of haploids in oat (*Avena sativa* L.) by means of pollination with maize (*Zea mays* L.)

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Doubled haploids (DHs) became important in crop breeding because homozygous lines can be made in one generation. Haploid production is a basic step for obtaining DH plants. However production of oat DHs still remains inefficient in comparison with other crops, e.g. wheat or triticale. The aim of the study was to improve the method of oat haploid production by pollination with maize. Oat and maize plants were grown in a controlled conditions in a greenhouse (16h photoperiod, 21/17°C day/night). Nineteen oat genotypes (derived from polish breeding stations) differ in drought tolerance, concentration of starch and fat in grains were used in the experiment. Influence of two auxins: 2,4-dichlorophenosyacetic acid (2,4-D) and dicamba on growth and development of caryopsis and haploid embryos were investigated. Oat florets were emasculated 2 days before anthesis and after that they were pollinated with fresh maize pollen collected at

15 minutes intervals. Next 2,4-D and dicamba were applied to pistils of oat. The growth regulators showed significant differences in ovaries enlargement. These after dicamba treatment were bigger then those after 2,4-D, although there was no significant differences in embryos production. The frequencies of enlarged ovaries and excised embryos strongly differed between genotypes. In average 85% of pollinated by maize ovaries were enlarged and 9% of them produced haploid embryos. These embryos were rescued on the TL3 and 190-2 media modified with maltose. After ca 3 weeks 30.5% green plants were obtained from all embryos. There were no albino plants, which are frequently observed in androgenic cultures.

1.37.

Purification and characterisation of protochlorophyllide oxidoreductase A:NADPH from *Arabidopsis thaliana*

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The light-triggered reaction of Pchlide to Chlide reduction is the main step in chlorophyll biosynthesis pathway in Angiosperms. This reaction needs a special enzyme protochlorophyllide oxidoreductase (POR), which is light- dependent. Three isoforms of this enzyme: POR A, POR B and POR C were found in *Arabidopsis thaliana*. Knowledge about this proteins is still not sufficient (Masuda et al., 2003).

Open reading frame of *Arabidopsis thaliana* PORA was cloned into pGEX4T1 plasmid. The obtained vector pGEX4T1 PORA was transformed into *Escherichia coli* strain BL21 and protein was overexpressed with 1M IPTG for 4 hours at different temperatures. The process of purification of POR A was optimized by using sonication, lysozyme and GST affinity chromatography. The protein activity was investigated at each step of its purification, from the step of the supernatant from *Escherichia coli* lysate up to the samples of isolated proteins. Purified protochlorophyllide and NADPH was added to the reaction mixture. Fluorescence spectra at 77K were measured before and after a flash of light. A relative increase of the fluorescence intensity at around 680 nm was examined for chlorophyllide, which is the product of the reaction. A quantitative analysis of the amount of the product was performed for acetone extract of the investigated samples.

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1.38.

In search for the mechanism of C4 photosynthesis intermediate exchange between *Kranz* mesophyll and bundle sheath cells in grasses

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C4 photosynthesis involves cell-to-cell exchange of photosynthetic intermediates between the *Kranz* mesophyll (KMS) and bundle sheath (BS) cells. This was believed to occur by simple diffusion through plentiful plasmodesmatal (PD) connections between these cell types. The model of C4 intermediates' transport was elaborated over 30 years ago and was based on experimental data derived from measurements made at the time. Recent advances on plasmodesmatal ultrastructure throw doubt on these assumptions, so we proposed that simple diffusion-driven transport of C4 intermediates between KMS and BS cells through the plasmodesmatal microchannels is not adequate to explain the C4 metabolite exchange during C4 photosynthesis (Sowiński et al., 2008).

The aim of this study was to search on the alternative mechanisms, involving the participation of desmotubule and/or active mechanisms as either apoplasmic or vesicular transport. Six grass species were used as the experimental material: *Digitaria sanguinalis*, *Echinochloa cruss-galli*, *Panicum miliaceum*, *Panicum maximum*, *Bouteloua curtipendula* and *Eraggrostis neomexicana*. Plants were grown in a climate chamber (24°C/22°C day/night, photoperiod of 14/10 h) until 4-th or 5-th leaf stage. To evaluate participation of different mechanisms of transport in the C4 metabolite exchange we used several methods, including an *in vivo* method of ^{14}C-photosynthate transport, microautoradiography, immunogold cytolocalisation of proteins and confocal microscope observations.

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1.39.

Immunofluorescence of the microtubular cytoskeleton of the embryo-suspensor in *Alisma plantago-aquatica*

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The differentiation of the embryo-suspensor (consisting of basal cell and a few chalazal cells) in *Alisma plantago-aquatica* L. were investigated in comparison with the development of embryo-proper. After fertilization, the zygote divides once, giving rise to a smaller apical cell and a larger basal cell (BC). The BC undergoes no division, becomes much enlarged and forms the basal cell of the suspensor. The apical cell develops into the embryo-proper and chalazal suspensor. Changes in the pattern of organization of the microtubular cytoskeleton were visualized after preparation (Bohdanowicz et al., 2005).

During the initial stage of development of suspensor BC, the microtubules formed a delicate tubulin network. Some of micro-

tubules formed irregular bundles in the cortical cytoplasm of the cell. In the fully differentiated BC, the microtubules were found to localize from micropylar to chalazal apex of the cell. The microtubules formed a dense intricate network. At the micropylar pole of the suspensor BC, a large amount of tubulin material was present. Numerous of these tubulin filaments were distributed longitudinally or transversally to the long axis of the cell. The large nucleus of the suspensor BC was located centrally. The microtubules were observed distributed around the nucleus surface. A distinct tubulin arrays were localized at the chalazal pole of suspensor BC. At all stages of suspensor basal cell development in the embryo-proper cells an extensive cortical network of microtubular cytoskeleton was observed. These observations suggest that the microtubules may have a crucial role in the development of suspensor basal cell in *Alisma plantago-aquatica*.

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1.40.

Changes of CDPK genes expression during embryogenesis in *Panax ginseng rolC*-expressing cell culture 2c3

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Embryogenic 2c3 cell culture was obtained as the result of transformation *Panax ginseng* callus cells with *Agrobacterium rhizogenes rolC* oncogene. We determined that inhibitors of Ca^{2+}-channels ($LaCl_3$, verapamil, and niflumic acid) certainly lowered the quantity of 2c3 somatic embryos (Kiselev et al., 2008). It proved the influence of calcium-dependent signal system on plant embryogenesis. Protein kinases inhibitors W7 and H7 also lowered 2c3 somatic embryos quantity. Next, we analyzed *CDPK* genes expression, protein products of which are the main cytoplasmic calcium sensors in plant cell (Cheng et al., 2002). Changes of *CDPK* genes expression were analyzed using frequency analysis of RT-PCR products and real-time PCR (Kiselev et al., 2008). We used ginseng cell cultures with different *rolC* gene expression level (2c2- low *rolC*-expressing, 2cR2- high *rolC*-expressing), and different stages of 2c3 somatic embryo development. The expression of *CDPK* genes in 2c3 was the lowest among all analyzed cultures, and 1.2-1.5 times less comparing with the control callus culture. Further we analyzed the expression level of individual *CDPK* genes.

CDPK expression in 2c3 embryos lowered at the expense of inhibition of three gene subfamilies *PgCDPK1a*, *PgCDPK1b*, and *PgCDPK3a*, while the expression of *PgCDPK2b* and *PgCDPK2c* gene subfamilies increased. Further, *CDPK* gene expression was analyzed in 2c3 somatic embryo development at different stages. The expression level of *PgCDPK2d* subfamily members (*PgCDPK2d1, PgCDPK2ds*, and *PgCDPK2dL*) increased significantly at the early stages of 2c3 embryo development. Therefore we suggested *PgCDPK2d* members play a role in somatic embryos development. The kinase domain of these genes was subjected to insertions and deletions. The observed transcriptional and post-transcriptional modifications of *PgCDPK2d* genes could contribute to the formation of *rolC*-initiated somatic embryos. Ultimately, it was shown *rolC* gene expression and embryogenesis is capable to change expression of definite *CDPK* gene forms.

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1.41.

Bioinformatics analysis of four *Arabidopsis thaliana* proteins potentially involved in cytoskeleton-dependent vesicle trafficking

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An essential, yet still poorly understood, element of the cell wall-plasma membrane-cytoskeleton (WMC) continuum is its internal part. Here, the cytoskeleton, anchored to the walls, is responsible for many processes, among them endo- and exocytosis, organelle positioning, cytokinesis or membrane trafficking. For a long time the very existence of endocytosis in plant cells was neglected as the membrane trafficking against turgor pressure was considered impossible. However, recent numerous reports indicated clearly the dynamics of endocytic activities in plant cells, although the full knowledge about this process in plants is still far from complete.

For this research, four *Arabidopsis thaliana* proteins (At4g32160, At3g15920, At1g15240, At2g15900) were selected, which are suspected to play a role in cytoskeleton-dependent membrane trafficking. To predict the most probable functions of those proteins, several bioinformatics analyses were performed.

At4g32160 and At3g15920, contain two important domains: PX and SPEC/Coil-Coiled, and this might suggests that they are able to simultaneously interact with membranes and with cytoskeleton. Judging from the data on animals and yeasts cells, we assume that they might be responsible for endocytic vesicle mobility driven by actin polymerization. The main function of PX domain is the binding of membrane phosphatidylinositols, like PI(3)P or PI(4,5)P2. It seems that PXs present in At4g32160 and At3g15920 have all the required amino acids to fulfill such function. We also suppose that these domains have the ability to bind P(3)P, which is typical for the endocytic vesicles membranes. On the other hand, domain SPEC and coiled-coil region, are suspected to interact with the actin cytoskeleton.

The modular structures of two other proteins: At1g15240 and At2g15900 contain four domains: transmembrane domain, PXA, PX, and Nexin_C. The presence of Nexin_C domain suggests that these proteins belong to a family of sorting nexins, and are responsible for sorting of the endosomes. The occurrence of transmembrane and PX domains suggests in turn the putative connection with or the anchorage to the membranes. PXA domain is usually associated with PX, fulfilling similar functions.

A series of experiments with the use of functional genomics approaches to confirm and investigate further the functions of these four proteins is now under way.

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1.42.

Geometric relation between the size of apical meristem and the lateral organ primordia size is not the only factor determining floral phyllotaxis in *Magnolia*

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According to some models (Richards, 1948) the quality of phyllotactic pattern depends upon the ratio of organ size to the size of meristem (P/M ratio). Intrinsic mechanisms, balancing cell proliferation rate, cellular growth and the size of the cell poll sequestered for the future lateral organ, should be decisive for the stability of phyllotaxis. Any change in the proportion of these parameters should lead to phyllotactic transitions and to increase in phyllotactic diversity. These theoretical assumptions were put into test by studying floral meristems of *Magnolia*, already known to exhibit greater phyllotactic diversity than any other plant model system (Zagórska-Marek 1994).

Floral apices representing four taxa of *Magnolia: M.* × *salicifolia, M. stellata, M. denudata* and *M. acuminata*, were collected at different stages of their development and measured. Our study revealed that the two parameters: the primordia size and the size of the apex itself, changed considerably together with the developmental phase of flower growth. The elements of perianth, usually positioned in trimerous whorls, were initiated as large primordia on the relatively small meristem. Initiation of very small stamen primordia followed, accompanied by the abrupt increase in the meristem size. Small values of P/M ratio contributed to the exceptional richness of phyllotactic patterns identified in this zone. At the moment when the size of the meristem started to diminish, due to the determinate character of the apex growth, the primordia of carpels started appearing. These were bigger than the preceding stamen primordia, and arranged usually in a new, specific manner, bringing up entirely new spectra of patterns. Values of P/M ratio calculated for each meristematic zone with the specific identity of floral organs, were characteristic for each investigated taxon, but comparison of these values with theoretical data surprisingly showed much greater variability of natural phyllotactic patterns than theoretically predicted. This was especially clear for a carpel zone. Our results imply that besides the ratio of the organ primordia size to the size of the meristem, there are other factors, perhaps even different, than those of purely geometric nature, which determine the initiation site and consequently the pattern of primordia spacing on the meristem's organogenic surface.

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1.43.

ABA regulation of InFCA transcriptional activity in *Pharbitis nil*

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Experiments carried out in a short-day plant *Pharbitis nil* have been allowed to identify a number of genes involved in flowering mechanisms. Apart from the photoperiodic pathway elements, the genes involved in the functioning of autonomous pathway, among them *InFVE* (*multicopy suppressor of ira 1*) and *InFCA* (*flowering locus ca*) were also described. The function of the protein encoded by them is repression of transcription activity of the key flowering inhibitor *FLC* (*flowering locus C*). Two separate mechanism of this repression has been described on the basis of experiments in *A. thaliana*. In one of them FCA interacts with another protein FY (FLOWERING LOCUS Y) causing inadequate premature splicing and polyadenylation of *FLC* transcript. The results obtained from *A. thaliana* investigations have been suggested that this process is also affected by a plant hormone ABA. Therefore we decided to study the effect of this hormone on expression of homologous gene to *FCA* isolated from *Pharbitis nil*.

Expression pattern of *InFCA* has been examined during inductive photoperiod in the cotyledons of 5-day old seedlings treated at the begging of darkness with 1 mM ABA or NDGA an inhibitor of abscisic acid biosynthesis. Effect of ABA on the expression of *InFCA* in seedlings cultivated onto subinductive conditions has been also investigated. A significant increase in expression level of *InFCA* after ABA application in both inductive and subinductive conditions suggests participation of this hormone in the control of transcriptional activity of *InFCA*. The results obtained suggest the ABA participation in the controlling of the *InFCA* transcription activity.

1.44.

Activity and transcript level of aldehyde oxidase during embryo development and seed maturation of *Pisum sativum*

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Aldehyde oxidase (AO; EC 1.2.3.1) is a molybdenohydroxylase that catalyzes the last step of two plant hormones biosynthesis, that are indole-3-acetic acid (IAA) and abscisic acid (ABA). Both hormones regulate processes during pea embryo development (1-17 DAA; days after anthesis) as well as seed maturation (20-42 DAA). In developing pea seeds, five AO activity bands (AO-1,2,3,4,5) were detected after native PAGE with indole-3-aldehyde as substrate. Only three of them oxidized abscisic aldehyde (AO-3,4,5). AO-1 was present at the highest activity level from 2 to 10 DAA, slightly lower from 11 to 13 DAA and disappeared at 17 DAA. AO-3 activity was low at 1-3 DAA, gradually increased to maximum level at 9 DAA (early-cotyledon stage), subsequently gradually decreased to a very low level at 20 DAA, and appeared again at 35 DAA. AO-4 and AO-5 activity bands were present only

from 20 DAA, when protein reserves started to accumulate. While AO-4 was present in mature dry seeds, AO-5 disappeared when intense desiccation took place (35 DAA). During embryo development the AO activity was almost exclusively observed in seed coat. When the seed maturation started, the seed coat revealed only AO-3 activity band, cotyledons AO-4 and AO-5 bands, while the embryo axis AO-3 band. The transcript level of three *PsAO* genes was examined during seed development by RT-PCR. The expression profile of *PsAO-3* coincided with the activity changes of AO-3 isoform and the product of heterological expression of *PsAO-3* gene in *Pichia pastoris* oxidized the abscisic aldehyde, thus it is predicted that *PsAO-3* gene encode the homodimeric AO-3 isoform. The transcript levels of *PsAO-1* and *PsAO-2* genes were the highest during embrogenesis, and gradually disappeared by day 20 DAA for *PsAO-1*, and by day 35 DAA for *PsAO-2*. Considering the obtained results it can be assumed that AO-3 isoform is most probably implicated in synthesis of ABA involved in embryogenesis and subsequent arrest of embryo growth. AO-4 isoform might be implicated in synthesis of ABA engaged in storage reserves accumulation, while AO-5 and AO-3 synthesis of ABA responsible for acquisition and maintenance of desiccation tolerance and of seed dormancy. AO-1 isoform most probably is implicated in biosynthesis of IAA regulating embryogenesis (1–8 DAA) and subsequent embryo growth (9–17 DAA).

1.45.

Nuclear activity in the sperm cells of *Hyacinthus orientalis* L. Does sperm cell nucleus carry to zygote anything else beside chromatin?

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The transcriptional state and distribution of splicing machinery elements and rRNA were investigated in sperm cells and zygote of *Hyacinthus orientalis*. The sperm cells were obtain *in vitro* growing pollen tubes and the zygotes, after pollination of the pistils. Nascent transcripts were detected using immunofluorescence techniques followed the incubation of the material with BrU. Pre-mRNA splicing molecules (TMG snRNA and SC35 protein) were localized using immunofluorescence techniques. Transcripts of rRNA were detected using fluorescence in situ hybridization (26S rRNA probe and ITS rRNA probe).

The obtained results have shown that the division of the generative cell occurred in pollen tubes after 8–12 h of growth in the medium containing molecules from pollinated pistils. Just after their origin, the young sperm cells were shown to be transcriptionally active, although the level of the nascent transcripts was markedly lower than in pollen tube nucleus. In the nucleus of both, the pollen tube and sperm cells, molecules involved in pre-mRNA maturation have been found. At later steps of pollen tube growth (24–36 h) both, vegetative and sperms nuclei were transcriptionally silent and elimination of the splicing machinery elements was indicated. These results suggest that in the sperm cells pre-mRNA maturation process proceeds. Analysis of the ITS rRNA and 26S rRNA localization showed that the sperm nuclei are devoid of the nucleolus.

Just after fertilization the restart of RNA synthesis and high levels of splicing molecules were indicated in the zygote. At this time of development in the nucleolus of the zygote low level of rRNA nascent transcripts was detected only around NOR. During first embryonic interphase the second nucleolus was forming and increased level of ITS and 26S rRNA was observed.

Basing on these results we postulate that in *H. orientalis*: (1) sperm cells don't deliver into zygote any male transcripts, splicing molecules and nucleolar molecules, (2) zygotic genes are activated just after fertilization and factors for the processing of new "somatic" transcripts are synthesized further in the development, (3) the activation of male NOR genes take place after restart of the nuclear transcription.

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1.46.

Hydroxyurea-induced replication stress up-regulates cyclin B1 and creates intrachromosomal gradients of chromatin condensation

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Although root meristem cells of *Allium cepa* exposed to the prolonged incubation with 0.75 mM hydroxyurea (HU; an inhibitor of ribonucleotide reductase) accumulate mainly in late S- and G2-phases of the cell cycle (as evidenced by Feulgen-DNA cytophotometry), a small amount of elongated cells with atypical mitotic divisions has been continuously observed within the whole period of replication stress, some of them indicating apparent symptoms of premature chromosome condensation (PCC). Induction of the PCC-like state is restricted merely to a subpopulation of cells with almost completely replicated DNA. While a considerable drop in the frequency of M-phase cells reflects the secondary effect of the block imposed upon DNA synthesis (S-phase checkpoint), a minor fraction of PCC cells has to be viewed as a consequence of mechanisms which override the DNA stress-response pathway and permit to proceed towards premature mitosis regardless of incomplete nuclear DNA replication. Together with cells showing typical features of an unscheduled mitotic division, some PCC-cells reveal spatio-temporal gradients of chromatin states characteristic for various periods of S-phase (evidenced using BrdUrd) and an abnormal pattern of mitotic condensation, characterized by a gradient of chromatin states (spanning within individual chromosomes from interphase to metaphase). While anti-β tubulin-stained PCC-cells revealed mitotic spindles with regular arrays of microtubules, the most common site of preprophase bands in cells showing progressive gradients of chromatin condensation correlated well with a discrete area of chromatin positioned close to the transitory region between the decondensed nucleoplasm and the structures typical for initial stages of prophase.

To elucidate an unusual mode of mitotic behavior in cells exposed to prolonged HU-treatment, there is a need to accept functional predominance of mitotic activators (such as cyclin-dependent kinases) over those factors (such as ATM and ATR kinases), which efficiently promote replication checkpoint control mechanisms to prevent cells from entering nuclear division. By using immunocytochemical methods, we have demonstrated that, prolonged HU-treatment (compared with the control cells), brings about a considerably increased accumulation of cyclin B1 and that intracellular gradients of this protein may provide direct link between a specific spatio-temporal distribution of the threshold concentration of the active M-phase CDKs and a wide range of structural changes extending from interphase to mitosis.

Session 2

Plant-microbial interactions

PLENARY LECTURES

2.1.

Using pathogen effectors to investigate host resistance mechanisms

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Plant pathogens use small molecules and also proteins to render their hosts susceptible. Many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial type III secretion system (T3SS) to investigate effectors from filamentous pathogens such as oomycetes. We are using T3SS delivery of oomycete effectors from *Pseudomonas* sp. to investigate the effector complement of the downy mildew pathogen *Hyaloperonospora parasitica* (Hpa). I will report recent data on Hpa effector functions and on the use of the Solexa/Illumina sequencing instrument to advance our understanding of Hpa pathogenicity. We are using Illumina paired read sequencing and Velvet software (Zerbino and Birney, Genome Research, 2008) to assemble sequences of multiple races of another oomycete pathogen, *Albugo candida*, which is particularly effective at shutting down host defence. The analysis of its effectors is likely to provide very interesting new insights into host defence mechanisms. In addition, we are using T3SS delivery of oomycete effectors to investigate the molecular basis of pathogen/host specificity and non-host resistance. An update on recent progress will be presented.

2.2.

Dissecting grass-endophyte literature

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Endophyte-grass symbiosis is considered to be a classic example of microbe-plant symbiosis in which the fitness of the microbial symbiont and its host plant are closely linked, and thus, presumed to align the interests of partners toward mutually beneficial cooperation. In the symbiosis fungal hyphae grow intercellularly and asymptomatically throughout the above-ground tissues of the host grass. While growing into the developing inflorescence and seeds, the fungus is vertically transmitted from maternal plant to offspring. Grass endophytes are generally considered to be mutualists because (i) the fungus subsists entirely on the host's resources, and the fitness of an endophytic symbiont that has lost or limited opportunities for contagious spread by spores depends largely on fitness of the host plant, and (ii) the host receives benefits through increased resistance to herbivores, pathogens and drought and flooding stress, and enhanced competitive abilities. Using meta-analyses, I reveal that the reasons for the strong mutualistic stamp of endophytes is, however, largely historical and system-based. Although ecological, evolutionary, mycological and agronomical studies involving fungal endophytes in grasses have proliferated within past decades, the conceptual framework for endophyte-grass interactions has largely been based on endophyte-plant-herbivore studies of two, economically important, artificially selected and introduced agricultural grass species, tall fescue and perennial rye grass. Consistent with conventional wisdom, the meta-analysis indicates that endophytes slightly increase grass resistance to herbivores. However, endophytes appear not to affect plant performance or competitive ability. Furthermore, the positive effects of endophytes on the host grass appear to be dependent on genetic variation in the host and endophyte, and be more pronounced in high nutrient soils. These results suggest that the agronomic grass model systems fail to capture the breadth of variability inherent in the wild grass-endophyte populations and communities. I propose that endophyte-grass interactions are much more complex than described in past literature. Like any other biological species-species interactions, endophyte-grass interactions may involve selfishness, cheating and power-struggle between the partners leading to a continuum of the interactions from antagonistic to mutualistic and occasional mutualism breakdown.

2.3.

Plant-microbe interaction in the rhizospere – a metabolomic approach

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Plants and microbes communicate in the rhizosphere via secreted small molecules. A non-targeted metabolomic approach was applied to analyze the complex pattern of compounds possibly involved in interorganismic signaling. *Arabidopsis thaliana* seedlings were co-cultivated in hydroponic culture with the mutualistic root endophyte, *Piriformospora indica*, or the pathogenic Oomycete, *Phytophthora infestans*, for which *Arabidopsis thaliana* is not a host plant. Upon co-cultivation for two weeks, methanolic extracts were prepared from roots and leaves of the plantlets, as well as of fungal and Oomycete mycelia. In addition, the culture medium was reduced and pre-fractionated by solid-phase extraction. All extracts were analyzed by non-targeted metabolite profiling using UPLC-ESI-QTOF-based mass spectrometry, which has the potential to detect a broad spectrum of mostly secondary metabolites. Differential metabolite patterns were detected in culture media, roots and mycelia of co-cultivated versus individually grown plants and fungus or Oomycete. Most recent results on the metabolite-based analysis of these mutualistic and pathogenic interactions will be presented.

ORAL PRESENTATIONS

2.4.

Relationships between arbuscular mycorrhiza development, NtEXPA5 expression and microtubules rearrangement in *Nicotiana tabacum* L. mycorrhized roots

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Arbuscular mycorrhiza (AM) is the widespread mutualistic symbiosis in plant kingdom, known to increase nutrient uptake from the soil and increase plant resistance against biotic and abiotic stressors. In these associations, a key structural process is the development of arbuscules, where bidirectional exchange of phosphorus and carbohydrates take place. As fungi hyphae are separated from the host cell cytoplasm by a host-derived membrane and interfacial matrix, this apoplastic compartment besides the role in nutrient transfer must play also a role in controlling fungi spread inside the root cells. We investigated the spatial pattern of microtubules – cytoskeleton elements strictly connected with cell wall synthesis and immunolocalized an expansin NtEXPA5, extracellular protein modifying the cell wall matrix. During AM development microtubules have been tightly correlated with large arbuscule branches. Expansin has been immunolocalized mainly in the interfacial matrix of growing hyphae tips, arbuscule ramification, and around small, active arbuscule branches. This results point to not only an important role of both expansin and microtubules in proper AM development, but also suggests that microtubule rearrangement and expansin distribution are in some way correlated.

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2.5.

Full-size ABC transporters from the ABCG subfamily in *Medicago truncatula*

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ATP binding cassette (ABC) proteins occur in all living species. Most of them are involved in a trans-membrane transport of a great variety of molecules. These proteins possess a well defined modular type of organization and distinctive domains which are well conserved among all family members. Full size ABC transporters belonging to the ABCG subfamily are unique for plants and fungi. There is growing evidence that certain of these proteins play a role in plant defense or signaling systems. Genes encoding full size ABCG proteins in *Arabidopsis thaliana* and *Oryza sativa* were tentatively organized into several groups (Jasiński et al., 2003). Although legumes are the third largest plant family in the world and the second most important crop family, our knowledge concerning legume ABC transporters is limited. Recently, domain-based clustering analysis made for *Lotus japonicus* has predicted the presence of at least 12 ABCG proteins in this organism (Sugiyama et al., 2006). Here we identify and classify 19 genes coding full size ABCG proteins in *Medicago truncatul*a (Jasiński et al., 2009). We found that majority of these genes are well expressed in roots. Expression of several is induced upon pathogenic or symbiotic infection and a few are expressed in specific for legume root nodules. The data presented are the first glimpse of evidence concerning full size ABCG transporters in this model legume plant thus providing a scaffold for further studies of their physiological function and possible role in the modulation of plant interactions with other organisms.

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2.6.

Development of syncytia induced by potato cyst nematode in roots of transgenic plants with silenced expression of susceptibility genes

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Potato and tomato lines with enhanced resistance to *Globodera rostochiensis* obtained via post-transcriptional silencing of *cel7* and *cel9C1* endoglucanase genes and *LeEXPA5* expansin gene were generated. These plants were used for microscopic examinations to analyse cytological and histological patterns of syncytium development.

In all examined transgenic lines syncytia were induced in cortical parenchyma cells and spread toward vascular cylinder where pro/cambial and pericyclic cells were incorporated. In *cel9C1*-silenced potato line all parenchymatous vascular cylinder cells were incorporated into syncytium at 7 DAI. No divisions of pericyclic cells leading to neither the formation of regular peridermis-like cover tissue surrounding syncytia, nor differentiation of pro/cambial cells into new conductive elements occurred around syncytia. Syncytia were relatively small and composed of several slightly hypertrophied cells. Syncytial elements were interconnected by few and narrow cell wall openings. At the ultrastructural level syncytial cytoplasm proliferated and became electron dense, but central vacuoles were usually preserved. Syncytial nuclei enlarged and acquired amoeboid shapes. Syncytial cell walls remained thin, but at different locations irregular cell wall depositions and numerous paramural bodies were formed. Similar reactions were also observed in *cel7*- and *LeEXPA5*-silenced lines, but fewer cells were incorporated into syncytium and peridermis-like layer was formed. In contrast to syncytia induced in endoglucanase-silenced lines, in *LeEXPA5*-silenced lines syncytial cytoplasm became electron-translucent and necrotised since 5 DAI.

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2.7.

Specific phosphorylation of conserved eukaryotic protein SGT1 is an essential element of plant pathogen defense

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SIPK, a MAP kinase that is rapidly activated in response to pathogen challenge, is involved in induction of defense-related genes and HR cell death. The ubiquitin ligase-associated co-chaperone protein SGT1 has been shown to be required for plant immune responses, cell death and hormone signaling. Despite the knowledge that both SGT1 and SIPK orthologs are required for R-gene mediated resistance, the functional relationship between these two signaling molecules has remained unexplored. Using mass spectrometric (LC-MS-MS/MS) analysis, we showed that in tobacco SGT1 undergoes specific phosphorylation by SIPK in the canonical MAPK target-SP-motif. Heterologous transient complementation assay in *Nicotiana benthamiana* was used to study the significance of this phosphorylation in the ability of plants to mount effective defense against pathogen infection. Site-directed mutations that alter this motif result in impaired TMV resistance, indicating a functional role for SGT1 phosphorylation in controlling resistance to viral infection. Consistent with this, transgenic tobacco plants that are altered in SIPK expression are also compromised in their resistance to TMV.

The phosphorylation site lies within a highly conserved domain of SGT1 referred to as SGS (SGT1-specific), the disruption of which leads to loss of many SGT1 activities in plant stress signaling. The same canonical MAPK phosphorylation motif is present in most known plant SGT1 proteins, and in the yeast *Saccharomyces cerevisiae* Sgt1, but is absent from the metazoan orthologs. Phosphorylation can affect various properties of proteins: subcellular localization, ability to form complexes, stability, conformation, binding of divalent cations. Currently, we explore which of them are regulated by posttranslational modification of SGT1 and may be related to SGT1 function in plant defense signaling.

POSTERS

2.8.

Induction of certain defense-associated genes is dampened during immunization of tobacco with hydrogen peroxide against localized viral symptoms

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The hypersensitive type of resistance (HR) to plant pathogen infections is associated with the accumulation of reactive oxygen species (ROS). Earlier we have demonstrated that low concentrations (5–10 mM) of hydrogen-peroxide (H_2O_2) can suppress cell- and tissue death caused by various biotic stresses in tobacco (Hafez et al., 2004).

Here we show that immunization of tobacco to local symptoms caused by *Tobacco mosaic virus* (TMV) with low concentrations of H_2O_2 results in suppression of the number and size of HR-type necrotic lesions. On the other hand, viral concentrations do not change in the H_2O_2-treated plants as assayed by ELISA. Furthermore, immunization of tobacco with H_2O_2 dampens the induction of certain defense-associated genes that encode proteins necessary for the storage of salicylic acid (SA), a plant resistance inducer. Induction of this type of genes, such as a SA methyltransferase (*NtSAMT*) and an UDP-glucose:SA glucosyltransferase (*NtUDPG-Tr*) during TMV-infection is considerably weaker in H_2O_2-treated plants than in control (water-treated) plants as assayed by qRT-PCR. This could possibly indicate that immunization with H_2O_2 against a localized viral infection may operate through increased mobilization of free (unbound) salicylic acid.

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2.9.

Draft genome sequence analysis to identify regulatory mechanism of Type III Secretion System in *Pseudomonas syringae* pv. *tabaci* strain IBB1

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Pseudomonas syringae is a model plant pathogenic bacterium which uses Type III Secretion System (TTSS) to inject effector proteins and others virulence factors into host cells. The effectors serve as factors disrupting cell defence and metabolism to benefit bacterial growth. In our previous studies, using LC-MS-MS/MS profiling, we have identified a strain of *P. syringae* pv. *tabaci* (referred to as IBB1) that is capable to secrete 17 effectors in culture, while other *P. syringae* strains tested secrete only a few effectors at the same conditions.

To find factors contributing to this phenomenon we decided to analyze genome sequence of the interesting strain. The sequencing was performed using 454 Life Sciences (Roche) technology. The assembling process yielded: sequence of chromosomal DNA 5,93 Mbp in size, with near 100% coverage in comparison with previously published sequence of *P. syringae* pv. *phaseolicola* 1448A (Ac no. NC_007273) as a reference sequence, sequence of plasmid DNA (near 100% coverage, Ac no. NC_005773) and small part of sequence of a second plasmid (Ac no. NC_007275). Since the two *P. syringae* strains show important differences in pathogenesis process, we expected the repertoire of the effectors had diverged significantly. Surprisingly, the draft genome sequence analysis showed very strong similarity between IBB1 and 1448A strains, including the genes encoding effectors. Comparison of the genome sequences revealed, however, differences in the sequences coding for proteins belonging to TTSS regulatory machinery. Preliminary investigations indicate that those differences can underlie the capability of strain IBB1 to secrete large number of the effectors in the inducing medium. In our further research we address the question how the sequence divergence may affect the TTSS regulation.

2.10.

rosR and *pssA* genes are involved in exopolysaccharide production, symbiotic competitiveness and clover nodulation in *Rhizobium leguminosarum* bv. *trifolii* strains

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Rhizobium leguminosarum bv. *trifolii* produces large amounts of exopolysaccharide (EPS) which plays an important role in the establishment of effective symbiosis with clover plants. The *pssA* gene encodes the glucosyl-IP-transferase engaged in the first step of EPS repeating units synthesis (Janczarek et al., 2009). The *rosR* gene codes for a positive transcriptional regulator involved in the regulation of EPS biosynthesis (Janczarek, Skorupski, 2007). Mutation in *pssA* resulted in a total lack of EPS and *rosR* mutation substantially decreased the amount of produced EPS. Both mutants induced nodules on clover but the bacteria were unable to fix nitrogen. Defective functions of *pssA* and *rosR* mutants were fully restored by wild type copies of the respective genes. Introduction of multiple copies of *rosR* and *pssA* genes on the broad-host-range plasmid vector pBBR1MCS-2 into five isolates of *R. leguminosarum* bv. *trifolii* resulted in significantly increased growth rates, EPS production and the number of nodules on clover roots. Significant increase in fresh and dry shoot mass of clovers and nodule occupation was also observed. Additional copies of *pssA* and *rosR* genes caused nearly twofold increase of competitiveness of tested strains in relation to the wild type parental strains. Our data suggest that increased amount of EPS beneficially affects *R. leguminosarum* bv. *trifolii* competitiveness and symbiosis with clover.

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2.11.

Early flax response to *Fusarium*

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Fusarium is the most common flax pathogen causing serious plant diseases and in most cases leading to plant death. The identification of the plant genes, which respond to infection, and generation of suitable transgenic plants is the approach that has been used in this study. Forty-seven flax genes have been identified by means of cDNAs subtraction method as those, which respond to pathogen infection. Subtracted genes were classified into several classes and the prevalence of the genes involved in the broad spectrum of antioxidants biosynthesis has been noticed. By means of semi-quantitative PCR and metabolite profiling, the involvement of subtracted genes controlling phenylpropanoid pathway in flax upon infection was positively verified. The data from transgenic flax analysis confirmed protection effect of phenylpropanoid compounds against *Fusarium* infection. We identified the key genes of this compounds synthesis that enabled us to create plants with modified levels of phenylopropanoids thus confirming the important function of those genes in flax resistance to fungi infection. To our best knowledge this is the first report to describe genes and metabolites of early flax response to pathogens studied in a comprehensive way.

2.12.

Purification, molecular cloning and characterization of antimicrobial proteins from scots pine seedlings

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The expression of antimicrobial peptides (AMPs) is a universal mechanism by which plants respond to a wide spectrum of biotic and abiotic stress factors. The family of plant AMPs is very diverse and includes chitinases, ribosome-inactivating proteins, inhibitors of serine and cysteine proteases, lipid-transfer proteins, defensins and etc.

In this work we describe a novel protocol for the purification of antimicrobial peptides from Scots pine seedlings. Using cation-exchange chromatography on phosphocellulose column, we purified nearly to homogeneity two proteins which were identified by mass spectrometry as defensin and lipid-transfer protein. We demonstrated their growth inhibitory effects against a group of phytopathogenic fungi. We found that Scots pine defensin inhibited the growth of *Fusarium* sp., *Botrytis cinerea* and *Heterobasidion annosum* mycelium at concentration < 1 μM, and caused morphological changes of hyphae.

Furthermore, we report for the first time molecular cloning and characterization of defensin 1 cDNA from Scots pine. To elucidate the role of Scots pine defensin in plant's response to various environmental stresses we have produced recombinant protein in bacterial expression system. We found that the antifungal

activity of recombinant pine defensin is similar to that of endogenous protein. Finally, we have generated specific polyclonal antibodies against defensin that would allow us to study the expression of PsDef1 in different organs of Scots pine at various stages of development and in response to pathogenic factors.

2.13.

Endogenous defence receptors coincide with PAMP-perception systems

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Pathogen-derived molecules (PAMPs) serve as recognition patterns through binding to plant plasmalemma receptors. Upon of PAMP-binding membrane depolarization represents one of the first symptoms to be recognized. It is believed that the presence of microbial pathogens is communicated between plant cells through so called endogenous peptide elicitors such as AtPep1, AtPep2, AtPep3. Here we examined PEP-induced membrane potential changes in mesophyll cells of *Arabidopsis thaliana*. Following application of these peptide elicitors, dose-dependent membrane potential changes were evoked via activation of chloride-channels, reaching a maximum of 79 ± 10 mV (n=13) with 10 nM AtPep1. On the top of AtPep1-induced membrane potential changes further depolarizations were observed in response to the bacterial elicitors; flg22 or elf 18. Among all elicitors tested, flg22 was the most efficacious one, resulting in depolarizations of 98 ± 17 mV(n=12), while the action of elf18 (80 ± 20 mV n=12) was comparable to AtPep1.

BAK1 is a receptor-like kinase reported to form a complex with the flagellin (flg22) receptor FLS2 during flg22 perception. *Bak1-4* mutants exhibited attenuated responses to all elicitors employed, generating depolarizations of 47.2 ± 12.4 mV (n=4), 42.7 ± 5.4 mV (n=4), 47.8 ± 15.7 mV (n=6) in response to AtPep1, flg22 and elf18, respectively. Moreover, depolarizations could be triggered literally one on another. We thus conclude, that the three receptor systems examined here (namely PEPR, FLS, EFR) although acting independently from each other, share a common receptor subunit represented by the BAK1 kinase, capable of augmenting the action of each of them.

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2.14.

What're you gonna do if you catch me? Black spot disease of *Brassica oleracea* from transcriptome profile changes to cell death

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Black spot is the one of the most devastating disease of *Brassica* crop caused by *Alternaria brassicicola*, the necrotrophic fungus responsible for temporary epidemics on the cabbage plantations all over the Europe. This fungus breaks the defense barriers of the host plant mainly through its phytoalexins detoxification. The process of *A. brassicicola* recognition during compatible/incompatible interaction remains unknown. However, the role of RLKs (*Receptor-Like Kinases*) and TNL (TIR-NB-LRR) receptors in the recognition by plant of necrotrophic fungi has been confirmed recently. Another question without the answer is, how plant cell dies during attack of necrotrophic fungi and disease development? Is it "active" cell death (programmed cell death)? What kind of signaling pathways are responsible for spreading or not the cell death signal and what kind of genes are involved in?

Here we show changes in the global transcription profile of *Brassica oleracea* at early stages (12, 24 and 48 hpi) of *A. brassicicola* infection (DNA microarray) and expression changes of some important genes such as defense responsive, pathogenesis-related and associated with the host cell death (*Real-Time* PCR). Our research focuses particularly on the symptoms of cell death, especially chlorophyll degradation and DNA damage examined by TUNEL assay and DNA electrophoresis, respectively.

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2.15.

Direct observation of microbial distributions in the rhizosphere and near-root zones: combining plastic film sampling with modern molecular, chemical and structural techniques

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Plant growth promoting rhizobacteria (PGPR) inhabit rhizosphere region as well as the surfaces of plant roots, and play an important role in plant health and crop productivity. Biological, chemical and physical interactions between members of the community and with plants occur over a wide range of physical scales,

making the investigation technically challenging. Although the physical structure of the soil and root surfaces is recognized to play a significant role in the distribution of bacteria and the formation of local microbial assemblages (cenosis), direct investigation of the interaction between structure and community is hindered by an inability to recover samples without disturbing structure. Here we describe a plastic-film technology which is compatible with modern microbial techniques such as fluorescent microscopy, FISH, DNA isolation and direct PCR amplification. In preliminary *in vitro* tests, we demonstrate bacterial attachment and detachment using the soil bacterium *Pseudomonas putida* KT2440, and the rhizosphere bacterium *P. fluorescens* SBW25 (both are regarded as PGPR). Bacterial attachment was found to be enhanced by pre-treating the films in soil water. Genomic DNA could be isolated from SBW25 attached to film samples using a genomic isolation kit, and direct PCR amplification of the 16S DNA sequence was possible using standard PCR conditions and Taq polymerase. Both flexible and more rigid plastic films have been tested in soil and rhizosphere microcosms, where fluorescent microscopy has been used to assess the interactions between GFP-tagged SBW25 and the native soil microbe community. Preliminary CT (Computerized Axial Tomography) scans suggest that the exact positioning of the film with regard to soil pores, aggregates and plant roots is possible. This combination of modern molecular, chemical and structural techniques presents a powerful new tool with which to examine microbial communities in soil and the rhizosphere.

2.16.

The search for bamboo endophytes: on the crossroads of different methodologies

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Plants are constantly involved in interactions with a wide range of bacteria, living in the rhizosphere (rhizobacteria), on the surface of the leaves (epiphytes) or inside the plant tissues (endophytes). Endophytes have been indicated to present in most healthy plant tissues, including flowers, fruits, leaves, stems, roots and seeds. Moreover, at least some of these endophytes prove beneficial for the plant, impacting on different aspects of plant physiology, such as stimulation of plant growth, nitrogen fixation and induction of resistance to plant pathogens. Unfortunately, little is known about the large majority of the ecological relations between bacteria and plants – which is even more deplorable given the potential role of bacteria in the establishment and the success of different phytotechnical approaches such as biofertilization, bioremediation, sustainable agriculture and energy production.

A plant taxon that is widely acknowledged as an important crop worldwide, is the group of the bamboos. In contrast, however, there is a lack of knowledge regarding our knowledge on its bacterial endophytic communities. Combining three approaches, (1) direct cultivation, (2) DNA extraction and analysis and (3) in situ microscopy, we set out to observe the endophytic community composition in *Phyllostachys atrovaginata* and *P. humilis*.

PCR amplification using 16S rRNA flanking primers was attempted both of the microbial communities which were isolated and grown in pure culture, as well as of the DNA isolated directly from cut plant tissue. Sequence analysis indicated that among the endophytes of the shoots of *P. atrovaginata*, the following bacteria (or close relatives) are present: *Bacillus amyloliquefaciens, B. subtilis, Mycobacterium palustre, M. lentiflavum* and *M. avium.*

The direct observation method used inert plastic films as carriers for direct endophytic microbial assemblages formation. The films were adjoined closely to plant tissues inside columns and nodes for a period of four months. Afterwards, the films were taken out and analysed with different types of light and confocal microscopy. Combined with a cytochemical analysis based on confocal microscopy and scanning electron microscopy, significant differences of microbial populations in nodes and stems tissues could be shown.

2.17.

The ultrastructural comparison of compatible and incompatible potato – and tobacco – PVY^{NTN} interaction

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Potato plants cv. Rywal with hypersensitivity gene *Ny-1* (Szajko et al., 2008) infected with PVY^{NTN} reacted with local necroses 3 days after infection (incompatible response). Potato virus Y particles were found in epidermis, mesophyll, phloem parenchyma and xylem tracheary elements during HR and compatible response. The virus spread systemically outside the infected leaves despite rapid and localized defense hypersensitive reaction. During HR in potato cv. Rywal tissues virus particles were observed already 10 hours after infection (Otulak, Garbaczewska, 2007), whereas cytoplasmic inclusions were noticed first time 24 days after infection. In compatible tobacco cv. Samsun and potato cv. Igor tissues response to PVY^{NTN} inoculation systemic necrosis were presented 15 days after infection. Ultrastructural studies showed that ER may take part in PVY replication and also inclusion formation. The observed cytopathological lesions and virus particles indicate that cell nucleus and mitochondrion participate in PVY life cycle. In HR as well as in compatible response PVY particles were found in plasmodesmatas and also in vascular tissues. Virus particles and cytoplasmic PVY inclusions observed in phloem and xylem cells gives ground to conclude that even in case of resistant potato variety PVY is able to systemic transport through phloem and xylem.

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2.18.

Ultrastructure of *Nicotiana tabacum* cv. Samsun cells infected with CDV

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An emerging virus, Colombian Datura virus (CDV; genus *Potyvirus*), might present a risk to solanaceous crops. It was first identified in ornamental plants (*Brugmansia* spp.) imported from Columbia into the USA in 1967 (Kahn, Bartels, 1968). In 2004, it appeared in field-cropped *Nicotiana tabacum* in three Central European countries: Poland, Germany and Hungary (Schubert et al., 2006).

Nicotiana tabacum cv. Samsun was the model plant mechanically inoculated with Colombian Datura virus. The ultrastructural and cytological studies of infected leaves showed necrotic cells in all types of blades and petioles tissues. CDV particles and cytoplasmatic inclusions were found in the protoplasts of infected cells. They were connected with endoplasmatic reticulum cisternae and/or with mitochondrial external membranes. Often mitochondria were the most changeable organelles. Viral filamentous particles were also present inside nucleus of some infected plant cells. Those localisations were suggested that nucleus, mitochondrion and endoplasmatic reticulum participated in CDV life cycle. Besides, the virus particles and its protein inclusions were detected in floem and xylem elements. This data suggest that all vascular tissues participated in long distance transport of CDV in infected plants.

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2.19.

Localisation of actin and tubulin cytoskeleton in syncytia induced by *Heterodera schachtii* in roots of *Arabidopsis thaliana*

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Cyst nematodes such as *Heterodera schachtii* are obligatory sedentary endoparasites of plant roots. They are able to induce development of syncytium which are large multinucleate feeding site created by fussion of neighbouring cells with initial cell (de Almeida Engler et al., 2004). Syncytium is the main site of molecular interactions between the plant and developing nematode. Characteristic features of syncytia are: hypertrophy of incorporated cells, formation of cell wall openings, proliferation of cytoplasm and decrease of vacuole volume.

The plant cytoskeleton is a highly dynamic and versatile intracellular scaffold composed of microtubules and actin microfilament. It plays important role in many aspects of plant cell growth and development. Both the microlubule and actin cytoskeleton in plants are known to rearange when numerous, diverse external stimuli are applied (Vantard and Blanchoin, 2002).

To find out whether cytoskeleton changes occurred during development of syncytia the immunolocalisation of actin and tubulin was conducted. The microtubule and actin cytoskeletons are concominantly affected in syncytia. Microtubules and actin microfilaments interact with each other structurally and functionally and probably are regulated by common mechanisms.

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2.20.

Novel hosts for transient expression of recombinant proteins among Australian species of *Nicotiana* genus

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Obtaining of foreign proteins from plants by *Agrobacterium*-mediated transient expression has several advantages in comparison with stably transformed plant systems. The main preference is a potential for production of high amounts of foreign proteins during short time. Plant host species may affect strongly the production of recombinant proteins (Sheludko et al., 2007), but at the present time a limited number of species are commonly used for transient expression assays, first of all *Nicotiana benthamiana*. Here we report about novel perspective hosts for transient expression selected among Australian representatives of *Nicotiana* L. genus – *N. excelsior* and *N. cavicola*. We tested these species for accumulation of reporter proteins (green fluorescent protein (GFP), β-glucuronidase (GUS), thermostable lichenase (LicBM3) from *Clostridium thermocellum*) as well as other foreign proteins – desaturases (DesA and DesC) from *Synechocystis* sp. fused with LicBM3 and human interferon α2b (INF).

Plants were infiltrated with *A. tumefaciens* harboring the gene of interest (*gfp*, GUS, *licB, desAlicBM3, desClicBM3*) under control of 35S CaMV promoter or viral-based transient expression system (Marillonnet et al., 2004) (genes – *gfp, inf*). In all experiments the p19 suppressor of PTGS was used to enhance transient expression (Voinnet et al., 2003). The GFP content was calculated by spectrophotometric measurements; enzymatic activities of GUS, LicBM3, DesALicBM3 and DesCLicBM3 were proved using color reactions with substrates; biological activity of INF was demonstrated and calculated using titration method (capability to delay the replication of vesicular stomatitis virus in mammalian cell culture). Total soluble proteins (TSP) were calculated using Bradford method.

The transient expression and accumulation of foreign proteins was observed in all tested systems. Reporter proteins GFP and GUS were successfully expressed in *N. cavicola leaves*. The content of GFP protein was 12.6 ± 6.5% TSP (viral-based system) and 6.0 ± 1.5% TSP (gene under control of 35S CaMV promoter). In *N. excelsior* leaves were successfully expressed *gfp, GUS, licBM3, desAlicBM3, desClicBM3* and *inf* genes. The content of GFP protein was 32.1 ± 14.2% TSP (viral-based system) and 3.7 ± 1.7% TSP (gene under control of 35S CaMV promoter). Additionally, biologically active pharmaceutical protein human interferon α2b was successfully expressed in *N. excelsior*. To the best of our knowledge no quantitative data on active IFN α2b

transient expression was reported. The activity of INF was 20.6 ± 7.8 × 10^2 IU/ml (approximate 20-30 ng/g fresh weight) and maximal activity was 32 × 10^2 IU/ml (approximately 30–50 ng/g FW).

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2.21.

Mesembryanthemum crystallinum – Botrytis cinerea interaction depends on the photosynthetic metabolism

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Till now, *Mesembryanthemum crystallinum* L. has been studied for its plasticity in the response to abiotic stress factors accelerating the switch from C_3 photosynthesis to Crassulacean Acid Metabolism (CAM), however, the scarce data on the response of C_3/CAM plants to biotic stress are available. *Botrytis cinerea*, a necrotrophic fungus, showed the ability to infect *M. crystallinum* in the C_3 and CAM states. The microscopic inspection of inoculated leaves revealed that the response of C_3 and CAM plants to *B. cinerea* differentiated shortly after inoculation, and in CAM plants the decreased rate of conidia germination in comparison to C_3 plants take place. Detection of response of photosynthetic apparatus to infection with this fungus at 3, 24 and 48h post inoculation was carried out on leaves of different whorls using chlorophyll fluorescence imaging technique. Stimulation of photosynthetic processes after 24h in infected C_3 plants, and just after 3h in CAM plants was observed. The signal induced by pathogen in photochemistry processes of infected leaf was translocated to systemic leaves, before necrotic lesions surrounded by a chlorotic halo developed. The results provide evidence for the involvement of photochemistry processes in the induction of defense mechanisms among the whole plant. Comparing our previous analysis of changes in activities of antioxidative enzymes with measurements of photosynthetic processes, it could be concluded that activation of antioxidative enzymes occurs slower than changes in the photosynthetic processes. Moreover, changes in metabolism of carbohydrates and phenols (the part of non-enzymatic antioxidative system), before induction of enzymatic antioxidants take place. The outcome of plant-pathogen interaction depends on the co-regulation of the photosynthetic mode of carbon assimilation and antioxidative system.

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2.22.

Flavonoid preactivation of *Rhizobium leguminosarum* bv. *trifolii* improves symbiosis with clover (*Trifolium pratense*)

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The *Rhizobium*-legume symbiosis is dependent on the exchange of numerous molecular signals between bacteria and their plant host. Flavonoids secreted by plant roots into rhizosphere play an important role in early steps of symbiosis. They induce the expression of rhizobial *nod* genes, resulting in synthesis of chitolipooligosaccharides (Nod factors). Mixtures of flavonoids may affect survival of rhizobia in the rhizosphere and influence their competitiveness (Cooper, 2004). Nod factors secreted by bacteria elicit multiple responses in the root epidermis that lead to the nodulation of the appropriate host plants (Spaink, 2000).

In this work, we studied the effect of flavonoid preactivation of *Rhizobium leguminosarum* bv. *trifolii* (*Rlt*) on clover nodulation and growth. Three pairs of *Rlt* strains were chosen and clover seedlings were inoculated with mixed cultures of two strains. Four experimental groups were formed for each pair of strains: in I and II group only one strain was flavonoid-preactivated; in III group both strains were flavonoid-preactivated; in IV group none strain was preactivated. For preactivation, the strains before clover inoculation were grown in the presence of clover seeds exudate. Clover plants were grown (4 weeks) under laboratory conditions, then nodule number was estimated and shoots and roots were weighted.

The beneficial effect of flavonoid preactivation of *Rlt* strains on clover growth was observed in the case of preactivation of both strains (III group) and wet mass of roots and shoots was significantly greater in comparison to clover inoculated *Rlt* strains without preactivation. In some cases, the increase of wet root or shoot mass was also visible when only one *Rlt* strain was treated with flavonoids before clover inoculation. Moreover, the increase in nodule number was observed after flavonoid preactivation, but this effect was less pronounced. We concluded, that *Rlt* strains treated with flavonoids, one of a signal factor in rhizobia-plant interactions, may increase the symbiotic efficiency.

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2.23.

Mycorrhizal fungi and deficit irrigation as a new water saving strategy for tomato plants

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World water supplies are limited and therefore water-saving practices should be explored. Plant water relations, yield, and fruit quality of fresh tomato (*Lycopersicon esculentum* Mill. cv. Atut) in response to partial root-zone drying (PRD) and root injections with endomycorrhizal fungi were investigated in potted plants in a glasshouse. Three main irrigation treatments were imposed – control (fully irrigated, FI), and two forms of dynamic deficit irrigation – partial root-zone drying (DPRD), and common deficit irrigation (DDI). Under DDI, a deficit volume of irrigation water was applied to both sides of the split roots. Under the DPRD regime, the same volume of irrigation was applied, but alternately to one side of the root system, switched every 6 days (1 PRD cycle). Deficit irrigation was applied in dynamic form during 66 days (11 PRD cycles), which means that every cycle decreased amounts of water applied from 85 to 45% of FI, on average 62% of FI. Half of plants from every treatment were injected with endomycorrhizal fungi to improve water supply in drought stress. Mycorrhizal fungi improved water use efficiency (WUE) in both alternating and common deficit irrigations as compared to DPRD and DDI without the injection. Water saving was caused by partial stomata closure which however did not limit the photosynthetic efficiency and the main signal closing stomata during deficit irrigations was a decrease of water potential. Mycorrhizal fungi ameliorated the disadvantageous effects of deficit irrigations on yield but could not prevent its considerable reduction as compared to FI. It is interesting that mycorrhiza significantly increased yield of fruits and improved WUE of fully irrigated plants. Both forms of deficit irrigations significantly raised osmotic potential and total acidity of fruit sap. The pH of fruits decreased in FI and DDI injected with endomycorrhiza compared to treatments without mycorrhiza.

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2.24.

Characterisation of hypersensitive response of potato to PVY infection

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A prototypic plant pathogen interaction system used to study disease resistance is tobacco and tobacco mosaic virus (TMV). In this pathosystem, the resistance response comprises two phenomena, known as the hypersensitive response (HR) and systemic acquired resistance (SAR). The mechanisms that are involved in HR and SAR are not completely understood.

We studied resistance response of *Solanum tuberosum* ssp. *tuberosum* cv. Rywal to PVY infection. We identified a novel locus *Ny-1*, that determines the HR response of *S. tuberosum* cv. Rywal plants to PVY. The *Ny-1* locus has been mapped (Szajko et al., 2008) on potato chromosome IX, linked by 2cM to the locus *GP41*.

In contrast to most characterised potato cultivars, Rywal develops local HR reaction after PVY inoculation. These plants, are resistant to known: $^{N,\ O,\ NTN}$PVY strains, when grown in growth chambers at 20°C. Such resistance response successfully limits virus replication and its spreading. Using RT-PCR approach we were able to detect presence of PVY particles only in the inoculated parts of plants. The HR and virus localisation correlates with an increase in salicylic acid (SA) biosynthesis and PR gene induction. Additionally, these reactions are abolished when plants are growing at constitutive ≥ 28°C temperature or in transgenic *nahG* plant with reduced level of SA.

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Session 3

Mitochondria and chloroplasts in cell metabolism

PLENARY LECTURES

3.1.

Relationships between mitochondrial respiratory pathways and photosynthesis in the control of plant growth and defence

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Chloroplasts and mitochondria play important roles in cellular energy and redox metabolism connected to photosynthesis and respiration. They are important sources of reactive oxygen species. Hydrogen peroxide is produced by electron transport processes in the chloroplasts and mitochondria, and by photorespiratory glycolate metabolism in the peroxisomes. The mitochondrial electron transport chain oxidizes NADH produced during photorespiratory and respiratory metabolism. To analyze the impact of redox metabolism on metabolic integration and oxidative signalling, we have used phenotypic, metabolic and transcriptomic analysis of tobacco knockout mutants lacking mitochondrial complex I. Aspects of these studies and key conclusions will be described. In particular, it will be shown that mitochondrial pyridine nucleotides status is an important determinant of plant growth and development, and of stress tolerance through effects on the carbon-nitrogen metabolism, and on gibberellin metabolism and associated signalling pathways.

3.2.

Mitochondrial control of cellular redox

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Plant mitochondria are profoundly integrated into the cellular metabolic systems by consuming and producing metabolites adhering to various processes, like photosynthesis, nitrogen metabolism and stress resistance. Especially, mitochondria produce or consume redox compounds like ascorbic acid, superoxide, NADPH and NADH, and are therefore involved in establishing the redox homeostasis of the cell (Rassmusson et al., 2008).

Central to this function, plant mitochondria contain energy bypass enzymes which allow respiratory electron transport without inherent linkage to proton pumping and ATP production. These include alternative oxidases and external NAD(P)H dehydrogenases, which oxidise cytosolic NADH or NADPH and reduce ubiquinone. In potato and *Arabidopsis*, NDB1 is an external calcium-dependent NADPH dehydrogenase, the *Arabidopsis* NDB2 is a calcium-stimulated NADH dehydrogenase and NDB4 a calcium-independent NADH dehydrogenase (Rassmusson et al., 2008). The enzymatic system thus potentially allows specific tuning of cytosolic NADH and NADPH reduction levels. Analyses of transgenic *Nicotiana sylvestris*, modified to overexpress and suppress NDB1, showed that the external NADPH dehydrogenase is able to specifically modify the cellular NADPH level. A specific change in stem apex was linked to modified metabolite ratios, expression of meristem identity genes, and a bolting time phenotype, thus indicating the presence of redox control for stem development (Liu et al., 2008; 2009).

Further work aim at determining how alternative NAD(P)H dehydrogenases and oxidases in conjunction may mediate redox scenarios for plant life functions in relation to developmental programmes and fluctuating growth conditions.

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3.3.

On the mechanism of regulation of excess energy dissipation in plant photosystem II

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In oxygenic photosynthetic organisms, reaction centre complexes catalyze electron transport from water to CO_2 using light energy absorbed by tetrapyrrole pigments bound to so called "antenna proteins". A major challenge in performing this type of photosynthesis consists on the difficulty of operating "one electron" transport through a multi-step pathway in the oxygen-rich chloroplast. In fact, synthesis of ROS, mainly singlet oxygen and superoxide anion and consequent photoinhibition is an intrinsically unavoidable consequence of photosynthesis. This process must be prevented and/or controlled in order to avoid photo-destruction and cell death. Mechanisms involved include chlorophyll (Chl) triplet quenching, ROS scavenging and controlled heat dissipation of excess Chl singlet excited states. Reverse genomics and ultrafast-spectroscopy led to the proposal that the transient formation of carotenoid radical cations, followed by charge recombination might be the underlying mechanism of energy dissipation while three proteins belonging to the LHC superfamily could be the site hosting this reaction. The dynamic association of Light harvesting and reaction centre proteins within grana membranes plays a fundamental role in triggering excess energy dissipation. These finding have been described in the references below.

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ORAL PRESENTATIONS

3.4.

Photophosphorylation and CF_1-ATPase activity in *Brassica chinensis* L. plants grown under light-emitting diodes and different light intensity

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Light quality and quantity play a crucial role in the development and function of the photosynthetic apparatus. The influence of growth light intensity on photosynthetic light-driven reactions has been studied in a wide range of works, but there are much fewer works focusing on the influence of incident light spectrum on these processes. We studied the photophosphorylation, electron transport, trans-thylakoid proton gradient on isolated thylakoid membranes and CF_1-ATPase activity in the 4-th leaf of 27-days-old Chinese cabbage (*Brassica chinensis* L.) plants. The plants were grown under two illumination sources with different emission spectra: high-pressure sodium (HPS) lamps and red (650 nm) and blue (470 nm) light-emitting diodes (LEDs) with a red : blue photon ratio of 7 : 1. The plants were illuminated with two photosynthetic photon flux levels: nearly 400 μE and about 100 μE. The electron transport activity and ΔpH value were nearly the same in all the plants studied. However, photophosphorylation and CF_1-ATPase activity were significantly different. When plants grew at 400 μE, non-cyclic photophosphorylation was more than two-fold higher under HPS lamps than under LEDs, while cyclic photophosphorylation was nearly the same. At 100 μE non-cyclic photophosphorylation was more than four-fold higher under LEDs than under HPS lamps, due to it being lower than at 400 μE under HPS lamps and higher under LEDs. Cyclic photophosphorylation at 100 μE was nearly three-fold higher in plants grown under LEDs than under HPS lamps. CF_1-ATPase activity showed the same patterns, but less pronounced. CF_1 from plants grown under LEDs also reacted differently to activation conditions during measurement and adding dithiothreitol to the reaction medium than CF_1 from plants grown under HPS lamps. Our results suggest that under the red-blue LED spectrum, the development and/or regulation of the chloroplast ATP-synthase occurs differently than under broadband HPS lamp spectrum, while electron transport and ΔpH are hardly affected by this difference in spectra.

3.5.

Alternative respiratory pathway: A way to reduce imbalance between source and sink activity in the sink-limited plant *Erythronium americanum*

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Feedback-inhibition of photosynthesis by carbohydrate accumulation is a well known process, which can lead to the induction of leaf senescence. However, mechanisms that allow plants to cope with recurrent surplus of carbon, in conditions of imbalance between source and sink activity, have not received much attention. We investigated the response of sink growth and metabolism to the modulation of source activity using elevated CO_2 and elevated O_3 growth conditions in *Erythronium americanum*, a sink-limited plant. Sink activity (bulb) was monitored via slice and mitochondrial respiratory rates, sucrose hydrolysis activity, carbohydrates and biomass accumulation throughout the growth season, while source activity (leaf) was monitored via gas exchanges, rubisco and phosphoenolpyruvate carboxylase activities, carbohydrates and respiratory rates. Elevated CO_2 increased net photosynthetic rate by increasing substrate availability for rubisco, whereas elevated O_3 decreased net photosynthetic rate mainly through a reduction in rubisco activity. Despite this modulation of source activity by treatments, neither plant growth nor starch accumulation were affected. Sucrose synthase activity was higher in the sink under elevated CO_2 and lower under elevated O_3, thereby modulating the pool of glycolytic intermediates. Alternative respiratory pathway was similarly increased under elevated CO_2 and decreased under elevated O_3 in the sink. This modulation was seen both with the activity and capacity of the pathway, as well as with the alternative oxidase abundance. Furthermore, the modulation of the alternative pathway occurred mainly when malate was used as substrate for mitochondria, suggesting a linking role for pyruvate between carbon availability and alternative oxidase activation.

In this sink-limited species, the alternative respiratory pathway appears to balance carbon availability with the sink capacity, thereby avoiding early feedback-inhibition of photosynthesis in conditions of excess carbon.

3.6.

Searching for molecular factors associated with homeotic male sterility caused by the *Brassica nigra* cytoplasm in cauliflower

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Cauliflower plants carrying the cytoplasm of black mustard (*Brassica nigra*) may exhibit male-sterile phenotype manifested by transformation of stamens into petal-like organs. In our research, we compared such plants with those cauliflower accessions which carried the normal and Ogura cytoplasms. With the use of vectorette PCR we found 4 mitochondrial loci which differentiated the normal and *nigra* cytoplasms – *ccb206, nad1, nad3* and *nad7*. Their polymorphic character was confirmed in Southern blotting experiments which also indicated that these sequences were physically linked in the mitochondrial genome. Sequence analysis of the *ccb206, nad1* and *nad7* loci allowed design of specific amplicons which proved to be useful for routine identification of the plasmotype. Northern hybridization showed that transcription pattern of *ccb206* and *nad1* sequence was altered in plants with the *nigra* cytoplasm. Additionally, RT-PCR analysis of the *nad3* sequence detected the mRNA species that was preferentially accumulated in the *nigra* cytoplasm. Mitochondrial protein composition was analyzed using Blue Native electrophoresis and showed a few qualitative and quantitative differences between the studied cytoplasms.

We also studied mRNA accumulation of the selected floral organ identity genes in flower buds of male-sterile and male-fertile plants. This analysis showed that male-sterile plants with the *nigra* cytoplasm exhibit reduced transcript level of *APETALA3* and *PISTILLATA* orthologs. This result agreed with partial feminization of 3-rd whorl organs found in flower buds of the male-sterile plants. We also found that cauliflower curds with a coarse texture displayed elevated accumulation of *AGAMOUS, APETALA3* and *PISTILLATA* mRNA.

POSTERS

3.7.

Adaptation of rubisco to crassulacean acid metabolism (CAM)

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the assimilation of inorganic CO_2 into organic molecules. This enzyme is subjected to a wide range of regulatory mechanisms operating at nearly all stages of protein expression. The aim of this study was to elucidate the effects on RubisCO imposed by the induction of Crassulacean acid metabolism in the C_3-CAM intermediate and halophyte *Mesembryanthemum crystallinum* plants. Since a diurnal performance of CAM is associated with appearance of dramatic variation in the internal CO_2 level (minimum – at night and maximum around midday), CAM plants seem to be a suitable model to evaluate a CO_2 dependent impact on RubisCO. In CAM plants at the onset of the day activity of Rubisco at full activation was the lowest. This supports earlier data on decreased involvement of photorespiration in this time of the day revealed by drop in catalase activity (Niewiadomska and Miszalski, 2008). This limitation of RubisCO activity was not accompanied by any decline in the content of RubisCO protein, suggesting a posttranslational effect. Later during the photoperiod RubisCO activity and the level of RubisCO subunits increased both in C_3 and CAM plants. Plastid run-on transcription analyses revealed that in CAM plants in comparison to C_3-control plants at midday transcription of the *rbcL* gene was enhanced. The increase in transcription rate coincided with a higher level of the *rbcL* transcript as revealed by RT-PCR method (Niewiadomska et al., submitted). Altogether, the data suggest that a high CO_2 concentration may have a stimulatory effects on the transcription of the *rbcL* gene, whilst, a low CO_2 level appears to slow down both transcription and activity of RubisCO.

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3.8.

Oxidation of an adjacent methionine residue inhibits regulatory seryl-phosphorylation of pyruvate dehydrogenase

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A Met residue is located adjacent to phosphorylation site 1 in the sequences of mitochondrial pyruvate dehydrogenase E1α subunits. When synthetic peptides including site 1 were treated with H_2O_2, the Met residue was oxidized to methionine sulfoxide

(MetSO), and the peptides were no longer phosphorylated by E1α-kinase. Isolated mitochondria were incubated under state III or IV conditions, lysed, the pyruvate dehydrogenase complex (PDC) immunoprecipitated, and tryptic peptides analyzed by MALDI-TOF mass spectrometry. In all instances both Met and MetSO site 1 tryptic-peptides were detected. Similar results were obtained when suspension-cultured cells were incubated with chemical agents known to stimulate production of reactive oxygen species within the mitochondria. Treatment with these agents had no effect upon the amount of total PDC, but decreased the proportion of P-PDC. We propose that the redox-state of the Met residue adjacent to phosphorylation site 1 of pyruvate dehydrogenase contributes to overall regulation of PDC activity *in vivo*.

3.9.

Revealing the structure and function of prohibitins in the mitochondria of *Arabidopsis thaliana*

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Prohibitins (PHBs) are evolutionary conserved proteins. In mammals and yeasts, two homologous proteins (named Phb1 and Phb2) form a 1 MDa complex located in the inner mitochondrial membrane. Both proteins are interdependent – deletion of one prohibitin gene leads to a rapid degradation of the other prohibitin protein. In yeasts, PHB complex interacts with complex composed of m-AAA protease subunits. The size and ring shape of PHBs complexes suggests that they act as membrane scaffolds defining mitochondrial subcompartments with different functions.

PHBs in *Arabidopsis thaliana* are represented by 5 proteins: AtPHB3, AtPHB4 (homologs of Phb1), AtPHB1, AtPHB2 and AtPHB6 (homologs of Phb2). Our results show that all five PHBs form two complexes with molecular weight of 1 and 2 MDa in the mitochondria of *A. thaliana*. The larger complex, apart from PHBs, contains also m-AAA proteases represented by two proteins: AtFtsH3 and AtFtsH10. In order to establish the role of PHBs in plants we investigated double mutant of *A. thaliana* with T-DNA inserts in *AtPHB2* and *AtPHB6*. Growth of *phb2/phb6* plants is severely reduced in compare with wild-type plants. Diameter and biomass of rosettes are decreased more than 57% and 76%, respectively. Overall level of PHBs in *phb2/phb6* mitochondria is unaffected indicating that absence of AtPHB2 and AtPHB6 proteins is compensated by increased abundance of the remaining PHBs. Lack of PHB complex in mammalian cells results in depletion of mitochondrial DNA. Contrary to this, in *phb2/phb6* plants we observed 60% increase of mtDNA level, which was not accompanied by increased level of mtDNA-encoded transcripts and proteins. We found also that level and activity of complexes of oxidative phosphorylation are also unaffected. Our results indicate that (1) PHBs interact with m-AAA proteases forming large 2 MDa complex; (2) absence of AtPHB2 and AtPHB6 proteins cannot be completely compensated by increased level of remaining PHBs resulting in severe growth reduction of *phb2/phb6* plants; (3) phenotype of *phb2/phb6* plants does not result from deficiency of the oxidative phosphorylation system.

3.10.

Biogenesis of cauliflower mitochondria in abiotic stress conditions – molecular and physiological study

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Plant mitochondria play important role in stress response. The proper biogenesis of plant mitochondria during stress requires coordinate expression of mitochondrial and nuclear genomes. However, the available data on the influence of abiotic stress on the pattern of plant mitochondrial proteome is still limited. The aim of our study was to analyze the level of chosen mitochondrial proteins in cauliflower submitted to cold or heat stress and to estimate the connection between molecular and physiological data (rate of photosynthesis and respiration, chlorophyll content), and also ultrastructure of mitochondria in stress conditions. All analyses were performed on mitochondria isolated from 3-month-old cauliflower inflorescences by differential centrifugation and gradient purification. We noticed the significant increase of the steady-state level of AOX, mitochondrial Hsp 17.6 and dehydrin-like proteins in most tested stress conditions, as well as the increased pool of some mitochondrial matrix enzymes, like serine hydroxymethylase. On the other hands, the level of components of respiratory chain and cytochrome c maturation proteins was less affected in investigated stress conditions, with the exception for cyt. *c*, cyt. c_1, VDAC1 and COXII. The rate of photosynthesis and respiration of cauliflower leaves was significantly affected by all tested stress conditions. Cold stress, contrary to heat treatment resulted in the increase of chlorophyll content and the rate of photosynthesis in cauliflower leaves. The rate of leaves respiration was decreased by both tested stress conditions. The alterations in the ultrastructure and organization of mitochondria in apical cell layer of cauliflower inflorescences after stress treatment were less significant, but also will be discussed.

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3.11.

Downregulation of atdeg2 leads to a distinct pheotype

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Homozygous -*deg2* mutant was established by self-fertilization of plants derived from seeds of SALK-118424.39.64 T-DNA insertion line, in combination with PCR-based genotyping. It was demon-

strated that the mutant was depleted in both mRNA and the protein product, namely serine-type chloroplast protease AtDeg2.

To investigate the phenotypic effect of reduced AtDeg2 accumulation leaf morphology and chloroplast ultrastructure was analyzed by scanning and transmission electron microscopy, respectively. When grown under long day conditions the mutant plants exhibited a reduction of leaf size which was most evident with respect to leaves representing three older whorls (out of four analyzed) of 2–3 weeks-old plants (the reduction of the leaf size was mainly due to the descrease in the leaf length). As a consequence the density of trichomes distribution over the leaf surface was considerably higher in mutant vs wild type plants. The leaves accumulated less chlorophyll per area unit and due to his the mutant plants had slightly pale appearance.

Chloroplasts of mesophyll cells of 2–3 weeks-old leaves of mutant yielded considerable changes in their ultrastructure with respect to the wild type ones. Namely, they were two times larger, had larger starch grains and wider grana stacks. The amount of plastoglobuli per chloroplast was, in turn considerably smaller.

We conclude that *AtDEG2* product is essential for chloroplast biogenesis and whole plant development.

3.12.

Quantum efficiency of biomass production in three diatom species

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Phytoplankton cells can be exposed to highly variable light conditions due to vertical mixing within the water column where diatoms dominate in well mixed, nutrient-rich waters. Their flexibility in the adjustment of the photosynthetic apparatus in response to light and in particular, the high capacity for non-photochemical quenching (NPQ) of chlorophyll fluorescence is of great advantage under these highly dynamic light conditions. However, amongst diatom species significant differences in the characteristics of NPQ have been found in dependence of their living habitat. In this study, we compared the influence of different NPQ characteristics on the photosynthetic activity and finally on the quantum efficiency of biomass production in three diatom species (*Phaeodactylum tricornutum, Skeletonema costatum* and *Cyclotella meneghiniana*) under fluctuating light conditions. Surprisingly, despite the very different capacities for NPQ it was observed that the quantum efficiency of oxygen evolution was comparable in all three species under fluctuating light conditions. Differences were found in the ability to dissipate excessively absorbed energy via the so-called alternative electron pathways. This process has been kept down-regulated in *P. tricornutum*, but was up-regulated in *S. costatum*. Finally, *S. costatum* had a considerably higher quantum efficiency of carbon-related biomass production in comparison with the two other diatom species which could be due to a lower energy content of the biomass.

3.13.

Mitochondrial respiration, energy metabolism and redox status of *Arabidopsis* leaves under different nitrogen nutrition

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When supplied as an exclusive N source ammonium causes symptoms of toxicity. During last few decades it has been suggested that stress symptoms (e.g. chlorosis or retardation of growth) observed under sole ammonium nutrition can be related to various metabolic events. Recently it was proposed that the major reason of plant sensitivity to NH_4^+ is oxidative stress (Zhu et al., 2000). In the light, chloroplast NADPH synthesis exceeds ATP production. During NO_3^- nutrition an excess of reducing power generated in chloroplasts is consumed in NO_3^- assimilation but under ammonium nutrition chloroplasts must cope with the excess of reducing equivalents which can be exported and oxidized by mitochondrial respiratory chain. An increased mitochondrial respiration during NH_4^+ nutrition may result in higher formation of reactive oxygen species (ROS) (Guo et al., 2005)

Mitochondrial electron transport chain has a branched structure. Additional plant-specific components (ND_{in}, ND_{ex} and AOX) of mitochondrial chain do not pump the protons and it is thought that one of their roles might be the dissipation the excess of reducing power and decreasing the formation of reactive oxygen species in the cell. Increased transcript abundance of the respiratory chain alternative pathways in response to rapid changes in NH_4^+ concentration was recently documented in *Arabidopsis in vitro* cultures (Escobar et al., 2006). *Arabidopsis thaliana* grown in hydroponic cultures on NO_3^- or NH_4^+ medium were used in these studies. Changes in metabolite concentrations and activities of enzymes involved in N metabolism were observed. Influence different N forms on adenine and pyridine nucleotides concentration, mitochondrial respiration (including AOX) and enzymatic antioxidants were determined. The role of mitochondria under ammonium nutrition is discussed.

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3.14.

Blue Native Polyacrylamide Gel Electrophoresis – as a method of mitochondrial proteome analysis

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Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) is a method allowing separation of protein complexes in a gradient polyacrylamide gel under native conditions.

In our investigations plant mitochondria were solubilized with a mild detergent – dodecylmaltoside (DDM). DDM extracts protein complexes from the mitochondrial membranes in the native form.

In BN-PAGE protein mobility results from the bound dye Coomassie Blue G250 which is added both to the cathode and loading buffer. Moreover, the bound dye molecules make proteins visible during electrophoresis. Protein complexes separated with BN-PAGE can also be visualized using in-gel activity assay. Alternatively, protein complexes resolved in BN gel are separated in the second dimension using standard SDS-PAGE. In our experiments the 2D gels were subjected either to silver or colloidal blue staining. We used BN-PAGE to trace differences in the mitochondrial proteome composition between cytoplasmic male-sterile (CMS) and male-fertile plants of carrot (*Daucus carota* L.) and sugar beet (*Beta vulgaris* L.). Isolation of mitochondria was based on differential centrifugation followed by centrifugation in a 3-step sucrose or Percoll density gradient.

Comparison of carrot petaloid male-sterile Sp-cytoplasm and male-fertile normal N-cytoplasm revealed that:

- occurrence of sterilizing cytoplasm correlated with reduced accumulation of respiratory complexes I (NADH dehydrogenase) and V (ATP synthase);
- this correlated with lower enzymatic activity of complexes I and V;
- in male-sterile plants two specific proteins were found with molecular weight of 41 kDa; in plants with the normal cytoplasm these proteins were substituted with the ones of 38 kDa.

In sugar beet comparison of the Owen sterilizing cytoplasm (S) and the normal cytoplasm (N) showed that:

- both cytoplasm sources did not differ with respect to enzymatic activity of respiratory complexes I, IV and V;
- three proteins differentiated the CMS and fertile lines: two proteins with molecular weight of 30 kDa were detected only in S-cytoplasmic plants, another protein with molecular weight of 25 kDa was present in both male-sterile and male-fertile lines – however, in the former its accumulation was higher.

Our results provide a starting point for further, more detailed analysis of mitochondrial proteins involving immunoblotting and mass spectrometry

Session 4

Stress tolerance in plants

PLENARY LECTURES

4.1.

Regulation of plant resistance to host-adapted pathogens

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Plants have evolved a multi-layered surveillance system that enables them to respond to pathogen attack. The aim of my group is to understand processes controlling resistance to host-adapted pathogens once they have overcome recognition barriers at the cell surface and become invasive. An important post-invasion (basal) resistance layer restricts the growth of virulent pathogens in the absence of host cell death. This low level immune response depends on the balance of plant hormones and can be amplified by receptor-mediated recognition of specific pathogen effectors (normally leading to localized programmed cell death). We have identified a number of components of the resistance machinery in *Arabidopsis* and are using gene expression and metabolite profiling, protein biochemistry and cell biology approaches to understand better the modes of action of intracellular immune receptors and defence regulatory complexes. I will describe our findings so far and discuss the molecular and spatial control of one TIR-NB-LRR type immune receptor RPS4 that recognizes a bacterial type III effector, AvrRps4. Of particular interest is understanding how RPS4 connects with the downstream nucleo-cytoplasmic basal defence regulator EDS1 to trigger resistance.

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4.2.

Finding regulatory genes at the interface between abiotic and biotic stress

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Plants have to cope with a multiplicity of challenges in their natural environment for which they must develop tolerance. Does this mean that regulatory genes such as those coding for transcription factors can determine tolerance to unconnected stresses? Or are multiple genetic loci required to produce the combination of resistance phenotypes observed? I shall describe our very recent research which says that single regulatory genes can control a series of diverse responses to stress. Our approach has been to consider first how the plant perceives the stress at the physiological level. This means, for example, that a typical heat stress applied by many researchers may actually be perceived as a dehydration stress. Therefore, regulatory genes classically associated with one stress may, in reality, have a role in promoting tolerance to apparently unconnected stresses. Another example is that several types of environmental challenge elicit the accumulation of reactive oxygen species (ROS) as a common cellular response. ROS then trigger a set of defence mechanisms that ameliorate the effect of the environmental challenge. However, not all cross tolerance to multiple challenges can be explained by either of the above phenomena. I shall provide one example where a heat shock transcription factor (HSF) is important in determining seed yield under a range of soil water deficits, promotes a large increase in basal resistance to several pathogens as well as eliciting heat stress tolerance. In doing so I hope to illustrate the molecular basis of how this transcription factor may achieve control of diverse functions. These range from altered defence gene expression to how the HSF achieves an influence upon lifetime traits associated with growth and development.

4.3.

Control of plant senescence and stress responses by chloroplasts – involvement of dual-targeted Whirly1

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Senescence is the last phase of development preceding death of whole plants or plant organs such as leaves. Though senescence is an age dependent process, it is also affected by various environmental factors. Various stress situations (drought, temperature, light) might induce senescence prematurely. Chloroplasts which are degraded during senescence of photosynthetically competent tissues are at the same time sensors of the environmental situation. Moreover, in response to stress chloroplasts produce several signal compounds that are known to accelerate senescence such as jasmonic acid, abscisic acid, salicylic acid and ROS. Hence, chloroplasts are cellular integrators of environmental information and transduce this to the nucleus where gene expression is adjusted to the actual demands.

Although plastid-to-nucleus signalling is a well-known phenomenon, the factors involved in this retrograde communication are elusive. Recently, we could show that the DNA binding protein Whirly1 is located in plastids and the nucleus of the same cell (Grabowski et al., 2008). It is hypothesized that Whirly1 is sequestered in the organelles and might be relocated from one compartment to the other upon environmental or developmental clues (Krause and Krupinska, 2009). Investigations on *Arabidopsis* Whirly1 knockdown mutants and barley RNAi plants suggest that Whirly1 is a repressor of senescence and cell death. A model on the mechanism of senescence repression by chloroplast derived Whirly1 is presented.

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ORAL PRESENTATIONS

4.4.

Evidence for light wavelength-specific systemic photoelectrical signalling and cellular light memory in *Arabidopsis thaliana*

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Plants are able to integrate inputs, process and prioritize their outputs to survive and propagate in a hostile environment. Here we show that systemic acquired acclimation is associated with wavy changes in the non-photochemical quenching, reactive oxygen species levels and photoelectrical signalling. In this study we find, that signalling cascades from the chloroplast, initiated by quantum-redox changes in PSII and in its proximity in response to conditions that promotes EEE also play the role in regulation of the photoelectrical signalling in plants. Photoelectrical signalling is transduced, at least in part, by the bundle sheath cells, is light wavelength specific and its systemic propagation speed is depending on *ASCORBATE PEROXIDSASE* 2 gene expression. Our results suggest that photoelectrical signalling could be a component of SAA signalling. We also find that leaves are able to physiologically and specifically memorize excess of blue (450 nm) and red (650 nm) light episodes. This memorized information is used, for example, for improving their immune defences.

These findings have broad implications in the study of plant light acclimatory responses, plant-microbe interactions, bacterial pathogenesis and diseases, but also in the molecular eco-physiology of plant behaviour.

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4.5.

Implication of metabolic-dependent changes on reducing power in the determination of ozone risk threshold for higher plants

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Tropospheric ozone is one of the most important air pollutants affecting crop and forest vegetation. Under long term exposure of plants to low ozone concentrations biochemical processes are affected prior to any detectable symptoms of injury. Until recently, the European critical level of ozone exposure associated to biomass loss was based on the seasonal sum of the external concentrations of the pollutant above 40 $nL.L^{-1}$ (AOT 40). A new concept, taking into account the effective ozone flux in the leaf through stomata and the internal defense capacity of the foliar tissues is proposed (Musselman et al., 2006; Wieser and Matyssek, 2007). Among defense processes, we suggested to consider some parameters linked to the production of the reducing power which is essential to sustain the functioning of the detoxifying systems (Dizengremel et al., 2008). In this report, we present preliminary results on poplar and wheat species exposed to chronic ozone fumigations. In the leaves of both species, the pools of NAD(P)H appeared quite well maintained. This result could be the consequence of a stimulatory effect on the activity of enzymes implied in the biosynthesis of the reduced pyridine nucleotides. The stimulation of the phosphoenolpyruvate carboxylase (PEPc) activity in these tissues validates this hypothesis: PEPc is involved in the biosynthesis of malate, a mobile form of reducing power in the cell. In addition, we have shown that the activities of cytosolic enzymes involved in NADPH biosynthesis (NADP malic enzyme, glucose-6-phosphate dehydrogenase) were stimulated in different plant species exposed to a chronic ozone fumigation. We are running additional experiments to precise the level of regulation of these enzymes (transcriptional, translational or post-translational). The question will thus arise on how to use these metabolic changes as indicators to improve the risk assessment for plants exposed to ozone.

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4.6.

Stress-induced morphogenic responses in plants: plants deal with stress in their own time

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Plants have several different ways of reacting to a wide range of oxidative stressors. One specific way, generally overlooked, comprises (a) an inhibition of cell elongation (particularly in exposed parts of the plants), (b) localised stimulation of cell division and (c) complex alterations in cell differentiation status. This stress-induced morphogenic response (short: SIMR) is brought about by heavy metals, nutrient deficiency and UV irradiance and is therefore thought to imply a carefully controlled mechanism, mediated by reactive oxygen species, and reminiscent of the plant phenotype induced by hormones, in particular by a redistribution of auxins.

Several possible mechanisms are being proposed, in which either hormones and stress act independently, or by way of a coordinated effort of auxins and ROS. Possible targets include cellular redox state, peroxidase action, microtubule organisation or auxin metabolism. Moreover, the existence of such a wide range of different possible responses brings up several questions regarding the rationale behind this complexity. This leads to a novel definition of stress, where the plant-environment interaction should be viewed in terms of a thermodynamic model, in which not the specific pathway, but the achieved metabolic state is biologically conserved.

From an ecophysiological point of view, the stress-induced morphogenic response is postulated to take part in a general acclimation strategy, whereby a plant growth is redirected in order to avoid the stress as much as possible.

4.7.

Effect of temperature on carotenoid content and composition in cyanobacterium *Synechocystis* PCC6803

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Temperature is the one of most critical environmental factors which enable growth and development of living organisms. Carotenoids are among the most potent molecules that are able to protect photosynthetic and other energy generating processes against various stress effects. Our earlier studies with *pgsA* mutant of mesophilic cyanobacterium *Synechocystis* PCC6803 demonstrated the increase in the content of myxoxanthophyll and echinenone upon stress conditions induced by phosphatidylglycerol depletion (Domonkos et al., 2009). Also, myxoxanthophyll has been found to be preferentially synthesized at low temperatures (Varkonyi et al., 2002). Here, quantitative HPLC-based pigment separation technique has been applied to study the carotenoid content and composition in wild-type PCC6803 strain grown at different temperatures (15–39°C) at standardized light conditions (60 μE $m^{-2}s^{-1}$). Carotenoid derivatives were identified on the basis of both their absorption spectra and their retention times. A significant increase (approx. 2-fold) of the relative myxoxantophyll content in cells grown both at lowest (15°C) and highest (39°C) temperature was observed, in comparison to optimal temperature conditions (25–30°C). In contrast, the relative concentration of β-carotene decreased noticeably both at low and high temperatures. These results point to the importance of myxoxantophyll in protection against a stress caused by extreme temperature conditions.

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4.8.

Hydrogen peroxide generation in leaf veins

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It is well documented that a majority of hydrogen peroxide of the leaf is present in the proximity of leaf veins. Experiments with *Arabidopsis thaliana* plants treated with excess light suggested that increased H_2O_2 production in veins might occur via the chloroplastic Mehler reaction (Fryer et al., 2003). However, hydrogen peroxide is also enriched in veins under non-photooxidative conditions. The goal of this study was to analyze the possible sources of H_2O_2 under non-photooxidative conditions by comparing catalase and superoxide dismutase activities in veins and intervein areas of tobacco leaves. High levels of H_2O_2 in the tissue close to the mid rib is accompanied by a lower activities of catalase and the chloroplastic form of superoxide dismutase. This might imply that photosynthesis and photorespiration are not the main source of H_2O_2 in this part of the leaf. This is supported by a histochemical analysis which revealed that accumulation of H_2O_2 is predominantly confined to the xylem tissue (Ślesak et al., 2008). Analysis of hydrogen peroxide levels revealed that in the veins, in contrary to the interveinal regions, H_2O_2 is primarily associated with the plasmamembrane and cell wall debris (Niewiadomska et al., 2009). Altogether, this data suggests that veinal H_2O_2 is mainly generated in the apoplast in association with lignification and is not an indication of oxidative stress. On the contrary, in veins in comparison to the midvein leaf areas of *M. crystallinum* and *N. tabacum* leaves higher values of potential PSII quantum yield indicate a higher resistance to photooxidative stress (Niewiadomska et al., 2009). We hypothesize that preservation of PSII activity in the veins ensures remobilisation of the structural metabolites which is of special importance during senescence.

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POSTERS

4.9.

Intact anthracene inhibits photosynthesis and stimulates Hsp70 synthesis in algal cells

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Intensive batch cultures (30°C, 2.5% CO_2, 80-120 μmol × m^{-2} × s^{-1} PAR) were performed to examine the effect of polycyclic aromatic hydrocarbon (PAH), anthracene (ANT), on green algae (*Chlamydomonas reinhardtii, Desmodesmus subspicatus*). ANT inhibited the growth of algae populations as well as the intensity of photosynthesis and influenced chlorophyll *a* fluorescence parameters measured by OJIP test. ANT diminished the performance index (P.I.), the yield of primary photochemistry (Φ_{Po}), the yield of electron transport (Φ_{Eo}) the efficiency of moving the electron beyond Qa^- (ψ_0) and the fraction of active oxygen evolving complexes (OEC). The fraction of active PSII reaction centres (RC/CS_0) in the treated samples dramatically dropped. The most pronounced change in ANT-treated cells was the stimulation of energy dissipation parameter (DI_0/RC). The results lead to a conclusion that the inhibition of photosynthesis in green algae cells may be one of consequences of unspecific ANT-membrane interaction, resulting from hydrophobic character of the hydrocarbon. SDS-PAGE and western blotting enabled us to show, that ANT stimulated synthesis of Hsp70[cytosolic] and Hsp70B[stromal] in concentration- and time-dependent manner. It is known, that exposition of plant cells to anthropogenic pollutants (heavy metals, herbicides etc.) causes synthesis of Hsp70 to defend against stress consequences (Torres et al., 2008). However, very little is known about the role of heat shock proteins in protection of plants from PAH-induced stress. In the light of our findings it could be supposed that overproduction of Hsp in ANT-treated cells may result from damages of the structure of intracellular proteins.

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4.10.

Phenol and lipid compounds of *Rhododendron* L. different species while acclimation to forest-steppe climate condition of Ukraine

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Ontogenetic development of rhododendrons, as "introduced", substantially depends upon the climatic terms of natural habitat of their growing. The evergreen representatives (*Rh. fortunei, Rh. ponticum, Rh. amesiae*) of *Rhododendron* L. species, introduced in the climatic terms of Kiev, were investigated in the period of 2007-2009. Beginning of vegetation period (begins in April) was characterized by the active steryl glycoside synthesis maximal accumulation of which is observed in a period of the mass flowering of *Rh. ponticum, Rh. amesiae, Rh. fortunei* (May – June) connected to the increase of activity of metabolic processes. The considerable decline of steryl glycoside content was observed in the period of fruit ripening, which is in November for *Rh. amesiae* and *Rh. fortunei*, and in October for *Rh. ponticum*.

The characteristic plant response to the stress factor action is anthocyanin and flavonoid synthesis. All species explored had growth of anthocyanin content from May till November, in particular in *Rh. ponticum* and *Rh. fortunei* in May and June, and in *Rh.amesiae* in September and October. The insignificant anthocyanin content was observed during winter months.

The flavonoid content at the beginning of vegetation achieved a spring maximum at all species explored. Insignificant flavonoid content decline in summer months we connect with the of synthetic process decline in the drought conditions.

Temperature falling down in autumn period was accompanied at all species by the accumulation of flavonoids and anthocyanins connected with plant adaptation which continued till November. Increase of free sterol and sulfoquinovosyldiacylglycerol (SQDG) content in photosynthesizing tissues of *Rh. ponticum* at the sharp increase of temperature (to to +27°C) in summer months (June – July) was revealed.

While the decline of temperature in November sterol content increased sharply in *Rh. fortunei* and *Rh. ponticum*, which was stabilized in *Rh. fortunei* in a next month, but was high till April in *Rh. ponticum*. The SQDG accumulation was also registered in *Rh. ponticum* beginning from October and during all winter period.

The decline of monogalactosyldiacylglycerol content, digalactosyldiacylglycerol (DGDG) and SQDG accumulation in *Rh. fortunei* and *Rh. ponticum* was revealed. DGDG quantity was relatively high and SQDG content grew insignificantly in *Rh.amesiae*.

Leaf phenol and lipid composition study allows to characterize *Rh.amesiae* specie as more adaptable in climatic conditions of forest-steppe of Ukraine.

4.11.

EPR study of Cr(VI) phytoremediation by *Callitriche cophocarpa* (Sendtn.)

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Callitriche cophocarpa Sendt. (*Callitriche polymorpha* Lönnr.) – a widespread, aquatic macrophyte was found to be promising phytoremediator of hexavalent chromium contaminants in aquatic systems. In the last work the high accumulation capacity of Cr by shoots of this species was demonstrated (Augustynowicz et al., 2009). In this study the remediation of strongly toxic Cr(VI) anions by shoots and roots of *Callitriche cophocarpa* was measured *in vivo* with the low frequency electron paramagnetic spectroscopy (L-band EPR). This method enabled to detect a signal of unstable intermediate of Cr(V) followed by Cr(VI)→Cr(III) reduction (Kaszycki et al., 2005). This redox reaction is considered to be useful mechanism of Cr(VI) detoxification due to the lower redox potential and mobility of Cr(III) ions. The kinetic of Cr(V) production was measured during 5 hours. Both roots and shoots were able to reduce Cr(VI) ions effectively at the 1 mM concentration reaching a close-to-plateau state after about 4 hours. However, shoots exhibited considerably higher reduction capacity. To estimate vitality of 1 mM Cr(VI)-treated shoots, it was also

examined the kinetic of modulated chl *a* fluorescence (F_v/F_m) of leaves. Though after 5 hours the decay of chl *a* fluorescence was observed to an average 68% of the beginning value, the plants still exhibited significant photosynthetic activity. Since there is little data available about chromium phytoremediation by shoots our results point to *Callitriche* as a good model plant for further investigation of heavy metal remediation mechanisms by photosynthetically active tissues.

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4.12.

Response to cadmium of tobacco expressing *pAhHMA4::AhHMA4* depends on air humidity

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Modification of heavy-metal uptake and distribution is a major concern in biofortification and phytoremediation. In an attempt to receive plants with increased Zn uptake and translocation to shoots, *AhHMA4*, P_{1B}ATPase known to be responsible for Zn hyperaccumulation and hypertolerance in *Arabidopsis hallerii* (Hanikenne et al., 2008), was expressed under its constitutive promoter in tobacco. However, since Cd-hypertolerance appeared to be also dependent on *AhHMA4* (Hanikenne et al., 2008), in this study we examined whether transgenic tobacco accumulates cadmium in altered amount compared to wild type plants. Experiments were performed on 5 week-old plants grown on 1/4 Knop's medium supplemented with 0.25 and 4 μM cadmium under low (40%) and high (80%) humidity. Expression of the transgene (by RT-PCR), Cd accumulation in roots and shoots (by ASA) and the level of tolerance (assessment of the appearance) were determined.

The *AhHMA4* transgene under its own promoter was expressed in roots and shoots of tobacco plants at similar levels regardless of applied Cd^{2+} concentration.

It appeared that plants grown in the presence of 0.25 μM Cd^{2+} under low humidity (30%) accumulated in roots less cadmium than WT (65±17 μg/g DW and 165±23 μg/g DW respectively). The shoot/root Cd concentration ratio in *AhHMA4* and WT plants was 1.48±0.16 and 0.81±0.10 respectively. However, the differences were much less discernable when plants were exposed to higher cadmium (4 μM). Under such conditions shoot/root Cd concentration ratio was 1.52±0.07 (transgenic) and 1.28±0.08 (WT), whereas root Cd concentration reached 455±34 μg/g DW and 607±36 μg/g DW respectively.

On the other hand, when plants were subjected to cadmium under high humidity (80%), the pattern of metal accumulation, as well as the concentration in root and shoot tissues, were at the similar level. Our study demonstrates that contribution of the transgene to modification of cadmium accumulation in the host tobacco plant depends on both external cadmium concentration and on the air humidity.

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4.13.

Modification of plasmodesmata ultrastructure in maize leaves at low temperature

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In maize, as in other C4 plants, short distance transport of photosynthates goes through numerous plasmodesmata crossing the suberin lamella impermeable to aqueous solutions Sucrose, the end-product of photosynthetic carbon metabolism, before being loaded into the companion cell/sieve element (CC/SE) complex, also moves symplasmically from the site of synthesis, the KMS cells, to vascular parenchyma (VP) cells. As we have found earlier (Bilska and Sowinski, submitted), low temperature slows down the transfer of photosyntates on the path to vein, as the result of KMS/BS and BS/VP plasmodesmata closure. The aim of this work was to study the effect of chilling on maize KMS/BS and BS/VP plasmodesmata ultrastructure in detail. To do that, we visualise 3D structure of plasmodesmata on the basis of electron microscope micrograms, as well as cytolocalised some proteins suggested to be involved in the mechanism of plasmodesmata closure.

The chilling-tolerant maize (*Zea mays* L.) line KW 1074 and the chilling-sensitive CM 109 were used as the experimental material. Plants were grown in a growth chamber (24°C/22°C day/night) till the 3-rd leaf stage, and then half of the plants were transferred to a cold chamber (14°C/12°C) for either four hours or 28 h and the other half was kept as before as controls. Then plant material was fixed according to the standard procedures for either immunogold protein cytolocalization and electron microscopy. The result of the study is the 3D model of changes of maize KMS/BS and BS/VP plasmodesmata at low temperature.

4.14.

Protease activities during cold acclimation of perennial ryegrass (*Lolium perenne* L.)

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Perennial ryegrass (*Lolium perenne* L.) is a forage grass species with good quality and relatively high frost tolerance. During a process of cold acclimation (CA), that increases the level of plant frost tolerance, a rapid reconstruction of existing regulatory pathways in cells occurs. Protein degradation, dependent on proteolysis, is one of the most essential components of plant responses to low temperature stress. In the presented research, two *L. perenne* plants with different levels of frost tolerance – higher

frost tolerant (HFT) plant with T_{EL50} = -15.13°C and lower frost tolerant (LFT) plant with T_{EL50} = -11.42°C (after 21 days of CA) – were selected to compare activities of proteolytic enzymes during three weeks of CA. The protease activities were studied by performing proteolytic enzyme assays at pH = 7.8. It was clearly visible that significant differences in the protease activities between the selected plants appeared after five days of CA. Furthermore, after three weeks of CA the activities of proteolytic enzymes increased 3-times in the HFT plant and decreased slightly in the LFT plant, compared to these enzyme activities detected in both plants before CA. Further research will be of help in better understanding of the existing differences in proteolytic enzyme activities between *L. perenne* plants distinct in the level of frost tolerance.

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4.15.

Antioxidant responses in tubers of potato cultivars differing in dehydration tolerance

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Potato (*Solanum tuberosum* L) is highly sensitive to soil drought and extracts less of the available water from the soil in comparison with other crops (Schlafleitner et al., 2007). Even very short period of water shortage has negative effect on consumptive and technological properties of potato tubers. Thus, tuber formation and development serve as the best measure of drought resistance (Watkinson et al., 2008). Our previous experiments showed that 19 d water withheld in the phase of tuberisation resulted in the reduction of final tuber yield even up to 50% (Cekin cv).

Antioxidative enzymes seem to be involved in diverse physiological processes such as cell elongation, lignification and plant defence mechanisms against environmental stresses. Among them peroxidase (EC 1.11.1.7) is suggested to be an enzyme responsible for tuberisation of potato plants and superoxide dismutase (EC 1.15.1.1) responsible for higher resistance of *curtilobum* potato plants to abiotic stresses as compared to other plants. Therefore, the question arises whether ten day soil drought applying in tuberisation phase of potato development affects activity and pattern of antioxidative enzymes like superoxide dismutase, peroxidase and catalase (EC 1.11.1.6). Analytic electrophoresis of tuber extracts under non-denaturating conditions (native PAGE) was performed according to Laemmli (1972). Total peroxidase activity was measured spectrophotometrically Blackwell (1988).

It has been shown that the higher dehydration tolerance of Tajfun cultivar was accompanied by the lower total peroxidase activity in tubers of plants growing throughout the experimental period in soil with optimal water supply. The total activity of peroxidase decreased with increasing water deficit at tuberisation phase independently of plant dehydration tolerance level. However, the electrophoretic pattern of peroxidase isoforms depends both on genotype and on sensitivity to water supply of potato plants.

Differential response of superoxide dismutase (SOD) activity in potato subjected to soil drought was observed. In tubers of resistant cultivar the MnSOD isoenzymes were non-detected and activity of Cu/Zn-SOD isoenzymes decreased with soil drought development whereas in sensitive cultivar the MnSOD isoenzymes activity increased and the activity of Cu/Zn-SOD isoenzymes was stable.

Catalase (class II) activity was only detected in sensitive (Cekin) tubers and it activity decreased as soil drought developed.

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4.16.

Rapid and slower response reactions of plant shoots on salinization and drought at the root zone

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Rapid (min) and slower (h) response reactions of leaves and stems of oat, barley, wheat, rice and buckwheat plants on increase and decrease in NaCl concentration and drought at the root zone have been studied using a highly sensitive method – laser interference auxanometry (Budagovskaya, 2007). Addition of NaCl in increased concentration to the root zone of plants caused a two phase response reaction of leaves: decrease and the following increase in their growth rate in each phase. Duration of the 1st phase was shorter than that of the 2nd. Growth rate of leaves was restored by the end of the 2nd phase (in few h after addition of NaCl). The 1st phase may be related to rapid adaptive reactions and changes in leaf turgor, the 2nd – to slower adaptive processes – de novo synthesis of protectors. Introduction of NaCl in high concentration caused stoppage in leaf and stem growth and shrinking of their tissues as result of dehydration. Reversal of water transport in roots under salinization has been demonstrated. Washing the roots of NaCl rapidly restored the turgor of leaves and increased their growth rate. Under drought conditions the growth rate of shoots decreased rapidly. Shrinking of leaf and stem tissues was observed after the stoppage of shoot growth under drought as well as at high level of salinization. The data obtained provide information on dynamics of response reactions of shoots on increase and decrease of NaCl concentration, drought and watering at the root zone.

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4.17.

Involvement of protein phosphatases pp2c in response of winter rapeseed (*Brassica napus* L.) to drought stress

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Environmental conditions in Europe have dramatically changed. Amount of atmospheric precipitation in our country, including Wielkopolska, has dramatically decreased in recent years and caused periodic drought seasons. This has a great influence on plants, especially on production of crop plants. Winter rapeseed (*Brassica napus* var. *oleifera* L.) is the most important oil plant in Poland and its seed yield has been diminished for drought conditions. Thus, it is of importance to understand mechanisms which are activated in plant cells to control and adapt to water deficiency. Complex networks of stress signal transduction are involved in metabolic reorganization and adaptation to new environmental conditions. Based on the knowledge accumulated for a model plant *Arabidopsis thaliana*, we study drought response mechanisms developped by *B. napus*. It is well established that

one of the major role in drought stress response play protein phosphatases PP2C such as ABI1, ABI2, HAB1 and HAB2. These are negative regulators of abscisic acid (ABA) signalization. During dehydratation, PP2C are responsible for stress signal propagation and metabolic changes like gene inactivation (ABI1), chromatin remodeling (HAB1) or ion channels regulations. This report concerns induction and repression of PP2C transcription during prolonged dehydratation and rehydratation of several lines DH of winter rapeseed. We identified interesting differences in the gene expression of four PP2C in genotypes under study. Distinct transcriptional profiles of ABI2 and HAB2 induction during drought stress and salt stress suggest their involvement in different steps of stress signal transduction. We also compared expression of PP2C-encoding genes with other marker genes known to be associated with dehydratation such as Rab18 and PLDα1. Contribution of PP2C homologues to a plasticity of the amphiploid *B. napus* response during water deficiency will be discussed.

4.18.

The xanthophyll cycle induction and chlorophyll fluorescence in leaves of *Plantago media* at adaptation to light regime of habitats

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The pigment complex plays an important role in adaptation of plant photosynthetic apparatus to environmental stress. Acclimation responses tend to maximize light capture under light-limiting conditions and to enhance the efficiency of protective mechanisms under excess light to avoid photooxidative damage. We monitored the duirnal dynamics in chlorophylls (Chl), carotenoids (Car) and Chl fluorescence in *Plantago media* plants growing in sun and shade environments on the South Tyman (62°45'N, 55°49'E). High light plants (HLP) grew on the open slope at high photon flux density (1500 μmol $m^{-2}s^{-1}$) and low light plants (LLP) inhabited the footslope in the dense grass stand.

The photosynthetic pigment content (per dry weight) in leaves of LLP was 1.5-2 times higher than in HLP. Pigment concentration daily changes in LLP leaves were observed. In the morning and afternoon, Chl and Car content in LLP leaves was higher by 45-50% as compared to that in the evening. The green and yellow pigment ratio was lower in HLP leaves. Zeaxanthin (Zea) was revealed in the carotenoid spectrum of HLP. Zea indicates the de-epoxidized state of the xanthophylls cycle (DEPS). Violaxanthin (Vio) and neoxanthin (Neo) were present in the carotenoid spectrum of the LLP leaves, and Zea was founded in trace quantities. At deficiency of irradiance, Vio and Neo contribute to light collection by the light-harvesting complexes. The maximal photochemical efficiency of photosystem II (Fv/Fm) in LLP leaves was not significantly different from that of HLP leaves. Fv/Fm value varied in the range 0.74–0.81. There were significant differences between the two leaf types in light-response curves for the electron transport rate (ETR), the ratio of photochemical (qP) and non-photochemical quenching of Chl fluorescence (qN). The LLP leaves were characterized by lower ETR and qP values. A correlation between an increase in qN and Zea formation under high light conditions suggests that Zea has a photoprotective function, dissipating excess energy by quenching in the photochemical system of HLP leaves. Our data characterize *Plantago media* as a physiologically plastic species, capable to grow in different habitats due to high tolerance of its photosynthetic apparatus to photoinhibition.

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4.19.

Antioxidants in germinability and desiccation tolerance of lupine seeds

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Seeds of yellow lupine (*Lupinus luteus* L. cv. Juno) were collected throughout their development on the mother plant to determine whether the ability to germinate and to tolerate desiccation is related to the level of free radicals and the changes of antioxidants. Electron paramagnetic resonance (EPR) – based analyses showed that development of lupine seed was accompanied by generation of free radicals with *g1* and *g2* values of 2.0049±0.0004 and 2.0029±0.0003, respectively. Free radicals level increased significantly 25 days after flowering and decreased thereafter. The amount of hydrogen peroxide was high in fresh immature seeds and decreased during maturation drying. Ascorbate accumulated in lupine embryos during early seed filling stage whereas glutathione content increased during late seed filling phase. During maturation drying the redox state of both ascorbate and glutathione pools shifted towards the oxidized forms. While superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities remained high at the early seed filling stage the activities of both dehydroascorbate reductase (DHAR) and glutathione reductase (GR) and that of catalase (CAT) increased before seeds reached physiological maturity and decreased thereafter. The changes of isoform patterns of antioxidative enzymes were observed during seed maturation. Immature lupine seeds tested immediately after harvest acquired the ability to germinate when were less than half-filled and reached high tolerance to desiccation just after physiological maturity. The physiological implications of the changes in antioxidative machinery for the acquisition of desiccation tolerance and seeds germinability are discussed.

4.20.

Periodic night-day chilling stress – the novel approach to studying cold-sensitive and cold-tolerant plants

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Low temperature is one of the most important factors affecting plant growth. It limits the geographical range of many economically important crops, especially if they are cold-sensitive. Long-term exposure of this kind of plants to low positive temperature induces several changes in the chloroplast structure and photosyn-

thetic parameters, combined with rearrangement of chlorophyll-protein complexes (Garstka et al., 2005, 2007). Therefore an attempt was made to analyze bean, a chilling-sensitive plant, and pea, a chilling-tolerant one, during periodic chilling stress. Both plant species were exposed to low temperature (5°C) at night while the day-time temperature in the climate room was optimal (22°C). Temperature changes weren't instant but occurred for three hours.

Several techniques were used for detailed analysis: chlorophyll *a* fluorescence measurements, in intact leaves *in vivo*, for detailed analysis of the functional activity of photosystem II, and rough consideration of specific components of the photosynthetic apparatus and molecular biology (gel electrophoresis, northern-blot of thylakoid proteins) and biochemical (HPLC-MS – ATP and lipids of thylakoid membranes, content of hydrogen peroxide, analysis of the mitochondrial transmembrane potential) techniques for comprehensive survey of possible correlations between this experimental model and the previous one (long-term chilling stress of cut leaves).

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4.21.

Changes in LEA proteins, soluble carbohydrates, antioxidants and ROS levels during loss of desiccation tolerance of germinating *Vicia hirsuta* seeds

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The process of loss of desiccation tolerance (DT) in germinating seeds of tiny vetch (*Vicia hirsuta*), classified as *orthodox*, was investigated. Between Oh and 72h of germination seeds were: (i) dehydrated over saturated solution of NaCl (75% RH- relative humidity), (ii) treated with polyethylene glycol (PEG) 6000 at -1,7 MPa osmotic potential, (iii) treated with PEG and then dehydrated as above. Germination test, electrolyte leakage, LEA proteins, composition and content of soluble carbohydrates, level of ROS: peroxide radical ($O_2^{\cdot-}$), oxygen peroxide (H_2O_2) and changes in ascorbate acid (ASA) and dehydroascorbic acid (DHA), enzymatic scavengers such as ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured as potential factors associated with DT. Comparing to dried seeds, germination rate of seed pretreated with PEG and dried remarkably increased starting from 48h of germination, and at the same time the electrolyte leakage decreased. Moreover moderately strong correlation between electrolyte leakage and MDA level was observed. Also the effect of PEG treatment was observed in decreased $O_2^{\cdot-}$ level in germinating seeds beginning from 54h. H_2O_2 level increased during germination in all used treatments. The content of low molecular antioxidants and enzymes activities revealed significant changes with decreasing trend. Two heat-stable proteins with molecular mass of 46 kDa and 32 kDa were detected using anti-K-segment dehydrin antibody. In general the level of identified proteins was decreasing as seeds were germinating, however drying and PEG treatment caused that up to 48h of germination the level of dehydrin-like proteins was as high as in dry stored seeds. During germination decreased level of D-galactosides: raffinose family of oligosaccharides (RFOs) and galactosyl cyclitols (Gal-C) was detected. PEG incubation and/or drying resulted primarily in increase of sucrose concentration, the main soluble carbohydrate in germinating seeds. These results confirmed that DT of tiny vetch seeds is multifactorial trait and that LEA proteins, soluble α-D-galactosides, sucrose and antioxidants are important components of protective mechanism determining DT in seeds.

4.22.

Engagment of enzymatic and non-enzymatic antioxidants in regulation of oxidative stress in layer of *Brassica pekinensis* (Lour.) Rupr. head

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Non-enzymatic antioxidants such as water-soluble vitamin C or lipid-soluble vitamin E as well as enzymatic antioxidants such as superoxide dismutases, catalase and ascorbate peroxidase contribute to defense system against oxidative stress and have been shown to fulfill many essential functions in living organisms. It is commonly known that vegetables such as cabbage are the main source of some antioxidants and therefore they become an interesting model for research leading to their better exploitation in agricultural.

Differences in intensity of oxidative stress and antioxidant system activity have been found in head of *Brassica pekinensis*. Leaves from a different layers of pekinensis cabbage heads have shown changes in H_2O_2 concentration. The highest level of H_2O_2 has been found in the central part of the cabbage head, while significantly lower concentration was noted in both outer and inner parts of the head. From all detected isoforms of superoxide dismutase only the activity of MnSOD has shown significant changes; it decreased gradually towards the central part of the head. Since MnSOD is known to be localized in mitochondria, we can suggest that in these leaves mainly mitochondrial metabolism is engaged in cousing oxidative stress. In the interior, etiolated layers of cabbage head higher level of ascorbate and ascorbate peroxidase was noted and it is probable that they may participate in lowering of oxidative stress in leaves in this part of cabbage head, while in the

outer, green light-exposed layers tocopherols and catalase are the main components involved in ROS scavenging.

On the basis of these results we speculate on the predominance of metabolic processes different layers of cabbage head.

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4.23.

Tocopherol effect on properties of model membranes of galactolipids extracted from plastids of wheat exposed to cadmium stress

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The aim of experiments was to find out whether any changes in tocopherol/galactolipids interaction can be connected with lipid composition of plastids of cells cultured on the media with/without cadmium presence. Lipids were extracted from winter wheat calli initiated from immature inflorescence. Surface pressure – molecular area isotherms were recorded with a NIMA (Coventry, U.K.) Langmuir trough (total area=300 cm^2). Surface pressure was measured using a Wilhelmy plate connected to the electrobalance. Equimolar mixed solutions of galactolipids (MGDG or DGDG) and tocopherol were prepared from the respective stock solutions.

The shape of the surface pressure – mean molecular area isotherms recorded for α-tocopherol and galactolipids spread separately at water/air interface suggests that these substances form monolayers in liquid expanded state. Lower values of the limiting area at the highest membrane packing registered for lipids cultivated on media supplied with cadmium are a consequence of their fatty acid composition – i.e. higher content of saturated acids. These lipids are also more stable against the collapse process in comparison to reference system (cultivating medium without Cd).

Addition of α-tocopherol to lipid monolayer (in molar ratio 1:1) decreases the differences between parameters of monolayer (the limiting area, the collapse pressure, the maximum compressibility modulus) formed by lipids extracted from calli cultivated on pure medium and on medium supplied with cadmium. Negative free energy of mixing obtained for equimolar mixture of α-tocopherol-galactolipid suggests that interactions between these compounds lead to formation of more stable systems.

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4.24.

Comparative effect of two selenium forms on nitrate reductase activity in maize seedlings

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The study was conducted in order to determine the effects of two selenium (Se) forms on nitrate reductase (NR) activity and biomass accumulation in maize (*Zea mays* L.) cv. Zlota Karlowa seedlings. Plants were grown 14 days in Hoagland's nutrient solution (pH 6.2) under controlled environmental conditions: temperature 25°C/20°C (day/night), 14/10 h photoperiod, and PPF 270 μmol m^{-2} s^{-1}. Selenium was added to the solution as selenite ($Na_2SeO_3 \times 5H_2O$) or selenate (Na_2SeO_4) to a final concentration of: 0 (control), 5, 25, 50 and 100 μM Se. Regardless of Se presence, NR activity was found to be higher in the maize roots than leaves. The roots of seedlings treated with the lowest selenite and selenate concentration (5 μM) had 19 and 54%, respectively, higher NR activities than control plants. However, under 5 μM Se treatment there was no significant changes in enzyme activity in leaves. Moreover, the seedlings treated with 5 μM of Se generally grew better than plants grown without Se addition. Enrichment of medium with lowest selenite rate increased fresh weight (FW) of the maize roots and shoots by 40 and 26%, respectively. The lowest selenate application induced 11 and 21% increase in roots and shoots FW, respectively. At the higher Se concentrations (25-100 μM) NR activity decreased progressively with increase in the amount of Se in nutrient solution, especially in roots where at presence of 100 μM selenite and selenate NR activity reached only 32 and 50% of control value, respectively. Moreover, maize seedlings exposed to selenite had significantly lower NR activities than those treated with selenate. The selenate application promoted plant growth also at 25 and 50 μM Se level, at which a 18-32% increase in fresh root and shoot biomass was observed. On the other hand, 25 and 50 μM selenite treatment reduced seedlings biomass by 18 and 64%, respectively. At 100 μM concentration both Se forms were phytotoxic, decreasing seedlings FW by about 90 and 40%, in the presence selenite and selenate, respectively. From these data it follows that selenite appears to be much more toxic for maize seedlings than selenate, which can be at least partially related to the changes in NR activity.

4.25.

Effect of aminooxyacetic acid and light intensity on anthocyanins, photosynthetic pigments and phenylalanine ammonia-lyase activity in seedlings of common buckwheat (*Fagopyrum esculentum* Moench)

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Effect of aminooxyacetic acid (AOA) – inhibitor of phenylalanine ammonia-lyase (PAL) on seedlings of common buckwheat (*Fagopyrum esculentum* Moench) was studiem. Germination of buckwheat seeds (cv. Hruszowska) was carried out during 4 days in thermostatic cabin at 24±1°C in darkness. From the seedlings were excised roots and hypocotyls with cotyledons were placed in water solutions of AOA (10^{-2} – 10^{-5} M). Treated with AOA excised seedlings were grown for next 4 days with a 16 h photoperiod. Chamber temperature was maintained at 24±2°C/16±2°C (day/night). Light was provided by 400 W high-pressure sodium lamps (Philips Agro). Part of seedlings were grown at light intensity 100 μmol m^{-2}·s^{-1} and another and another part at 200 μmol m^{-2}·s^{-1}.

AOA clearly decreased light-induced formation of anthocyanins and inhibited PAL activity in buckwheat hypocotyls, however enhanced anthocyanins level in cotyledons. The results are similar to obtained by Amrhein (1979). Light intensity had small influence on AOA decreased anthocyanins content. In buckwheat cotyledons AOA declined contents of chlorophylls *a* and *b*, as well as total carotenoids. The effect was much clear in higher light intensity.

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4.26.

The modification of plasma membrane H^+-ATPase activity from cucumber seedlings under cold

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Low temperature is one of the most important environmental factor limiting the growth of the plants. To adapt to cold stress, plants must perceive low temperature signal and transform it into biochemical responses. The primary site of low temperature injury is plasma membrane and cell membranes are directly involved in cold acclimation. Increase of permeability relating to membranes damage is observed in plants that have been subjected to cold. Thus, maintaining ionic balance and replenishing a loss of essential substances in repairing processes is an important issue in such conditions. Since active transport of solutes into the cell is dependent on proton motive force generated by H^+-ATPase, changes in its activity seem to be essential for plant adaptations to cold stress. In the present study we have examined alteration of plasma membrane H^+-ATPase activity in cucumber roots under low temperature. The plants were grown in low temperature for 3 or 6 days. Part of the plants after 3 days exposure to cold were transferred to control conditions (25°C) for next 3 days (post-cold, PC). The hydrolytic as well as transporting activities of plasma membrane H^+-ATPase were decreased in plants treated for 3 days with low temperature. However, the activity of proton pumps was higher in plants treated with low temperature for longer time (6 days) and in PC plants than in control plants. The level of plasma membrane H^+-ATPase mRNA was markedly decreased in roots exposed for 3 days to cold. Moreover, the increased H^+-ATPase activity in plasma membranes isolated from plants treated for 6 days with low temperature and from PC plants was positively correlated with a higher level of PM-H^+-ATPase (*CsHA3*) transcript. The Western blot analysis with the antibody against phosphothreonine showed that modification of the activity of plasma membrane H^+-ATPase under cold stress did not result from phosphorylation/dephosphorylation of the enzyme protein.

Taken together, the presented data suggested that modification of the plasma membrane H^+-ATPase activity under cold depends on the time of exposure plants to low temperature. Moreover the action of low temperature occurs at the gene expression level.

4.27.

Molecular organization of the LHCII complexes under Cu ions excess

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Cu is an essential micronutrient which plays a regulatory role in photosynthetic electron transport but its excess may change the structure and function of PSII. Our results demonstrate that treatment of *Secale cereale* plants with 50 μM Cu (as $CuSO_4 \cdot H_2O$) induces specific LHCII molecular organization responsible for effective dissipation of energy excitation. FTIR analysis show an increase in aggregated protein structure fractions giving rise of bands localized at 1626 and 1610 cm^{-1}. This specific LHCII aggregation may be based on trimeric organization stabilized by hydrogen bonds. The resonance light scattering spectra indicate that Cu-induced LHCII aggregation causes excitonic interaction between chlorophyll molecules and chlorophylls and xanthophylls producing energy dissipation. This energetic traps formation may be stimulated by changes of content of xanthophylls coupled with LHCII. Complexes isolated from Cu-treated rye leaves contain 9-*cis* violaxanthin fraction what enables contact of neighboring antennae. These modifications of LHCII molecular organization under Cu ions excess are a part of PSII adaptation to stress condition.

4.28.

Hormonal, hydraulic, and pH signals from chilled roots to shoots in maize plants

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Maize plants are often exposed to cool soil conditions in natural growth environment in the northern zones of their cultivation. Rapid and efficient root to shoot communication and an appropriate strategy in the coordination of root and shoot responses are vital for plant adaptation and survival under these conditions. The goal of the research was to determine the role of the export of abscisic acid (ABA) in the transpiration stream, of the xylem pH, and of the changes in root water potential (RWP) as active root-born signals in the root to shoot communication in maize plants under chilling stress of roots.

Research was performed on maize seedlings in a special horizontal split-root system making it possible to chill only the lower part of the root. At the 3–4-leaf stage this part of the roots was chilled up to 36 h by submersion in cool (8°C), aerated nutrient solution. During the treatment xylem sap was collected from freshly de-topped and pressurised roots. Concentration of ABA in xylem sap, roots and leaves was measured by ELISA and ABA delivery rates to shoots calculated from xylem sap flow.

The chilling of a part of the root did not cause any significant decrease in shoot water content. In spite of this RWP dropped significantly already after 2 h of root chilling and remained clearly below that of control plants for the whole treatment period. ABA levels in chilled roots increased after 4 h of treatment but much more so in the chilling tolerant genotype than in the sensitive one. Similarly, ABA concentration in xylem sap and ABA delivery rates via transpiration stream in treated plants were above those of control plants, especially in the chilling tolerant genotype. The higher ABA concentration in xylem sap as well as the higher delivery rate from roots to shoots as compared to control plants both caused a significant increase of ABA content in the leaves. The alkalization of xylem sap in treated plants additionally increased the trapping of ABA (anion trap) in xylem vessels and its transport to leaves.

4.29.

Timing of gene expression pattern in maize leaves treated with severe cold (<10°C)

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Maize yield is limited by its cold-sensitivity, molecular mechanisms of response to this stress are poorly understood. In this study, in search of fast response to severe cold, changes in the gene expression pattern during first 24 h after temperature decrease was studied by means of microarray technique.

We used oligo microarrays designed and produced by the Maize Oligonucleotide Microarray Project USA, comprising of ~48 000 oligos representing ESTs and cDNAs from The Institute for Genomic Research (TIGR). We used the CM 109 inbred line studied before in our group as a model chilling sensitive line in physiological and ultrastructural research. Plants were grown in a growth chamber (24°C/22°C day/night) till the third leaf stage. For chilling treatment, half of the plants were transferred to a cold chamber (8°C/6°C) at the end of photoperiod and the other half was kept as before as a control. Material was taken and frozen in liquid nitrogen every 200 min during dark period (3 samples) and 210 min during light period (4 samples). Every sample consisted of middle parts of three separate leaves.

Hybridizations were done in a reference design, in four biological replications with dye-swap between experiments. Isolation, purification and hybridization was done according to the procedure of the microarray supplier, with a minor modifications. The results of microarrays scanning were normalized (Lowess curve), controlled for quality (Principal Component Analysis, PCA), and statistically analyzed (analysis of variance, ANOVA) with JMP Genomics 6.0.3 (SAS Institute). The p-values were then corrected for multiple comparisons with the False Discovery Rate correction set at 0.01. The time sequence in gene expression will be shown.

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4.30.

Effect of double stress – UV-B radiation and allelochemical substance – on transcript level and activity of phenylalanine ammonia lyase (PAL) in two genotypes of cucumber

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The phenylpropanoid pathway is responsible for synthesis a large range of secondary products, which participate in plant response to stress factors (e.g. drought, salinity, high temperature, heavy metals, UV-B radiation) (Ali et al., 2006). First step of this pathway is catalysed by phenylalanine ammonia lyase (PAL, EC 4.3.1.5), that deaminates L-phenylalanine converting it to transcinnamic acid. In natural environment plants are subjected to many stresses, mostly acting simultaneously or sequentially.

The aim of the study was to demonstrate the influence of UV and allelochemical substance acting in combinations on PAL activity and its transcript level. Cucumber genotypes (cold-S – cold susceptible and cold-R – cold resistant) cultivated in a growth chambers, were exposed to UV-B (6 hours at 16 kJ $m^{-2}d^{-1}$ – additionally to PhAR light) and 2mM ferulic acid (FA) – supplied to vermiculite – and to a double stresses: UV-B plus FA acting simultaneously and sequentially. Under UV-B radiation and under both stresses applied simultaneously PAL was activated, but there was no effect under ferulic acid influence as a single stress. The level of PAL transcript was especially related to the genotype response. In cold-S genotype the ferulic acid decreased the transcript level as compare to control and UV radiated plants. Under double stress the effects were negligible. The response of cold-R genotype gene expression was stimulated under each of single stress as well as under double influence.

The activity of PAL in sequential stress combinations increased during the experiments in both genotypes, but in a different way. Additive effect independently of factor application sequencing was noted in cold-R plants but in cold-S ones similar effects were only when radiation stress was at first. Molecular mechanism of the response to sequential stresses is still in progress.

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4.31.

Comparison of Cd and Cu effect on the vacuolar ATPase in cucumber roots

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The sequestration of heavy metals within the vacuole is one of the detoxification mechanisms functioning in plant cells. Accumulation of metals could be mediated by primary (ABC transporters) or secondary (antiport with protons) active transporters. The driving force for the secondary transporters is generated by the vacuolar proton pumps: V-ATPase and V-PPase. It has been shown that activities and/or gene expression of tonoplast enzymes have been induced in response to environmental stresses including salinity, cold, anoxia or deficit of mineral nutrients. However, the regulation of vacuolar proton pumps under heavy metals remains still unknown. In this study we demonstrate the effect of cadmium and copper on the V-ATPase activity of tonoplast isolated from cucumber roots.

Six-day-old cucumber seedlings were transferred to the solution containing $CdCl_2$ or $CuCl_2$ (10 μM or 100 μM) for 2 and 24 hours. Both hydrolytic and proton pumping activities of vacuolar ATPase were significantly stimulated in tonoplast vesicles isolated from roots treated with copper whereas treatment of seedlings with cadmium decreased it. When Cd or Cu ions were included into the incubation medium the inhibition of ATP hydrolysis and ATP-dependent proton transport has been observed at metal concentration range 10–100 μM. To explain whether heavy metals could modulate the expression of genes encoding V-ATPase the levels of specific transcripts *CsVHA-A* and *CsVHA-c* were deter-

mined with semi-quantitative RT-PCR. It was shown that mRNA levels of genes encoding V-ATPase were similar in cucumber roots untreated (control) and treated with metals. The analysis of ATP concentration in cucumber roots showed that both Cd and Cu, when applied at 100 μM, diminished the level of substrate for V-ATPase. Western blot analysis with antibodies against phosphothreonine demonstrated that at least one of V-ATPase subunits may be phosphorylated in metal-dependent manner. Heavy metal-induced oxidative changes of tonoplast lipids were demonstrated by determination of the MDA content. Exposure of plants to 100 μM Cu but not Cd enhanced the lipid peroxidation in tonoplast fractions isolated from cucumber roots.

Concluding, treatment of cucumber plants with copper, contrary to cadmium, markedly stimulated V-ATPase activities. This activation was not a result of direct Cu effect on the enzyme protein and did not involve the gene expression level. We suggest that Cu ions modulate vacuolar ATPase activities indirectly via phosphorylation of some subunits or/and modification of vacuolar membrane fluidity and permeability.

4.32.

Heat stress response of short- and long-stem varieties of hexaploid triticale

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Response of two genotypes of hexaploid spring triticale (long-stem "Ulyana" and short-stem – "Bogo") under heat stress (40–42°, 3 h) was studied. According to value of the ratio of fluorescence intensity of two forms of protochlorophyllide (Pd 657/Pd 635) higher heat stability of the short-stem variety "Bogo" at a stage of etiolated seedlings was found out. The variety "Ulyana" was characterized by higher degree of aggregation Pd 657 in norm and its strong decrease at heat stress.

The green "Bogo" seedlings were differed from "Ulyana" plants by higher endogenous level of ABA, smaller size of leaves, coleoptiles and roots, and faster development. Two genotypes had the same chlorophyll content, whereas lower anthocyanins and carotenoids amounts were observed in short-stem variety. Green seedlings of the variety "Bogo" contained less products of lipid peroxidation as compared with the variety "Ulyana", that testified to the low level of the destructive processes in lipid matrix of cellular membranes. Activity of lipid peroxidation decreased in two genotypes after heat stress. At optimum temperature green seedlings of two varieties were not distinguished by the content of the soluble sugars presented mainly as monosaccharides. After heating the total amount of soluble sugars increased in both varieties approximately 1,5 times.

Strong differences in mechanisms of the stress response at level of energetic reactions in the protoplasts isolated from mesophyll of leaves were founded for different genotypes. Protoplasts of short-stem form were characterized by higher rate of oxygen evolution and absorption, and also higher relative ATP content per chlorophyll, at that 90% of ATP was detected in chloroplasts. Heating of protoplasts during 10 min at 40°C caused some growth of ATP content in short-stem variety (by 20 %) owing to activation of photosynthetic reactions, and in long-stem variety (by 15 %) – because of increase of cellular respiration.

Thus, the genotype dependence of stress response in long- and short-stem triticale varieties was shown.

4.33.

Interactions between reactive oxygen species and nitric oxide in defense responses of tomato suspension cultures to *Botrytis cinerea*

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Cross-talk among reactive oxygen species (ROS), nitric oxide (NO), and other signaling pathways in different kinds of plant-pathogen interactions has been extensively investigated recently. Generation of ROS such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) during so-cold "oxidative burst" has been considered a central event in activation of disease resistance. Studies of various plant-pathogen interactions revealed striking correlation between the profile of ROS formation and the outcome of interaction (resistance or susceptibility) in plants. Increasing evidence indicates that NO collaborates with ROS in plant disease resistance initiation and execution, however, it is still controversial, especially regarding their contribution to the defense responses to necrotrophic pathogens.

In this study we estimated of *B. cinerea* infection development and changes in ROS: superoxide, hydrogen peroxide and nitric oxide generation in tomato suspension cultures differing in susceptibility to the pathogen. The influence of elevated NO levels resulting from SNP (sodium nitroprusside) treatment on the outcome of the interaction and ROS generation was also studied.

Cell suspension cultures of tomato cultivars: -"Corindo"(C) – more susceptible to *B. cinerea* and "Perkoz"(P) – less susceptible were grown in Chandler medium supplemented with BAP (0,2 mg/cm^3) and 2,4-D (1mg/cm^3). Three-day old cell suspensions were taken to experiments; some of them were treated with SNP. Nontreated and SNP-treated cultures were inoculated with *B. cinerea* conidia. The rates of infection development were compared in the studied cell cultures in parallel with changes in ROS: superoxide anion, hydrogen peroxide and nitric oxide concentrations measured by spectrofotometric method and analysed with the confocal microscope using DAF-DA fluorescent staining.

B. cinerea infection proceeded slower in the suspension culture of cv. "Perkoz" than in the suspension culture of cv. "Corindio". Noticeable differences were observed in constitutive ROS and NO concentrations in these cultures: ROS and NO concentrations were higher in the less susceptible cultivar (P) than in the more susceptible one (C). SNP treatment markedly reduced *B.cinerea* infection development. Both kinds of tomato suspension cultures reacted to the treatment with SNP and then inoculation with *B. cinerea* with enhanced synthesis of hydrogen peroxide, superoxide and nitric oxide but these reactions were faster and stronger in cv. "Perkoz" than in cv."Corindo".

4.34.

Modification of the intercellular contacts in plant suspension cells through adaptation to environmental stress

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The cell wall-plasma membrane-cytoskeleton continuum is responsible for mechanical integration of plant cells. Long-term water deprivation leading to osmotic stress activates adaptory

mechanisms which enable cell to function more or less normally. During stepwise adaptation of tobacco BY-2 suspension cells to growth in the presence of various osmotically active agents, cell lines diverged into independent, osmoticum type-specific lines. The changes in growth patterns were accompanied by the alterations in the composition of cell walls. It was determined that cell walls of cells grown in the presence of ionic agents were homogenous, while longitudinal walls and cross-walls in cells adapted to ninionic agents were significantly different. In plants, cross-walls within cell files of axial organs exhibit specific properties that allow them to act as domains of contact and intense intercellular communication e.g. via endocytosis, and the sites of anchorage of cytoskeleton. Similar properties were also observed in cross-walls connecting cells grown in the presence of nonionic osmoticum.

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4.35.

Methyl jasmonate as potential activator of induced systemic resistance in tomato against *Fusarium oxysporum*

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Induced systemic resistance (ISR) of plant against pathogen is a widespread phenomenon that has been intensively investigated with respect to its potential use in plant protection. Among plant hormones jasmonates have been reported to play an important role; treatment of plants with these compounds increases resistance to fungal pathogens. The present study was carried out to determine whether methyl jasmonate (MeJA) (1) has an activity against fungus *Fusarium oxysporum f.sp. lycopersici in vitro* isolated from seeds of *Lycopersicon esculentum* Mill; (2) as a potential inducer of ISR is safe for treated plants; (3) had effect on enhanced tomato defense responses including antioxidant catalase, phenolic compounds and related enzyme phenylalanine ammonia-lyase (PAL), directly involved in biosynthetic pathway of phenolic compounds (4) and whether pretreatment of plant with MeJA provide significant protection against *F. oxysporum*. Application of MeJA to PDA medium at concentrations 0.01, 0.1 and 1mM markedly inhibited the spore germination, mycelial growth of *F. oxysporum*, so it may play role as a fungus development inhibitor. Pretreatment of tomato seeds with MeJA (0.01, 0.1mM) had no inhibitory effect on seed germination and seedling emergence; only at the highest concentration (1mM) this hormone had negative influence on these processes. Chlorophyll a+b and reducing sugars contents in 15 days -old seedling leaves slightly decreased only in response to MeJA at concentration 1mM. Thus, in MeJA (0.01, 0.1mM) treated plants main physiological processes are not disturbed. Moreover, pretreatment of seeds or seedlings with MeJA at all tested concentrations, also 1mM, increased contents and activities of various biochemical defencemarkers such as antioxidant catalase activity, total phenols including anthocyane accumulation and PAL activity. Seedlings from seeds pretreated MeJA at 0.1mM concentration and inoculated with *F. oxysporum* showed the best resistance against fungus, average severity index (ASI) was the lowest comparing to control plants and those pretreated with MeJA at 0.01mM. These data indicate that exogenous MeJA have antifungal activity *in vitro*, pretreatment of plants with this hormone at concentration 0.1mM provides significant protection against wilt disease caused by *F. oxysporum* probably through increasing the level of phenolic compounds, PAL and catalase activitity.

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4.36.

Effect of low light on structural and functional changes in chloroplasts induced by short-term heating

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Earlier we have shown changes in some structural and functional characteristics of pea chloroplasts induced by short-term (5 min) heating at dark at 45°C. Sizes of chloroplasts become less, grana structure is changed, photochemical activity of PSI and PSII was reduced.

White light of intensity in the range 5000–20000 lux shows a protective effect. The sizes of chloroplasts were decreased only by 5% instead of 12% at heating in dark. Distribution in yields of subchloroplast fragments obtained by digitonine becomes more similar to that observed for control. It indicates less damage of chloroplast ultrastructure. Changes in low temperature fluorescence spectra and excitation spectra of fluorescence detected at 735 nm demonstrate variations in organization of membrane pigment-protein complexes. The variations are appeared less when chloroplasts were heated at presence of low light. Low light influence on functional characteristics was as follows. Yield of PSII photochemistry was reduced only by 20-30% instead of 70-80%. For PSI it has been revealed, that all three components in the kinetics of P700 oxidation are found out as well as in the control. Intensity of P700 signal appears lower only by 40% instead of 80% as it is found out for dark heating.

Thus, low light reveals protective effect at chloroplast heating, both in relation of their structural and functional characteristics. But this factor is not able to prevent heat damage completely.

4.37.

Initial chcaracteristic of RbcX proteins from *Arabidopsis thaliana*

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In most cases, in the cyanobacterial RuBisCO operon, the *rbcX* gene is juxtaposed and cotranscribed with the *rbcL* and *rbcS* genes, which encode large and small RuBisCO subunits, respectively. It has been proved that the *rbcX* position is not random and that the RbcX protein is a chaperone for RuBisCO (Saschenbrecker et al., 2007). In *Arabidopsis thaliana* genome two genes encoding proteins of significant similarity to cyanobacterial RbcX have been found. Here we present that both genes are expressed in this higher plant. Quantitative RT-PCR experiment revealed that one of these genes in most cases is expressed at relatively stable level, while transcript level of the second one changes significantly under various stress conditions. Although there is no possibility to express properly folded RuBisCO from

higher plants in *E. coli*, we have shown that both recombinant RbcX proteins from *Arabidopsis thaliana* reveal chaperonin activity during assembly of RuBisCO from thermophilic cyanobacteria *Thermosynechococcus elongatus* BP-1 in *E. coli* cells.

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4.38.

The antioxidant systems in *Chlorella vulgaris* cells during phosphate deficiency

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Chlorella vulgaris Beijerink cultures with complete (control) or phosphate deficient (-P) liquid Knop medium were grown for 5 or 12 days. Cells from phosphate deficient cultures displayed symptoms of increased oxidative stress, e.g., higher level of hydrogen peroxide and of the lipid peroxidation products. In -P cells the activities of superoxide dismutase, ascorbate peroxidase, and the total ascorbate (ascorbate + dehydroascorbate) level were higher than in control cells. The 5-day old cultures, in contrast to 12-day old, from deficient medium displayed an enhanced excretion of vitamin C components (mainly dehydroascorbate) into the culture media. It is concluded that insufficient phosphate feeding, which limits growth and photosynthesis, intensifies the oxidative stress in cells. The efficiency of antioxidative mechanisms in *C. vulgaris* stressed cells may be limited by the absence or low activity of dehydroascorbate reductase, an enzyme in the ascorbate-glutathione cycle, that catalyzes the reduction of dehydroascorbate to ascorbate. The potentially low activity of dehydroascorbate reductase in 5-day -P cells, may be a causal factor resulting in a relative increased dehydroascorbate excretion into the media. After prolonged phosphate deficiency, i.e., up to 12-day P_i limitation, the ascorbate-glutathione cycle or the alternative pathway(s) with participation of monodehydroascorbate reductase which, directly utilizes NADPH as reductant, have an increased activity.

4.39.

Internalization of lead with low-esterified pectins in tip growing moss protonemata. Lead sequestration compartments

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Pb remobilization from the cell wall (CW) by internalization with low-esterified pectins (JIM5-P), was the main aim of the study. In addition , the final compartment for lead sequestration was identified. The object of the study was the tip-growing apical cell of *Funaria hygrometrica* protonemata – exposed to 1mMPb, 4h. Final Pb sequestration compartments were detected in pulse-chase experiments.

Pb exposition resulted in common internalization of JIM5-P from CW. Pb bound to JIM5-P was remobilized from CW, together with this compound and transported via the endocytic pathway into plant protoplast. The main compartments for final Pb sequestration were CW, its thickenings (CWT) and vacuole (V). In CW and CWTs Pb was separated from protoplast by the callose layer.

Detection of Pb internalization together with JIM5-P showed that Pb deposition in CW is not as safe for plant cells as previously supposed. Successful lead sequestration in the apoplast is possible because plant cells possess the mechanism(s) protecting them from Pb returning, e.g. separating removed Pb from the protoplast by the callose layer. The obtained results shed a new light, both on the heavy metal uptake problem and on the role of CW in plant cell tolerance strategy.

4.40.

Low-temperature and (+/-)menthol induced transmembrane potential changes in the liverwort *Conocephalum conicum* L.

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(+/-)Menthol belongs to the group of "cooling compounds", which are cold receptor agonists in animals (Patapoutian et al., 2003). To check if it evokes "cold sensation" in plants, the effect of (+/-) menthol on the transmembrane potential changes in *Conocephalum conicum* was examined. *Conocephalum conicum* is one of the philogenetically oldest terrestrial plants and, because of its excitability, it is a very convenient object of electrophysiological research. It generates action potentials (APs) in response to depolarizing current, illumination, and sudden cooling. Plant responses evoked by (+/-)menthol were compared with those evoked by cold stimuli. It has been shown that (+/-)menthol causes generation of one AP at 1 mM concentration and two or more APs at 10mM. A temperature drop always evokes only one action potential irrespective of the strength and rate of cooling. Incubation of plants in 11°C or 5°C standard solution in most cases does not inhibit APs evoked by menthol, and it does not always suppress AP responses to cold, either.

The liverwort with anion channels blocked by antrahence-9-carboxylic acid (A9C) and potassium channels blocked by tetraethylammonium (TEA) exhibits approximately 22 mV depolarization of the resting potential in response to 10 mM menthol. In the same conditions cooling evokes dose-dependent voltage transient responses (VT). Blocking of only potassium channels resulted in slow long-lasting higher depolarization by menthol (36 mV) and similar long-lasting depolarization caused by cooling. Those changes last during the action of both stimuli; after ceasing of cooling or menthol washout the resting potential returned to the initial value.

Differences in electrical responses to cooling and menthol indicate that different membrane transporters are activated by these two treatments.

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4.41.

Influence of lead and nickel ions on oxidizing processes in seedlings of winter rye

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Increasing input of heavy metals (HM) in an environment leads to soil pollution which, in turn, affects plants at a cell, tissue, organism and population levels. Toxic effects of HM are especially essential in the case of cultivated plants grown near to large cities. The central role in cell reactions to stress factors play oxidizing processes occurring as a result of enhanced formation of reactive oxygen species (ROS) – superoxide radical O_2^{-*}, hydroxide radical OH^*, hydrogen peroxide H_2O_2, and their derivatives. These ROS can react with many molecules in cells, leading to their oxidation. In order to study the relevance of HM ions in oxidative stress, winter rye (*Secale cereale* L., cv. Relay-race of Tatarstan) seedlings were grown on distilled water (Control) or on solutions of lead and nickel salts (Pb $(NO_3)_2$ and $NiSO_4$) at various concentrations (1 mmol/l; 100 and 10 μmol/l) in growth chamber with a 12 h photoperiod at a light intensity 200 μmol $m^{-2}c^{-1}$ at 22°C. After 7 days lipid peroxidations (LPO) as well as the rate of superoxide anion (O_2^{-*}) generation was determined in leaves of the seedlings.

It was revealed that both metals lead to increased LPO level in all variants. At lowest concentration of Pb the increase in the malone dialdehyde (an end-product the LPO) contents was 75% as compared with control, and in case of nickel it was only 25%.

In the case of superoxide anion, at the micromolar concentrations of HM, the rate O_2^{-*} generation was on control plants level (in case of Ni^{2+}) or a little above (in case of Pb^{2+}). However, the rate of O_2^{-*} generation decreased to 57% at high concentration of Pb^{2+}, probably, because of reduction in the oxygen-consuming and O_2^{-*}-generating enzyme activities.

Thus, oxidative stress symptoms in rye seedlings were caused by lead and nickel ions (in micromolar concentrations). These symptoms are: an increased LPO level and enhanced ROS generation. Stronger effect of Pb^{2+} in comparison with Ni^{2+} was found.

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4.42.

Heavy metals cross-talk in *Pisum sativum* L. root cells

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Heavy metals derived from various anthropogenic sources (industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps and mining operations), have progressively affected the environment and ecosystems. Of major concern with respect to plant exposure as well as human food-chain are the metalloids: arsenic (As), selenium (Se), and the metals cadmium (Cd), mercury (Hg), and lead (Pb). Heavy metals can influence on physical and chemical processes in living organism. One of the effects of heavy metals is increased generation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\cdot$), which results in oxidative stress. At high concentrations, ROS have negative influence on the function of all cell organelles. They damage major cell components: proteins, lipids and nucleic acids. Plant cells can tolerate ROS by endogenous protective mechanisms involving antioxidant molecules (ascorbate, cysteine, glutathione, phytochelatins and α-tocopherol) and enzymes (superoxide dismutases SOD; catalase CAT; ascorbate peroxidase APOX; glutathione reductase GR), imbalance between production and quenching of ROS leads to plant damage breaking down the defense system of cells.

The goal of our study was to broaden our knowledge about processes, which occur in cells of plant roots exposed to four different heavy metals. The heavy metals (Cd, Pb, Cu, Zn) absorbed by the roots brought about oxidative stress conditions through the ROS production for the *Pisum sativum* L. plants cultivated hydroponically for 96 h on a Hoagland medium with the addition of 100 μM: $CdCl_2$, $Pb(NO_3)_2$, $CuSO_4$, $ZnSO_4$.

The alterations in $O_2^{-\cdot}$ and H_2O_2 concentrations were monitored spectrophotometrically showing a rapid increase of $O_2^{-\cdot}$ production during the initial 24 h, and in case of H_2O_2 – during the 48 h of cultivation. The maximum level of $O_2^{-\cdot}$ we shown in plants treatment Cu and Cd ions and the maximum level of H_2O_2 -in plants exposure for Cu and Zn. The production of $O_2^{-\cdot}$ and H_2O_2 was visualized by means of fluorescence microscope technique. As stress intensity raised so did the activities of SOD, CAT and GR antioxidative enzymes. and of low-molecular antioxidants, particularly glutathione (GSH), homoglutathione (h-GSH) and cysteine substrate towards their synthesis. We shown changes in redox state (GSH/GSSG) in root cells in *Pisum sativum* L. grown with heavy metals: Pb, Cu, Cd, Zn.

4.43.

Dark-chilling induced lipoxygenase 6 association with thylakoid membranes in the common bean (*Phaseolus vulgaris* L.)

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It has been demonstrated before that low temperature induced changes in chloroplast structure and function of chilling sensitive (CS) plant species caused by arrangement of chlorophyll-protein complexes inside thylakoid membranes (Garstka et al., 2005, 2007). We found out that in CS common bean (*Phaseolus vul-*

garis L.) dark-chilling stress induces association with thylakoid membranes of the lipoxygenase 6 (LOX6), which is probably involved in oxylipin synthesis against wounding and non-host pathogen infection (Porta et al., 2008).

For detailed analysis of LOX6 we carried out both molecular biology (immunodetection, mass spectrometry and northern-blot) and microscopy (electron microscopy with immunogold labeling) techniques. Performed analysis has shown increased *PvLOX6* mRNA and LOX6 protein levels in thylakoids during dark-chilling. Furthermore we observed reversal changes in LOX6 molecular weight. Microscope images confirmed chloroplast localization of bean lipoxygenases.

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4.44.

Function and properties of root cell walls under salinity conditions

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Ion exchange properties and swelling capacity of suaeda (*Suaeda altissima* L. Pall), spinach (*Spinacia oleracea* L., Matador cv.) and chickpea (*Cicer arietinum* L.) root cell walls were investigated under different salinity condition. It was found that, there are 3 cation-exchange groups (two types of carboxyl groups and phenolic group) and one anion-exchange group (amine group) in the plant root cell walls. The total quantities of cation exchange (S_t^{cat}) and anion exchange (S_t^{an}) groups were determined, and the quantity of functional groups of each type (ΔS^j) was estimated, and the corresponding values of pK_a^j were calculated. The S_t^{cat} and S_t^{an} as well as ΔS^j were demonstrated to be are stable at any pH and electrolyte concentration (C^{Na+}) of the medium. The values of pK_a^j and ΔS^j indicate that the root cell walls of tested plants are identical in qualitative structure of ionogenic groups but vary in the quantity of each ionogenic group. It was shown that for all types of cation exchangeable groups arranged in cell wall structure the acid properties are enhanced by the increasing of electrolyte concentration. It was found that swelling of root cell walls changes with pH, C^{Na+} and strongly depends on plant species. In the pH and C^{Na+} range covered the swelling coefficient varies in the following sequence: chickpea spinach suaeda. The results indicate that for tested plant species such sequence of change in swelling is connected with different cross-linked degree of the polymers in cell wall structure, different summarized quantity of carboxyl groups and different total quantity of functional groups. Based on known data and experimental results of present study it was concluded that the change in cell wall swelling in response to variances of environmental or initial conditions leads to the change in volume water flow through the plant roots. The results of this investigation point to the fact that both the transport of mineral solutes within the apoplast and the volume water flow through the plants are highly sensitive to environmental influence on the ion exchange properties of plant root cell walls.

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4.45.

An attempt to understand the mechanisms of structural changes in cold-sensitive and cold-tolerant plants' chloroplasts under dark-chilling conditions

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Low temperature is one of the most important factors affecting plant growth. It is known that a long-term exposure of chilling-sensitive plants to low positive temperature induces several destructive changes in the chloroplast ultrastructure combined with rearrangement of chlorophyll-protein complexes (Garstka et al., 2005, 2007). Ultrastructural changes in chloroplasts caused by physiological fluctuations of temperature are not well-known yet. Therefore an attempt was made to analyze these changes in bean (a chilling-sensitive plant) and pea (a chilling-tolerant one) during periodic chilling stress. Both plant species were exposed to low temperature (5°C) at night while the daytime temperature in the climate room was optimal (22°C).

The observed changes were related to substantial differences in starch content, swelling of chloroplast lamellae, numbers and structures of grana, and the content of plastoglobules. Significant modifications were observed in bean. In control conditions bean chloroplasts showed barely distinguishable grana filling the chloroplast stroma. On the third day of dark-chilling stress an increase of unstuck thylakoid membranes was noticeable as well as emergent irregularity of grana composition. During the chilling process the content and size of plastoglobules increased and swelling, disruption and degradation of chloroplast membranes were also noticeable. Pea, the chilling-tolerant plant, underwent quite a similar structure rearrangement but on a very low scale and only after several days of stress. In pea, ultrastructural changes were suggested to represent an adaptive response of the plant rather than a destructive one, just like in long-dark-chilling stress described in previous study (Garstka et al., 2005, 2007).

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4.46.

Pharbitis nil metallothionein-like-gene type 2 and its potential in phytoremediation

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Because of the toxicity of heavy metal there is urgent necessity to develop efficent and cheap methods of soil remediation. The conventional methods of remediation are rather ineffective and expensive, and often may destroy natural habitats. As a result some alternative methods have arisen. One of this method is phytoremediation. This method comes in several forms like phytoextraction, phytostabilization or pytovolatilization (Padmavathiamma, and Li, 2007).

Metallothioneins (MTs) are low molecular weigh, cysteine-rich metal binding protein. They are one of the best-characterized heavy-metal binding ligands. MT genes have been found through-out the animal and plant kingdoms as well as in some prokaryote species. Based on the arragement of the cysteine residue in plants MTs are classified into four types. In animals MTs protect against cadmium toxicity, but their function in plants is still elusive. Exposure of plants to various heavy metals increases the expression of MT genes. Probably MTs confer resistance to heavy metals in intact plant cells. The manipulations of MT genes expression may be potential mechanism for increasing the remedial capacity of plants (Cobbett and Goldsbrough, 2002).

Primary results suggest that *Pharbitis nil* metallothionein-like-gene type 2 is involved in heavy metal detoxification and it may be used for creating transgenic plant with phytoremediation potential.

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4.47.

Heavy metals, redox perturbations and plant growth regulators alter the expression of newly identified PDR genes in cucumber

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The ABC transporters found in bacteria, fungi, plants and animals form one of the largest protein families that is involved in the transport of a wide range of substances across cell membranes. In plants the family is considered to be relatively large as in *Arabidopsis* and rice it is composed of more than 120 members. Among them, the proteins belonging to the PDR (*Pleiotropic Drug Resistance*) subfamily have been found in plants and fungi but not in prokaryote or animal species. Most of the PDR genes characterized so far in plants have been shown to encode proteins involved in the response to abiotic and biotic stresses. It was shown that some of them might be implicated in the transport of antifungal agents and auxin-related herbicides (2,4-D). The others seem to contribute in plant tolerance to heavy metals (Cd, Pb, Zn) as well as to redox perturbations. The complete or partial ESTs encoding PDR-proteins is known only for *Arabidopsis, Nicotiana*, rice and citrus plants so the function of these proteins in other plants, particularly in agricultural crops, is currently unknown.

In yeasts, all identified PDRs have been localized to the plasma membrane, so it is assumed that these proteins constitute an active excretion systems involved in the detoxification of yeast cells from various toxic compounds. Searching for such type of system in plant cells, we have indentified first two ESTs encoding PDR genes in cucumber. The amino acid sequences predicted on the basis of two cDNAs were named *CsPDR8* and *CsPDR12* according to their closest *Arabidopsis* homologs, AtPDR8 and AtPDR12. The highest expression of *CsPDR8* was observed in roots of both 5-day-old seedlings and 3-week-old plants, while *CsPDR12* expression level was equal in the roots and shoots of cucumbers. Heavy metal effect on the level of both transcripts was tested in plants grown in the presence of Pb, Cd, Cu, Zn, Ni or Mn. In addition to this, the expression of genes was analyzed under salt, osmotic and redox stresses as well as in response to the plant growth regulators (salicylic acid, ABA, IAA, kinetin) and week organic acids. All analyses were performed using real-time PCR and different reference genes were evaluated for the normalization. We assume that this work provides a basis for further functional studies of CsPDR8 and CsPDR12 in cucumber.

4.48.

Enzymatic and non-enzymatic antioxidants pattern in different leaf layers of white cabbage head

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Parallel to chlorophyll decline in the interior leave-layers of white cabbage head (*Brassica oleracea* var. *alba f. capitata*) take place the differentiation of the level of reactive oxygen species (ROS) as well as activities of antioxidant system (superoxide dismutase - SOD, catalase, peroxidases) and vitamins C and E. In outer, light-exposed layer high level of H_2O_2 was stated, while the highest level of superoxide radical only in inner part of cabbage head was found. Mn-SOD form activity was high in central and inner layers, and parallel, the highest catalase activity in outer and inner parts of cabbage head was found. These results point out to higher level of photorespiratory and respiratory processes, respectively, in these parts of the cabbage head. The another H_2O_2 scavenging enzymes – peroxidases, showed the highest level in light-exposed layer in comparison to central and inner layer. In outer layer of cabbage head the higher level of vitamin E in comparison to inte-

rior parts (central and inner) was stated suggesting their involvement in chloroplast protection. In contrast to this, the higher level of vitamin C in interior leaf-layers suggest its key role in plant development. Our results lead to the conclusions that the alteration of ROS level and chloroplastic, peroxisomal and mitochondrial processes probably due to the stage of tissue development as well as tissue specific stress response.

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4.49.

The local metal tolerance adaptations in different metallicolous populations of *Armeria maritima*

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Armeria maritima (Mill) Willd. (*Plumbaginaceae* L.) is a pseudometalophyte species for which the characteristic trait is a grate polymorphism. Its taxonomy cover three, well defined subspecies (ssp. *maritima*, ssp. *elongata*, ssp. *alpine*) and eight, morphologically ill-defined, transitional forms of the formers. Five of them (ssp. *halleri*, ssp. *bottendorfensis*, ssp. *hornburgensis*, ssp. *calaminaria*, ssp. *eifeliaca*) together with the unclassified population of Plombieres represent metallicolous ecotype of *A. maritima*. The study of similarities and differences between different metallicolous populations and closely related to them nonmetallicolous populations of *A. maritima* presents a source of valuable knowledge about local metal tolerance adaptations in plants.

In the experiment two different metallicolous populations (ssp. *halleri*, population from Bolesław near Olkusz, Poland and population from Plombieres, Belgium) were analyzed and one nonmetallicolous population (Manasterz near Jarosław, Poland), closely related to both metallicolous populations, were used as a control. Plants of all populations were received from seeds collected in natural habitant of each population. After 9 moths of growth in garden soil, under greenhouse conditions, plants were transferred to Hoagland nutrient solution and after 1 week of adaptation treated with Zn (1000 and 2000 μM), Cd (8 and 16 μM) and Pb (15 and 30 μM) ions, for 2 weeks. In harvested plants there were analyzed Zn, Cd and Pb ions accumulation and translocation between roots and shoots. As a control for this analyze plants of all populations growing on garden soil, without metal addition, were used. Additionally, roots and leaves plants of all populations treated with higher metal dozes (2000 μM Zn, 16 μM Cd or 30 μM Pb) were used for Zn, Cd and Pb ions localization at the ultrastructural level.

4.50.

Occurrence of poly(ADP-ribose) polymerase in *Phaseolus coccineus* (L.) plants exposed to cadmium

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Poly(ADP-ribose) polymerase (PARP) can contribute to both DNA repair and plant cell death. PARP is activated by DNA strand breaks or kinks. Using the immunogold methods, we decided to check the occurrence of this enzyme in *Phaseolus coccineus* plants treated with Cd in two growth stage of their primary leaves. The young plants were treated with 25 μM Cd when the seedlings were being transferred into the nutrient solution. The older plants were exposed to Cd after 10 days of their growth in the nutrient medium. The immunolocalization of PARP was assessed in the leaves of runner bean plants after the 12th day of their exposure to the metal.

In young control plants, very weak immunopositive reaction appeared. Single immunogold particles were localized in the chloroplasts and nuclei. In young plants exposed to 25μ M Cd, the reaction in these organelles was stronger in comparison with the control and the immunogold particles were also found in the mitochondria.

In older control plants, the immunolabelling towards PARP was observed in the nuclei, chloroplasts and vacuoles. In the leaves of older plants exposed to Cd, the immunogold particles were distributed in a similar way as in the control, but a small number of them was found in the vacuoles and nuclei. However, single particles were also observed in the electron dense cytoplasm and condensed mitochondria.

4.51.

Nitric oxide affects oxidative stress caused by cadmium in roots of soybean seedling

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The excess of heavy metals increases levels of reactive oxygen species (ROS) which can cause severe damage to plant cells. Therefore one of the metal-induced stress response is the activation of several elements of antioxidant system. Recently, it has been shown that nitric oxide (NO) can alleviate the oxidative stress caused by various stress factors, including heavy metals.

The primary aim of our study was to characterize the role of NO in metabolism of ROS induced in plant by heavy metals. In present work we have analyzed the effect of NO on the accumulation of superoxide anion radical ($O_2^{\cdot-}$) and expression of superoxide dismutase (SOD) on the levels of mRNA and enzyme activity

in roots of soybean (*Glycine max* cv. Navico) seedlings treated with Cd^{2+} ions.

To examine whether NO has an impact on antioxidant system in soybean roots, two- day old seedlings were pretreated with 10 μM sodium nitroprusside (SNP) for 6h and then were transferred to cadmium ($CdCl_2$) solutions for 6h, 24h and 48h. Two concentrations of the metal were used: 10mg/l and 25mg/l.

Seedlings treated with increasing concentration of Cd^{2+} ions showed enhanced O_2^- accumulation, especially during the first 24h of stress duration. The effect of NO on $O_2^{\cdot-}$ level varied, depending on the duration of stress. NO caused enhanced O_2^- accumulation in seedlings treated with cadmium during first 6h, and at the next time points (24h and 48 h) the level of O_2^- significantly decreased as compared to plants treated only with cadmium. The analysis of Cu/Zn-SOD and Fe-SOD activity showed an induction of enzyme in response to Cd^{2+} ions, which was most pronounced at 48h of treatment. In turn, NO donor application resulted in a marked increase of activities of both SOD isoforms, that were higher than in plants treated with Cd^{2+} only. The observed changes were accompanied by modulation of SOD at the transcript level.

Summing up, the diminished level of superoxide radical at the later stages of stress duration in SNP pretreated plants was correlated with enhanced SOD activity. It may be concluded that exogenous NO induce specific signaling pathway towards protective plant responses to heavy metal stress.

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4.52.

Heavy metal tolerance, accumulation and detoxification in *Pisum sativum* plants

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Increased pollution due to heavy metals place human health at risk and it is responsible for several environmental problems, including the decrease of microbial activity, soil fertility and crop yields. The accumulation of heavy metals in vascular plants provokes significant biochemical and physiological responses, modifying several metabolic processes; photosynthesis, reduces the respiration rate, affects membrane structure and permeability and upsets mineral nutrition. Toxic metallic ions penetrate cells using the same absorption processes of essential micronutrient ions. In *Pisum sativum* plants was observed the effects of heavy metal toxicity at the level of nutrients (P, K, Ca, Mg and Cu). Cu, Zn, Cd and Pb excess disturbed nutrition contents in pea roots. The presence of Cd caused decreased of potassium and phosphorus signal intensity comparing to control, and increase of signal intensity for calcium, magnesium and cooper. In plants exposed to lead was observed decrease of K, P, Ca, Cu intensity and increase only of Mg signal intensity comparing to control plants. The LA ICP MS analysis allowed determining metal distributions in roots tissues. The peaks represent cadmium and lead were mostly localized in the epidermis and endodermis of cross-section through hair roots zone in all treated plants. In pea plants treated with lead and cadmium ions over 80% of metal was accumulated in roots. The most of Pb was localized in insoluble fraction of cell walls and cell membranes. Defence of plants against heavy metals involves the activation of a range of various mechanisms resulting in the modification of ion mobility within the rhizosphere, active exclusion of heavy metals from cells or synthesis of bioligands. In plants treated with metals was observed increase of γ-glutamylcysteine synthetase activity, increase of GSH and hGSH level, biosynthesis of phytochelatins (PCs) and formation of PC-metal complexes The metal-phytochelatin complexes were determined using HPLC-ICP-MS technique. Identification of phytochelatins was carried out with ESI-MS spectra obtained for the whole sample. Based on the *m/z* ratios were identified reduced and oxidized form of phytochelatins (PC_2, PC_3 and PC_4). Moreover, ESI MS mass spectra confirmed presence of homophytochelatins in *Pisum sativum*.

4.53.

Effect of cadmium on gene expression and activity of adenylate and guanylate cyclase in *Arabidopsis thaliana* seedlings grown *in vitro*

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Cadmium (Cd) is an important environmental pollutant with high toxicity to animals and plants. Cd damages the photosynthetic apparatus, disturbs the carbohydrate metabolism, reduces the nitrate absorption and its reduction, interferes with water balance, and inhibits several enzyme activities. In addition, Cd-induced oxidative stress would also account for the cellular toxicity of this heavy metal.

According to our knowledge, there are no available information about effect of cadmium on cAMP and cGMP activity and their transcript levels in plants. Till now, intercellular cAMP and cGMP were determined as the response of cadmium stress only in rats salivary gland. However, the molecular mechanism used by plant cells to counter cadmium toxicity is not well understood.

In this work the level of mRNA of adenylate and guanylate cyclase was determined. The expression of these genes was investigated in 9-days old seedlings of *Arabidopsis thaliana* grown in sterile liquid culture with constant, uniform fluorescent light and temperature. Plants were treated with 50 μM $CdCl_2$ for 0.5, 1, 3, 6 and 10 hours. Content of mRNA was determined by real-time PCR.

The highest stimulation of adenylate cyclase gene expression was observed after 0.5 hour in seedlings and it was approximately twofold higher than in control. Next mRNA level decreased and after 10 h was fourfold smaller than in control. In case of guanylate cyclase gene expression a drop of mRNA level was noticed. After 10 h it was about threefold lower than in control plants.

More details which are concerned on effect of cadmium on adenylate and guanylate cyclase activity will be presented and discussed.

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4.54.

β-1,3-glucanase accumulation in roots of maize and soybean exposed to lead, cadmium and arsenic

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Most crop plants are sensitive to higher concentrations of heavy metals in soils. Such contamination can cause serious damages in plants including poor growth, defects in respiration and photosynthesis, inactivation of enzymes. On the other hand, plants possess a broad scale of different mechanisms that are connected with processes of detoxification. The activity of β-1,3-glucanase in roots of soybean (*Glycine max* cv. Korada) and maize (*Zea mays* L. cv. Quintal), treated with lead (500 mg.l^{-1} Pb^{2+}), cadmium (300 mg.l^{-1} Cd^{2+}) and arsenic (100 mg.l^{-1} As^{III}) was studied in context of heavy metal impact on plant tissue such as cell viability, lignification and callose content. Two days after application of heavy metals on roots, strong damage of plasma membrane was observed in all tested plants as a typical symptom of heavy metal toxicity. Further, enhanced lignification and callose deposition in roots indicated formation of a barrier against metal entrance. In roots of maize two additional isoforms of β-1,3-glucanase was detected with aproximatly molecular mass 34 and 36 kDa. Native PAGE showed that there was acidic isoforms of these enzymes. This suggests that plant glucanases might be involved in defense mechanisms of plants growing in heavy metal polluted soils.

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4.55.

Capacity of several shrubs species for PMs accumulation and efficiency of their photosynthesis apparatus

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Air pollutants caused by urbanization exert harmful effect on human health. One of the most dangerous of these pollutants are particulate matters (PMs). They can be suspended in the air even for weeks as aerosols, and when inhaled can have carcinogenic, allergic and mutagenic effects. It is estimated, that PMs reduce average life expectancy in E.U. by ~ 1 year and in Poland even by 2 years (EEA Report 1/2007). The only opportunity to lower/remove PMs from atmosphere in open space is via environmental biotechnology – phytoremediation in which plants are employed to clean up the environment. Common is opinion that air pollution has also negative effects on efficiency of photosynthetic apparatus but still there are no data towards such effects of PMs. In this study we attempt at evaluation of (i) efficiency of photosynthetic apparatus and (ii) deposition of PMs on leaf surface of shrubs growing in 2-3 locations differing in level of air pollution.

Forsythia × *intermedia, Hedera helix, Parthenocissus tricuspidata, Spiraea salicifolia*, and *Taxus baccata* growing in city center, university park and nursery were used. For all the species intensity of photosynthesis and transpiration, stomatal conductance, chlorophyll content and fluorescence of chlorophyll *a* were measured 3–4 times during growing season 2008. Also amount of PMs deposited on leaf surface were also determined (for 2 fractions and in three filter pore size categories).

Intensity of photosynthesis and transpiration, chlorophyll content and Performance Index were usually lower in shrubs growing in city center. Amount of PMs deposited on leaves shrubs growing in city center was usually greater and this well corresponded with lowered efficiency of photosynthetic apparatus of these shrubs. Results shows also that there were differences between species in capacity of PMs accumulation.

Shrubs growing in more polluted sites characterize lower efficiency of photosynthetic apparatus and it is most probably that it can be attributed to greater, in this location, PMs deposition on leaf surface.

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4.56.

How do chloroplasts cope with stress?

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Chloroplasts perform a wide variety of metabolic processes which include photosynthesis and amino acid and fatty acid biosynthesis. They conserve many bacterial systems implicating its origin from symbiosis between an ancestral host cell and a cyanobacterium-like photosynthetic prokaryote. Recently, a stringent control system similar to that of bacteria has been suggested to operate in chloroplasts of photosynthetic eukaryotes.

In bacteria, the stringent response is a pleiotropic regulatory mechanism triggered by various nutritional and metabolic stresses including amino acid starvation and limitation of carbon, nitrogen and phosphate. The effector molecules of this process are unusual hyperphosphorylated guanosine tetraphosphates (ppGpp). In *E. coli*, ppGpp synthesis and degradation are the primary functions of two proteins – RelA and SpoT respectively (Dąbrowska et al., 2006a). It was believed that the stringent response was limited to the prokaryote kingdom, however, plant homologues to these bacterial stress proteins (RSH proteins – RelA/SpoT Homologues) and products of their activity (ppGpp) have been recently identified in plants. What is more, levels of ppGpp increased markedly in plants subjected to abiotic stresses such as wounding, high salinity, acidity, heavy metal, drought and UV irradiation (Dąbrowska et al., 2006b).

Previously we have reported identification of three cDNA sequences coding for *Pharbitis nil* RSH proteins. The aim of the present work was expression profiling of *P. nil* RSH genes in response to heat shock and wounding as well as estimation of their mRNAs level in vegetative and generative organs of *P. nil* seedlings and adult plants.

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4.57.

The response of proline metabolism enzymes to lead in lupin roots

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Abiotic stresses cause significant increase in the proline concentration in a large variety of plants. In plants proline is synthesized from either glutamate and ornithine. The first two steps of proline bisynthesis from glutamate are catalyzed by pyrroline-5-carboxylate synthetase (P5CS, EC 2.7.2.11), whereas from ornithine by ornithine aminotransferase (OAT, EC 2.6.1.13). Proline degradation is catalyzed by proline dehydrogenase (PDH, EC 1.5.1.2), which is also a determinant enzyme in proline accumulation.

The purpose of our study was to investigate the effect of lead ions on the proline level and the activities of key enzymes involved in proline biosythesis and degradation in lupin roots (*Lupinus luteus*). The germinated seeds were transferred to dishes containining 5 ml of either bi-distilled water or one of the aqueous solutions of $Pb(NO_3)_2$ with the concentrations of Pb^{2+}: 50, 100, 150, 200, 250, 300 and 350 mgl^{-1}. The proline content in roots was determined by the method of Bates et al. (1973). The activity of enzyemes w assayed by the following methods: P5CS – Vogel and Kopac (1960); OAT – Mazelis and Fowden (1969); PDH – Miler and Steward (1976).

The activity of P5CS in roots reached the maximum when the application of lead was at the level of 100 mgl^{-1} and then it declined slowly with the increasing concentration of metal. The highest activity of OAT appeared at 50 mgl^{-1} Pb^{2+} whereas at 250 mgl^{-1} Pb^{2+} decreased below control level. Proline content in lupin roots treated with lead decreased dramatically at the lowest metal concentration. This effect was observed despite the fact that the activity of enzymes resposible for proline synthesis was elevated at the same lead dose. At higher lead concetrations further decrease in proline level was noticed. The activity of PDH, which is involved in proline oxidation, increased at concetrations between 100–200 mgl^{-1} Pb^{2+}. These data suggest that the low concentration of proline in lupin roots exposed to lead might be a result of both inhibition of proline synthesis or stimulation of its degradation by metal. Our study doesn't support the generalized view that proline accumulation is a common response of plants to abiotic stresses.

4.58.

Study on the influence of AhHMA4 expression in tobacco on Zn homeostasis

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Plant transformation is a common tool not only in gene function investigation but also in modification of physiological processes for biotechnological purposes. The emerging phenotype results both from the function of the introduced gene, and from the alteration of the expression of the host plant endogenes. Although the knowledge of the interaction between the transgene and the endogenes is rudimentary, it is believed that different genetic background of distinct species transformed with the same gene may contribute to frequently observed generation of contrasting phenotypes.

In our study *AhHMA4::AhHMA4*, a heavy-metal transporter belonging to P_{1B}ATPases, was used for tobacco (*N. tabacum* v. *Xanthi*) transformation. In *A. hallerii* it is required for zinc hypertolerance and hyperaccumulation. Its introduction to Zn-sensitive *A. thaliana* generated Zn- hypertolerant and Zn-hyperaccumulating phenotype (Hanikenne et al., 2008). However, expression in tobacco caused increased sensitivity to zinc excess which was not accompanied by enhanced zinc accumulation.

To reveal the mechanism behind the phenotype of transgenic tobacco and gain insight into plant Zn homeostasis, first we checked whether the expression of *AhHMA4::AhHMA4* in tobacco is regulated by Zn level in the medium. Secondly, we examined if the expression of *NtIRT1* was modified by *AhHMA4* expression. It is known, that *IRT1*, the member of ZIP family, mediates the uptake of Fe^{2+}, Zn^{2+} and Cd^{2+} in various organisms, therefore it was of interest to learn, whether its expression is modified in transgenic tobacco, and could contribute to the detected phenotype. All experiments were performed on transgenic and wild-type tobacco exposed to a range of Zn concentrations (0 μM, 0,5 μM, 10 μM) in Knop's medium.

Our results demonstrate that *AhHMA4* in transgenic tobacco, similarly as in *A. thaliana* and *A. halleri*, is dependent on zinc availability. Furthermore, we showed that Zn-dependent pattern of *NtIRT1* expression was different in both transgenic and wild-type tobacco Although the expression level was low in both plants grown in Zn-deficiency, after their transfer to 10 μM Zn it decreased in transgenics whereas remained unchanged in wild-type Xanthi.

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4.59.

Estimation of weeds resistant to herbicides using isothermal calorimetry and Raman spectrometry

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Derivatives of arylfenoxyacids and sulfofenylurea are active chemical compounds in herbicides named Puma and respectively Glean 75 WG. In connection to the fact that weeds have recently acquired greater resistance on herbicides the purpose of this work was to investigate the influence of fenoxaprop on metabolism of *Lolium rigidum* seedlings and sulfochloride on the leaf of *Centaurea cyanus* in biotypes of different degree of resistance on herbicides. General metabolism of plants was determined by the use of isothermal calorimetry. Additionally changes in chemical composition which occurred in leucoma of *Lolium* seedlings under the influence of fenoxaprop were identified by the use of FT-Raman spectrometry.

Lolium seeds of susceptible and resistant biotypes were germinated by 96 hours and afterwards seedlings were put to calorimetric measurements on water or herbicide by 72 hours. Fenoxaprop strongly affected the intensity of metabolism process course in both *Lolium* biotypes. The pattern of curves illustrating changes in specific heat production rate was in initial phase of growth on fenoxaprop similar in both biotypes. However in resistant biotype between 4 and 12 hour much intense increase in value of heat production rate than in susceptible biotype with relation to control (growth on water) was observed. In later phase of seedlings growth reduction value of heat production rate with relation to control was almost two times bigger in susceptible biotype in comparison to resistant one. However in case of seedlings of resistant biotype fluctuation and increase of heat production rate, as compared with susceptible one, were observed. It can indicate that in case of susceptible biotype fenoxaprop caused more intense retardation of growing process in comparison to resistant one.

The results of cluster analysis revealed substantial differences in chemical composition in leucomas of both biotypes. Fenoxsaprop caused visible changes in chemical composition of leucoma in comparison to control (growth on water) in both biotypes. However in case of resistant biotype above-mentioned differences caused by fenoxaprop were greater than in susceptible biotype.

4.60.

Representatives of Violaceae family developed adaptations to polluted environment

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In *Violaceae* some species are perfectly adapted to toxic metal concentrations in the soil. At least nine species and two subspecies from 3 genera *Hybanthus, Rinorea* and *Viola* are obligatory metallophytes, representing hyperaccumulation or accumulation strategy in respect to different metals (Pb, Zn, Cd, Cu, Ni). Several other species from the genus *Viola* (*V. tricolor* and *V. arvensis, V. palustris, V. adunca, V. langsdorfii, V. cuneata, V. glabella*) are recognized as facultative metallophytes, occurring both on contaminated and non-contaminated soils. Studies on plants well adapted to harsh environmental conditions can give an insight into mechanisms involved in plant adaptation and/or tolerance.

We investigated both obligatory (*V. lutea* ssp. *calaminaria* and ssp. *westfalica*) and facultative metallophytes (*V. tricolor, V. riviniana, V. reichenbachiana*). In the case of obligatory metallophytes studies were focused on relationships of these taxa with close relatives to reconstruct their origin. Based on molecular ITS markers *V. lutea* was indicated as an ancestral species of two obligatory metallophytes (Siuta et al., 2005; Hildebrandt et al., 2006).

Studies on facultative metallophytes allowed to recognized two different strategies species have developed: 1) selection of well adapted genotypes growing on calamine heaps (*V. tricolor*), 2) form hybrid, colonizing contaminated sites (hybrids *V. reichenbachiana* × *V. riviniana*). These were found based on detailed studies including morphology, physiology, genetics, cytogenetics, embryology, heavy metal concentrations in plant organs (Słomka et al., 2008). The role of mychorriza in adaptation to heavy metal in soils was also investigated.

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4.61.

Gene expression profiling in leaves of three maize inbred lines treated with moderate chilling (14°C)

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Maize is a chilling-sensitive species, however intraspecific diversity in the expression of that feature is observed, particularly under moderate chilling (10–15°C). In the present study, microarray analysis was employed to study that phenomenon at the transcript level, comparing the expression pattern in chilling-treated leaves of three maize lines differing in chilling sensitivity.

We used oligo microarrays designed and produced by the Maize Oligonucleotide Microarray Project USA, comprising of ~48 000 oligos representing ESTs and cDNAs from TIGR. We used three inbred lines of maize differing in chilling-tolerance: S68911 (chilling-tolerant), S50676 (chilling-sensitive) S160 (chilling-sensitive). The lines were selected on the basis of field observations provided at Smolice location and laboratory tests. Plants were grown in a growth chamber (24°C/22°C day/night) till the 3-rd leaf stage, and then half of the plants were transferred to a cold chamber (14°C/12°C) for 38 h (10h night1/14h day1/10h night2/ 4h of day2) and the other half was kept as before as controls. Material was then frozen in liquid nitrogen. Every sample consisted of middle parts of three separate leaves.

Hybridizations were done in a loop design, in three biological replications. Isolation, purification and hybridization was done according to the procedure of the microarray supplier, with a minor modifications. The results of microarray scanning were normalized (Lowess curve), controlled for quality (Principal Component Analysis, PCA), and statistically analyzed (ANOVA, analysis of variance) with JMP Genomics 6.0.3 (SAS Institute). The p-values were then corrected for multiple comparisons with the False Discovery Rate correction set at 0.01, as an additional condition. A global analysis of gene expression pattern will be shown, based on Gene Ontology approach.

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4.62.

The function of tocopherols in *Arabidopsis thaliana* plants growing at low light intensity

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Arabidopsis thaliana plants in different stage of development (seedlings, mature plants) and with differing in their tocopherol equipment: wild type (WT) containing α- and γ-tocopherols, vte1 with impaired synthesis of α- and γ-tocopherols and vte4 accumulating γ-tocopherol instead of α-tocopherol were grown under low light intensity and salinity stress (50-70 μmol $m^{-2}s^{-1}$ and 100 mM NaCl or 100-120 μmol $m^{-2}s^{-1}$ and 200 mM NaCl, respectively). In WT and both mutants (vte1, vte4) seedlings exposed to salinity decreased activity of Cu/Zn superoxide dismutase activity was found. Changes in photochemistry of seedlings described by chlorophyll a fluorescence parameters [Fv/Fm, Y(II), NPQ] go parallel to alterations in tocopherol synthesis. Our results point out that γ-tocopherol plays an important role in protection of seedlings exposed to salinity at low light intensities. Mature *A. thaliana* plants (WT, vte1, vte4) and line overexpressing γ-TMT methyltransferase (tmt) exposed to salinity at low light conditions induce compensatory mechanisms involving antioxidative enzymes such as superoxide dismutase and catalase. Salinity stress generally leads to stimulation of maximal photosynthetic efficiency of PSII. Moreover, these changes are accompanied by changes in plastoquinone pool level and by the lowering of superoxide radical level in plant tissues. Obtained results indicate that tocopherols play also other functions than protection against oxidative stress. The alteration in the structure and function of chloroplast membranes due to the lack of tocopherols may lead to the modification of plant responses to NaCl stress.

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4.63.

The participation of glutathione S-transferase in limiting oxidative stress within tissues of primary host of *Rhopalosiphum padi* L.

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Glutathione S-transferases (GSTs, E.C. 2.5.1.18) have been associated with detoxification of xenobiotics, limiting oxidative damages and other stress responses in plant tissues (Gong et al., 2005; Conn et al., 2008; Dixon et al., 2009). The purpose of conducted studies was to elucidate the participation of glutathione S-transferase in limiting of oxidative burst in leaves of the bird cherry (*Prunus padus* L.) infested by the bird cherry-oat aphid (*Rhopalosiphum padi* L.). It has been shown that aphid-infested leaves possessed a significantly higher levels of GST activity, in relation to the control (without aphids). Additionally, it was proved, that rise of the glutathione S-transferase activity was proportional to the density of observed aphid population developing on shoots of the bird cherry.

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4.64.

Expansin gene expression in tomato roots is modified by *m*-tyrosine – nonprotein aminoacid

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Allelopathy is due to the production of phytotoxins and is one of the components of plant/plant interaction. The delay and reduction of seed germination and/or inhibition of seedling growth are the first, visible symptoms of allelopathy stress. Root exudates of fine fescue grasses contain *m*-tyrosine – an allelopatic compound identified by Bertin et al. (2007). This nonprotein amino acid inhibits root elongation growth of *Arabidopsis thaliana*. Expansin is considered as one of a primary agent participating in cell elongation. Expansin mediate nonenzymatic extension of the plant cell wall in pH-dependent manner and participate in growth of the cell as well as in many processes requiring wall modification.

We investigated the effect of *m*-tyrosine on tomato (*Lycopersicon esculentum* Mill. cv. Ożarowki Malinowy) root elongation growth by analysing expression of expansin genes in roots of young seedlings. We studied expression pattern of

7 expansin genes (*LeEXPA1, 2, 4, 5, 8, 9* and *18*). Tomato seeds were germinated in distilled water in dark. Three-days-old equally germinated seedlings were transferred to *m*-tyrosine water solution (0.025 – 0.50 mM). Culture was prolonged up to 3 days in the darkness. To analyse the gene expression, total root RNA was isolated by phenol extraction method (Rybka et al., 2009). Seedling viability was determined by TTC-test.

Tomato root growth was inhibited by *m*-tyrosine in dose dependent manner, while hypocotyls were insensitive to this allelochemical. Based on semi-quantitative reverse transcription – polymerase chain reaction, we revealed that *m*-tyrosine modified the expression of 4 expansin genes. The strongest decrease of transcript accumulation was observed for *LeEXPA8* and *18* just after 2 days, and for *LeEXPA4* and *9* after 3 days of *m*-tyrosine treatment. This effect was dose dependent. In the same experiment no significant alterations were observed for *LeEXP1*, 2 and 5. Such expression pattern suggests that inhibition in tomato root elongation growth after *m*-tyrosine treatment may be due to modification in expansin genes expression. TTC-test showed that *m*-tyrosine does not influence viability of root cells of tomato seedlings.

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4.65.

Selected physiological processes and profile gene expression in *Arabidopsis thaliana* L. plants as affected by platinum

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Platinum despite of being noble metal it can be a source of pollution, might have negative impact on living organisms and its oxides are recognized as allergenic or carcinogenic. Modern cars equipped with catalysts made of Pt, Pd and Rh emit them into environment. Cis-platinum is an important antycancerogenic drug thus oncological hospitals are a source of Pt pollution too. In this work we attempt at: (i) evaluation of platinum uptake by *A. thaliana* plants and its distribution between roots and rosette, (ii) comparison of changes in selected physiological processes elicited by Pt(IV), Pt(II), cis-Pt, (iii) changes in ABA level, and (iv) monitor profile gene expression in plants as influenced by Pt ions in growing medium.

Arabidopsis thaliana L. plants were grown in continuously aerated hydroponic culture in weekly changed Hoagland's nutrients solution. Plant after six weeks of growing in Pt free medium were exposed for 10 days to $[Pt(NH_3)_4](NO_3)_2$, in concentrations: 5, 50, 330, 500, 660, 1 000, 5 000, 10 000 and 20 000 μg dm^{-3} added during nutrients change. $PtCl_4$ and cis-$[Pt(NH_3)_2Cl_2]$ were used in concentrations 330 and 660 μg dm^{-3}. For profile gene expression cDNA microarray technology (*Arabidopsis thaliana* Genome Oligo Set, Version 3.0) was applied. Amount of Pt taken up by plants increased along with its concentration in medium and at higher concentration up to 14% of total Pt in plants was transported to rosette. Platinum at higher concentrations had toxic effects on plants manifested by lowered efficiency of photosynthetic apparatus, reduced transpiration, RWC and biomass accumulation. Level of ABA increased significantly and gene expression was changed being both up- and down regulated. The number of differentially expressed genes was greater at higher Pt concentration. In comparable concentrations, among studied form of Pt, cis-platinum exerted most negative effects and Pt(IV) was more toxic than Pt(II).

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4.66.

Application of photoacoustic spectroscopy in monitoring of the plant

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The photoacoustic effect is the production of pressure modulations with and around a sample when it absorbs intensity-modulated light. In the case of photosynthetic samples in a gas volume, thermal expansion of the gas surrounding the sample and photosynthetic oxygen evolution are the major contributors to the acoustic wave. Since heat diffuses more rapidly than oxygen does, the photothermal and oxygen (photobaric) components of the photoacoustic signal can be separated. The photothermal part of the photoacoustic signal is reduced by a fraction equal to the part of the absorbed energy stored by the photosynthetic process as chemical energy. The oxygen component of the photoacoustic signal consists largely of oxygen evolution at PS II. Two O_2 consuming processes such as photorespiration and respiration as too slow to be recorded by the photoacoustic spectroscopy.

In the present paper we describe the possibility of application of the photoacoustic spectroscopy to quantify the efficiency of photosynthetic oxygen evolution, photochemical energy storage, time of photothermal signal creation in a photoacoustic cell (heterogeneity of PS II), the coefficient of oxygen diffusion through the cell wall, the sample depth profiling, the light-saturation curve, kinetics nonphotochemical quenching on different plants (ranging from green alga – *Scenedesmus armatus* to Scots pine – *Pinus silvestris*).

4.67.

Comparison of antioxidant enzyme activity in dehydration-tolerant and dehydration-sensitive seedlings of *Hordeum vulgare* L.

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The ability of seedlings of spring wheat to tolerate severe dehydration up to fourth day following imbibition is well known phenomenon (Blum et al., 1980; Miazek et al., 2001; Corbineau et al., 2004). At that time full coleoptile length is attained and in the following days, appearance of the first leaf coincided with development of seedling susceptibility to dehydration (Blum et al. 1980; Miazek et al., 2001; Farrant et al., 2004; Corbineau, 2004).

This ability is species specific and dehydration tolerance period for other species is either shorter or longer as in some orthodox seeds (Seratna and McKersie, 1984). Our recent finding that the 6-day-old seedlings of spring barley are unable to tolerate the same water deficit as compared to the 4-day-old ones made possible to compare the crucial responses of these seedlings to water deficiency that may explain, at least in part, such a difference. Since superoxide dismutase (SOD, EC 1.15.1.1) has been shown to be an enzyme responsible for higher resistance of potato to abiotic stresses, the activity and pattern of antioxidative enzymes using analytic electrophoresis of extracts under non-denaturing conditions (native PAGE) was performed according to Laemmli (1972). It has been shown that the activity of peroxidase, catalase and superoxide dismutase did not change with the age of seedlings. Severe dehydration of 4-day-old seedlings did not change the activity of SOD (measured spectrophotometrically) that remained on the activity level noted for control, turgid plants. However, activity of guaiacol peroxidase increased under dehydration even in 4-day-old seedlings. Six day old seedlings responded to the same water deficit in tissues by a 50% increase in SOD activity. The SOD activity profile of water soluble protein extracts controls revealed two additional activity bands in comparison to extracts from dehydrated seedlings. The obtained results indicate that dehydration tolerance induced shift towards senescence related oxidation processes that was more pronounced in the sensitive than in the tolerant seedlings.

4.68.

Influence of the outer external layer on the photosynthetic apparatus location and activity

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For the photosynthetic apparatus process formation and its activity the PAR radiation is required. In leaves the chlorophyll-content layers are located just beneath the one-cell epidermal layer, what the easy solar radiation to photosynthetic apparatus permits. In wooden organs like stems, branches or trunks, because of phelloderm activity, epiderm by periderm with almost impermeable cork is replaced. Existence of the multilayer death cell of cork as the external layer influences either on quality and quantity of transmitted radiation or on the atmospheric CO_2 source access to perform process of photosynthesis and after all on the photosynthetic apparatus location composition and activity inside such kind of organs.

The analysis of the water vapour resistance, quality and quantity of transmitted through the cork PAR radiation, chlorophyll localisation and photosynthetic apparatus activity of apple tree current-year stems were conducted with porometer Mk3 Delta-T, LI-1800 Portable Spectroradiometer (LI-COR Bioscience), confocal microscope Bio-Rad MRC 1024 (Bio-Rad Microscience, UK, Handy PEA chlorophyll fluorymeter (Hansatech) respectively.

The existence of cork as the external outer layer highly influences on the conditions for performance photosynthetic process inside current-year apple tree stems. The water vapour resistance was over 30 times higher and transmittance radiation in PAR range only 30% of the adjacent leaves. The highest chlorophyll concentration was observed in the bark but chlorophyll was easy visible in wood and even in the pith. The PS II chlorophyll fluorescence showed lower maximal quantum efficiency (*Fv/Fm*) in high light conditions but very similar with leaves PS II efficiency in low light conditions. All these data indicate that present of the cork causes that photosynthetic apparatus of the wooden organs is highly dark adapted and, with high cork impermeable, process of photosynthesis in such kind of organs is connected with internal CO_2 reasymilation rather then effective net photosynthesis.

4.69.

Changes of photochemical activities in response to Pb ions and growth light intensity in maize and pea chloroplasts

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Heavy metals are the one of the major abiotic stresses effecting plants growth, productivity and development. The aim of this study was to compare the sensitivity of pea and maize plants growing in low (LL) and high (HL) light intensities to Pb ions in relation to photosynthetic and respiratory activities. It is known that photosynthesis in C4 plants involves mesophyll (M) and bundle sheath (BS) chloroplasts, which differ structurally and functionally, but up to date little information is available about acclimation strategies of two types of chloroplasts to stress conditions. Seedlings of pea and maize were growing under low (LL) and high (HL) light intensity, 60 μmol $m^{-2}s^{-1}$ and 800 μmol $m^{-2}s^{-1}$, respectively. Lead was introduced into steam with transpiration stream [24h, 5 mM $Pb(NO_3)_2$].

The data show that photosynthesis was inhibited by lead similarly in LL and HL grown plants and that pea leaves were more sensitive to heavy metal than maize. At the same time, lead stimulated CO_2 evolution of pea and maize leaves from high and low light grown plants. Increased respiratory activity correlated with higher adenylate contents. The potential photochemical efficiency of PSII in LL and HL grown plants were lowered slightly by Pb^{2+}.

PSI activity was decreased by lead in pea chloroplasts and only in BS chloroplasts of maize, in both light conditions. In BS chloroplasts decrease of PSII activity was also observed.

Amount of LHCII proteins was lower in HL than LL grown plants and it was more evident in pea than maize chloroplasts. Lead induced the changes in the degree of PSI-LHCI complexes and in LHCII trimers as was shown by non- denaturating electrophoresis.

Our results suggest that response of photosynthetic apparatus on Pb ions differ in both species grown at the same light conditions. This process is more complex in C4 plants because of different strategies in M and BS chloroplasts.

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4.70.

Expression profiles of barley (*Hordeum vulgare*) *HVA1* and *SRG6* genes in response to spring drought stress

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The barley *SRG6* (*stress-responsive gene 6*) gene, encoding a regulatory protein, is induced by drought stress although its role

in stress tolerance have not been characterized yet. *HVA1* gene from barley encodes LEA (Late Embryogenesis Abundant) protein. Transformations with both genes resulted in enhanced drought resistance in transgenic plants (Xu et al. 1996; Sivamani et al., 2000; Tong et al., 2007).

The aim of this study was to establish the expression profiles of barley *SRG6* and *HVA1* genes under the drought stress treatment. Two spring barley genotypes susceptible to drought stress and two tolerant genotypes were exposed to drought at the four-leaf stage for seven days. Accumulation of *HVA1* and *SRG6* transcripts was determined for second leaves harvested each day of the stress treatment. Real-time RT PCR was used as the quantitation method. The results showed different patterns of the expression and different driving forces for triggering both genes expression in drought. *HVA1* expression was induced only when relative water content (RWC) in leaves decreased significantly and the level of expression was independent from soil water potential. The expression level at the same RWC level was higher in tolerant genotypes. On the other hand the accumulation of *SRG6* transcript was measurable even in control samples and increased with increased drought level. The *SRG6* gene is a hypothetical transcription factor and its expression was proved to be highly correlated with the photochemical activity of PSII (Rapacz et al., unpublished), which may be affected by stomatal closure triggered by decreasing soil water potential. Our hypothesis seems to be confirmed in the simultaneously conducted physiological experiment.

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4.71.

Reaction to elevated Zn concentrations in two ecotypes of *Dianthus carthusianorum* varying in heavy metal tolerance

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Dianthus carthusianorum (Caryophyllaceae) is a common plant growing throughout Poland and is also one of the dominant species on Zn-Pb waste heaps in Bolesław near Olkusz. The ecotype of *D. carthusianorum* populating these heaps is characterised by evolutionary evolutionarily conserved metal tolerance. In its natural environment it is exposed to high metal concentrations in the substrate (up to 40 000 mg Zn, 1650 mg Pb and 170 mg Cd per kg of the substrate) as well as to high insolation and water and mineral nutrient deficits. A reaction of this ecotype of *D. carthusianorum* (calamine ecotype) to elevated Zn concentrations (50-1000 μM) in the nutrient solution was analysed in relation to the ecotype found in an unpolluted site in Wólka Lubelska near Lublin (control ecotype).

The calamine ecotype exhibited enhanced Zn tolerance in comparison to the control ecotype, as determined by EC_{100}, fresh mass reduction and loss of root viability. EC_{100}, the lowest effective concentration of the metal totally inhibiting root elongation, was 2000 μM Zn for the control ecotype and 3750 μM Zn for the calamine ecotype. The calamine plants accumulated higher concentrations of Zn in their shoots and especially roots in relation to the control ecotype plants, and this accumulation was correlated with Zn concentration in the solution. The glutathione concentration in the roots and shoots of plants of the control ecotype increased whilst in the calamine ecotype it decreased with increasing Zn concentration in the solution. Zn did not induce phytochelatin production in the plants. Accumulation of organic acids, citrate and malate, in the plants was not correlated with Zn concentration; the content of citrate was higher in the calamine ecotype and the content of malate was similar in both ecotypes.

The results show that enhanced Zn tolerance of waste heap plants is not related to organic acids or glutathione accumulation. The analyses of the anatomical structure as well as tissue or subcellular Zn localization in the plants are now performed in order to explain metal resistance in the calamine ecotype of *D. carthusianorum*.

4.72.

Protein profile of trees growing on chromium-rich tannery waste

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Chromium contamination of the soil and water is a serious problem in areas of many industrial activities, like electroplating, wood preservation, mining, and leather tanning. In various conditions it may shift between two stable oxidation states: Cr (III) and Cr (VI). The latter is far more hazardous because of its solubility and mobility in the environment. Chromium enters the food chain via the plant organism. Uptake into the root cells can be passive (Cr III) or active (Cr VI), mediated by sulfate transporters. In living cells, chromium is reduced and stored in its stable form (Cr III) (Shanker et al., 2005). Recently there is a growing interest in using this ability of plants in remediation of contaminated areas, especially with trees that could effectively extract and/or stabilize metals, preventing them from leaching to groundwater.

In this study we evaluated changes in the proteome of aspen (*Populus tremula* L.) and osier (*Salix viminalis* L.) under the influence of chromium-containing tannery waste. For this purpose, leaves and fine roots were collected from shoot cuttings grown for 16–17 weeks in pots containing solid tannery waste alone or mixed at various ratios with unpolluted soil. Protein profiles were analysed by 2-D electrophoresis, and compared to identify Cr-responsive proteins in poplar and willow.

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4.73.

Involvement of Deg1 protease in the degradation of chloroplast proteins after short-time exposition of *Arabidopsis thaliana* and *Zea mays* leaves to photoinhibitory irradiance

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Short term changes in the intensity and quality of light leads to plant acclimatization that might be executed by structural changes of photosynthetic apparatus. However, in extreme cases can also lead to photoinhibition as result of the damage of key photosynthetic proteins by excess light. Precisely regulated repair processes involves the degradation of damaged proteins and synthesis de novo of new copies, in order to restore photosynthetic function. The exact proteolytic system participating during acclimatization of plants to light changes has so far not been identified in both C3 and C4 plants. The pull-down assay was used to find substrates of Deg1 protease from *Arabidopsis thaliana* (C3 type) and *Zea mays* (C4 type) chloroplasts, in response to short time treatment of the leaves photoinhibitory light (900 μmol $m^{-2}s^{-1}$). Identification of bound to Deg1 proteins were performed using mass spectroscopy technique or with the use of special antibodies (Western blotting). Preliminary results show that Deg1 protease can be involved in the degradation of the core and antenna PSII proteins. Our data suggest that not only D1 protein, as was earlier demonstrated (Kapri-Pardes et al., 2007), but also D2 and some antenna proteins e.g. CP29, can be the substrate of Deg1 protease.

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4.74.

Influence of inorganic and organic phosphorus supply on acid phosphatase activity and growth of oat (*Avena sativa* L.)

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Phosphate deficiency is common in most cultivated soils. Most phosphorus in soil exists as organic compounds, available for plants only when hydrolyzed by soil enzymes eg. phosphatases. Acid phosphatases (EC 3.1.3.2) are believed to enhance inorganic phosphate (Pi) uptake and thus are important component of plant response to Pi starvation (Żebrowska, Ciereszko, 2007). Two oat varieties (Deresz and Rajtar) were grown for 1–3 weeks on different nutrient media with contrasting phosphorus source: inorganic – KH_2PO_4 (control), organic – phytic acid and without phosphate (-P). Extracellular acid phosphatases activity was determined in root exudates and by *in vivo* stain (Gilbert 1999; Ciereszko et al., 2002). Intracellular acid phosphatases activity and inorganic phosphate content in shoots and roots were also investigated. Additionally, protein extracts from tissues were run on native discontinuous PAGE to determine acid phosphatases isoforms. Pi content decreased in the leaves and roots of all plants grown in the -P media; however Pi content in plants grown on phytic acid was similar to control plants. Phosphate deficiency affected growth of both studied varieties: significantly decreased shoot growth, and increased the ratio of root/shoot fresh weight. Whereas growth parameters of plants supplied with phytate were more similar to control plants. Phosphate starvation significantly increased the activity of extracellular and intracellular acid phosphatases in comparison to the control plants. Surprisingly, both secreted and internal acid phosphatases activity in plants grown on phytate was more similar to control than to -P plants. Our results indicate that some other enzymes (maybe phytases), not only nonspecific acid phosphatases may be involved in Pi release from phytate.

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Session 5

Structural and functional organization of plant genome

PLENARY LECTURES

5.1.

Interphase chromosome organisation and its dynamics in plants

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Eukaryotic chromosomes occupy distinct territories within interphase nuclei. The arrangement of chromosome territories (and of specific chromatin domains therein) are likely to be important in key events that occur within cell nuclei such as replication, transcription, repair and recombination processes. Our knowledge about interphase chromatin arrangement, mainly based on results obtained by means of various in situ labelling approaches, is still meagre. Nevertheless, it is emerging that phylogenetic affiliation of a species, cell cycle and differentiation status, as well as environmental influences may have an impact on, and may cause alterations of, interphase nuclear architecture. Most data regarding interphase structural organization in plants have been obtained for Brassicaceae (*Arabidopsis thaliana* and related species) and for cereal species. I will survey the present knowledge about interphase arrangement of Brassicaceae chromosomes concerning the relative positioning of chromosome territories, somatic pairing of homologues, and sister chromatid alignment in meristematic and differentiated tissues. Furthermore I will discuss the morphological constraints and epigenetic impacts on the nuclear architecture and the evolutionary stability of chromosome arrangement patterns as well as alterations of nuclear architecture during transcription and repair, in mutants with increased recombination activity, and in lines carrying transgenic tandem repeat arrays.

5.2.

Characterisation of AtNUFIP, a novel gene controlling the biogenesis of small nucleolar RNAs, sheds new light on RNA methylation and its impact on plant development

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RNA methylation directed by class C/D small nucleolar RNAs (C/D snoRNAs) is an important modification occurring on several residues of ribosomal RNAs and other transcripts in eukaryotes. However in plants few studies have addressed the factors controlling RNA methylation and their impact in development.

Here we report the identification and functional characterisation of the *Arabidopsis AtNUFIP* gene that controls C/D snoRNA biogenesis. In *Arabidopsis* more than 100 C/D snoRNAs have been identified. Most of them are encoded by clustered genes producing polycistronic precursors that are processed to liberate the C/D snoRNAs. *In vivo* all C/D snoRNAs are associated with four conserved core nucleolar proteins including fibrillarin which is the RNA methylase and the 15,5K protein which is the only C/D snoRNP core component directly interacting with the C/D snoRNA. Assembly of the C/D snoRNP is a complex process that requires accessory proteins. A key protein recently identified in human cells corresponds to NUFIP, that directs assembly of the C/D snoRNP via a direct interaction with the 15,5K protein.

Surprisingly, *Arabidopsis AtNUFIP* shows little similarity to human NUFIP except for a short motif that interacts with the 15,5K protein. We isolated and characterised two *AtNUFIP* T-DNA insertional mutants that produce truncated mRNAs. Analysis of these mutants revealed that *AtNUFIP* specifically controls biogenesis of the C/D snoRNAs and this is correlated with a reduction of methylation of their target residues in rRNAs. Remarkably we also found that the impact of *AtNUFIP* is tightly related to genomic organisation of the snoRNA genes.

We further show that *AtNUFIP* also controls the biogenesis of the scaRNPs that direct methylation of spliceosomal snRNAs and are predicted to be located in Cajal bodies.

At the developmental level, *AtNUFIP* mutants are viable but display severe leaf, flower and seed developmental phenotypes. This raises the question whether this is due to a global decrease of methylation of rRNAs or snRNAs, affecting protein translation or splicing respectively. Alternatively, this could be due to another unknown function of *AtNUFIP*. We are now developing different approaches to address these questions in Arabidopsis and confirm the impact of RNA methylation on plant development.

5.3.

Regulation of plant gene expression by alternative splicing

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The importance of post-transcriptional regulation of expression is well-recognised in plants but the role of alternative splicing (AS) in the production of functionally different proteins or in modulating transcript levels is poorly understood. We have developed an RT-PCR panel to monitor changes in alternatively spliced isoforms of ca. 300 *Arabidopsis* AS events focusing on events in transcription factors, RNA-interacting proteins and proteins involved in signalling or stress responses. With the labs of Andrea Barta (Vienna) and Artur Jarmolowski (Poznan) we have used the panel to examine how factors involved in mRNA biogenesis (e.g. cap-binding proteins) and splicing regulation (e.g. SR proteins and PTB-like proteins) affect alternative splicing. We are currently using the RT-PCR panel to examine the wider relationship between alternative splicing and nonsense-mediated decay (NMD) and are developing process-specific AS panels.

The NMD pathway targets mRNAs containing premature termination codons (PTC) for degradation. In both mammals and plants, mRNAs with an increased distance between the PTC and the 3' end of the mRNA (long 3' UTRs) and/or with an exon junction complex downstream of a PTC are targeted for degradation. NMD is thought to reduce the consequences of mutations or mistakes in expression, to reduce genomic noise and prevent production of potentially detrimental truncated proteins. In addition, NMD modulates the levels of AS isoforms which contain PTCs. Around 10% of the human transcriptome is turned over by NMD and 30% of human AS transcripts contain PTCs and are degraded by NMD.

In *Arabidopsis*, examples of regulation by alternative splicing and NMD include the genes encoding circadian clock proteins, AtGRP7 and AtGRP8. We are analyzing mutants of the NMD proteins, UPF1 and UPF3 (*upf3-1* and *upf1-5*). Impaired NMD causes an increase in abundance of PTC+ AS isoforms which are normally turned over by NMD. The 264 AS events analysed so far produced around 700 alternatively spliced transcripts of which approximately 150 increased in abundance. We estimate from these results that between 15-20% of alternatively spliced products in Arabidopsis are turned over by NMD. Control of expression by AS must be carefully regulated. To examine co-ordinated control of AS in genes in the same pathway, we are identifying AS events and generating process-specific RT-PCR panels.

ORAL PRESENTATIONS

5.4.

Protein-protein interactions within cap-binding complex (CBC) and with pri-miRNA processing machinery components in living *Arabidopsis thaliana* cells

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A 7-methyl guanosine (7MeG) cap structure is added to the 5' end of all RNA Polymerase II transcripts. The cap-binding complex (CBC) is composed of two subunits – cap-binding proteins: CBP20 and CBP80. The CBC binds to the cap structure of pre-mRNA and is involved in several aspects of RNA metabolism. The cap is also present in pri-miRNA transcripts. miRNA processing in plants requires SERRATED (SE), DICER-LIKE 1 (DCL1), and HYPONASTIC LEAVES 1 (HYL1). It is known that pre-mRNA and pri-miRNA transcripts share common processing components. Recently, the roles of the CBP20, CBP80 and SERRATED in both mRNA splicing and miRNA processing has been shown. The working hypothesis is that SE can be a mediator between the cap-binding complex and both the splicing commitment complex and the pri-miRNA processing machinery. We have analyzed in details the interaction between the *Arabidopsis thaliana* CBP20 and CBP80, using the yeast two-hybrid system, Fluorescence Resonance Energy Transfer (FRET) and Bimolecular Fluorescence Complementation (BiFC) assays. The N-terminal part of AtCBP20 is essential for interaction with AtCBP80. The interactions between AtCBC and AtSE or AtHYL1 were also analyzed using FRET and BiFC methods. We show here that AtCBP80 interacts with AtSE. It seems that SERRATED functions as a physical link between CBC (via AtCBP80) and distinct RNA processing machineries for pre-mRNA splicing and pri-miRNA processing.

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5.5.

Stability, nucleotide sequences and expression of the *rolC* and *nptII* genes in 15-years old cell cultures of *Panax ginseng*

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We showed that after 15 years of the cultivation of *Panax ginseng* transgenic cell cultures the *rolC* and *nptII* (neomycin phosphotransferase) gene was expressed at a high level (Kiselev et al., 2008). Further, we analyze in detail the nucleotide composition of the *rolC* and *nptII* genes (Kiselev et al., 2009). It has been established that the nucleotide sequences of the *rolC* and *nptII* genes underwent mutagenesis during cultivation. Particularly, 1–4 nucleotide substitutions were found per sequence in the 540 bp and 798 bp segments of the complete *rolC* and *nptII* genes, respectively. Approximately half of these nucleotide substitutions caused changes in the structure of the predicted gene product. In addition, we attempted to determine the rate of accumulation of these changes by comparison of DNA extracted from *P. ginseng* cell cultures from 1995 and 2007. It was observed that the frequency of nucleotide substitutions for the *rolC* and *nptII* genes in 1995 was 1.21 ± 0.02 per 1,000 nucleotides analyzed, while in 2007, the nucleotide substitutions significantly increased (1.37 ± 0.07 per 1,000 nucleotides analyzed). Analyzing the nucleotide substitutions, we found that substitution to G or to C nucleotides significantly increased (in 1.9 times) in the *rolC* and *nptII* genes compared with *P. ginseng* actin gene. Finally, the level of nucleotide substitutions in the *rolC* gene was 1.1-fold higher when compared with the nptII gene. Thus, for the first time, we have experimentally demonstrated the level of nucleotide substitutions in transferred genes in transgenic plant cell cultures.

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5.6.

Cytogenetic analysis of *Chenopodium album* aggregate

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Chenopodium album agg. consists of several species representing three different ploidy levels: diploid (2n = 2x = 18), tetraploid (2n = 4x = 36) and hexaploid (2n = 6x = 54). The species which belong to the aggregate create a lot of taxonomical difficulties because of marked phenotypic plasticity, parallel evolution and putative hybridization. Moreover, some of the species which belong to the aggregate include plants on different ploidy level, what make the study even more complex.

Selected species of the aggregate were analyzed using cytogenetic methods. Among them two species (*C. album* and *C. strictum*) included plant with different ploidy levels. Cytogenetic analysis indicated that two studied diploids: *C. album* and *C. ficifolium* have very similar genome size and comparative GISH (cGISH) revealed that the fraction of the repetitive DNA are very similar, although some differences in chromosomal organization of rDNA loci were observed. Among studied polyploids three tetraploids (*C. berlandieri, C. quinoa, C. strictum*) and five hexaploids (*C. album, C. giganteum, C. bushianum, C. strictum, C. opulifolium*) were observed. The tetraploids had similar genome size, karyotype length and number of rDNA loci (one pair of loci of 45S rDNA and two or three pairs of 5S rDNA loci). Also analyzed hexaploids revealed similar genome size, karyotype length and rDNA loci distribution (two 45S rDNA loci and four pairs of 5S rDNA loci). The only one exception was *C. opulifolium* with bigger genome size and fewer number of rDNA loci. GISH was used to find the relationships between diploid *C. album* and selected polyploid species of the aggregate. The evolutionary implications of these findings will be discussed.

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POSTERS

5.7.

Ribosomal genes, AT- and GC-rich chromatin in *Rhoeo*. Implications for chromosome rearrangements

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FISH-aimed cytogenetic investigation was performed in a permanent translocation heterozygote *Rhoeo* spathacea (2n=12). The results suggest the involvement of rRNA gene arrays in chromosome rearrangements. 45S rDNAs accompanied by CMA-bands were detected within NOR-bearing chromosome ends. In contrast to previous study (Golczyk et al., 2005), they were also detected within all the twelve transcriptionally silent pericentromeric regions. It is suggested here that subtelomeric 45S rDNAs and pericentromeric chromatin may have served as a source of the putative breakpoints generating whole-arm translocations and/or whole-arm inversions. The persistence of two cytologically well-defined chromatin domains (AT and GC-rich, 45S rDNAs) within all twelve pericentromeres suggests spreading and homogenization of their sequences during evolution of *Rhoeo* karyotype. Such processes can be facilitated by clustering of centromeres which brings proximal sites into contact (Schweizer et al., 1987; Cerda et al., 1999). To note, in *Rhoeo* profound centromere associations are formed in somatic and meiotic cells. It was revealed that the large 5S rDNA locus residing on each of 8E and 9E arms is a duplicated locus, consisting of two smaller loci separated by a short distance. The duplication may have been due to small inversion with one of the breakpoints within the already existing 5S rDNA locus. On each of the two other chromosome arms: 3b and 4b, in addition to the major telomere-adjacent 5S rDNA locus, the new minor one was found in the interstitial position. Each of the two interstitial 5S rDNA loci may have originated due to a large segmental inversion with one of the breakpoints within the telomere-adjacent major 5S rDNA locus.

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5.8.

Molecular cytogenetics and meiotic system in *Oenothera*

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Permanent translocation heterozygosity (PTH) – a widespread phenomenon in *Oenothera* (evening primrose) presents itself valuable tool for studies on genome rearrangements and their biological significance. In evening primroses complex genome rearrangements involved the non-homologous chromosomes and resulted in rings/chains at meiosis. PTH organisms breed true for meiotic rings due to the existence of the two non-recombining superlinkage chromosome sets, the so called Renner complexes (Cleland, 1972; Rauwolf et al., 2008) and due to special means eliminating homozygotes (Levin, 2002). The fact that PTH system has evolved also in other unrelated plant taxa indicates that it can serve as a fully successful alternative strategy in adaptive evolution. However, the driving forces for the origin of this phenomenon and basic cytological predispositions required for PTH to evolve remain enigmatic. The critical cytogenetic parameters subjected to selection are karyotype structure and specialized type of meiosis (Cleland, 1972). Both are poorly recognized in *Oenothera* (Cleland, 1972; Golczyk et al., 2008). Here we present results of a cyto-molecular study on *Oenothera* chromosomes and meiotic nuclei. The analyzed species were ring formers as well as bivalent formers. All the species had two 5S rDNA loci per karyotype and 4–6 45S rDNA loci. Based on the distribution of ribosomal gene clusters and chromosome measurements (length and chromosome arm ratio) general chromosome identification rules and standard karyotypes were presented. A special attention was also paid to chromocenters and their behaviour during meiotic prophase. The results indicate that association of chromosomes with the nucleolus plays an important role in centromere clustering and obtaining Rabl-polarization during leptotene. The structure of karyotypes and meiotic nuclei in *Oenothera* were discussed with regard to the theory of reciprocal translocations and a need for specialized type of meiotic prophase in PTH organisms.

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5.9.

Karyotyping of *Humulus japonicus* Siebold & Zucc. by FISH and C-banding/DAPI method

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Humulus japonicus (Japanese hop) from the family Cannabaceae is a dioecious, annual species with heteromorphic sex chromosomes (XX/XY_1Y_2) and sex determination system based on the X to autosome balance (X:A). Chromosome constitution of females is 2n=14 + XX, and of males is 2n= 14 + XY_1Y_2. Morphological similarity makes exact identification of conventionally stained sex chromosomes and autosomes in *H. japonicus* very difficult, so the satisfactory description of *Humulus* karyotype require more advanced methods of karyotype analysis. In our study we applied FISH with probes of rDNA (45S, 5S) and telomeric sequences as well as C-banding/DAPI technique in effort to better characterization of chromosomal complement in this species and to advance our knowledge of plant sex chromosomes. We constructed a fluorescent karyotype that can be used to distinguish almost all autosome pairs.The only exceptions were two largest autosome pairs, deprived of rDNA signals and similar both in size and DAPI-banding pattern. Sex chromosomes of *H. japonicus* showed unique banding pattern and different intensity of DAPI fluorescence. X chromosome was equipped with only one terminal brightly stained AT-rich segment, Y_1 with two such segments and Y_2 was completely deprived of DAPI marks. Both Y chromosomes were easily distinguished after C-banding/DAPI from the rest of chromosome complement by the enhanced fluorescence of their arms. The elevated DAPI fluorescence of Y chromosomes after this method suggests enrichment in repetitive sequences – the situation similar to that observed previously in *Rumex acetosa*, a model plant with the same sex chromosome system (Jamilena et al., 2008).

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5.10.

Excessive rDNA amplification in tetraploid forms of *Aconitum*

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The physical localization of the 5S and 25S ribosomal DNA (rDNA) sequences was examined by fluorescence in situ hybridization (FISH) in two diploid (*A. variegatum* and *A. degenii*) and two tetraploid (*A. firmum* and *A. plicatum*) species of *Aconitum* subgen. *Aconitum* from Poland. Combined data of rDNA loci distribution FISH signals and already known C-banding patterns (Joachimiak et al., 1999; Mitka et al., 2007) may in some cases provide good chromosome markers to clarify the karyotype structure in analyzed species. The general feature of the *Aconitum* karyotypes was the localization of both kinds of rDNA sites exclusively on the short arms of chromosomes and heterochromatic appearance of 25S rDNA clusters.

Two diploid species showed uniform, subterminal (25S) or pericentromeric (5S) localization of rDNA loci. There were only three 5S rDNA sites in the karyotype of both species, and eight 25S rDNA sites in *A. variegatum* and six in *A. degenii*. The number of rDNA loci in *A. firmum* and *A. plicatum* was higher than the sum of loci in the diploid species. The most striking feature of tetraploid karyotypes was the conservation of rDNA loci existing in diploids and the emergence of many additional 5S rDNA sites. The number of 5S rDNA clusters within the karyotype was up to 23 in *A. firmum* and 20 in *A. plicatum*. The rDNA distribution within chromosome tetrads suggests the contribution of additional ribosomal DNA sites to the diploidization of tetraploid karyotypes. The similar basal genome size (Cx) of diploid and tetraploid *Aconitum* lines suggests that the massive amplification of rDNA sequences was counterbalanced by the elimination of some other sequences during evolution of tetraploid species. This is the first report on the 5S and 25S rDNA distribution and nuclear DNA amount in *Aconitum*.

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5.11.

Genome structure in several representatives of the genus *Taraxacum* section *Ruderalia*

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Within the genus *Taraxacum* about 3000 species occurring all over the word are distinguished. Polyploid species are either obligatory or facultative apomicts, whereas diploid species reproduce sexually (van Baarlen et al., 2000; Marciniuk and Rudzińska-Langwald, 2008).

Among several studied species from the section *Ruderalia*, one species – *T. linearisquameum* turned out to be diploid (2n=16) and in some others (*T. cyanolepis, T. fulgidum, T. gentile, T. sinuatum, T. undulatum*) triploid chromosome number (2n=24) was observed. Having applied the C-banding/DAPI method of chromosome staining, in the karyotype of *T. linearisquameum* the authors distinguished a pair of nucleolar chromosomes, each of them possessing one large, fluorescent segment of heterochromatin. The karyotype of two other triploid species: *T. gentile* and *T. sinuatum* revealed two SAT chromosomes with fluorescent bands as well as one without strong fluorescence. This result suggests that in the two studied triploid agamic species there are two genomes similar to those observed in the diploid *T. linearisquameum* and the third genome of a different type.

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5.12.

Stilbene synthase gene expression in callus cultures of *Vitis amurensis* with different levels of resveratrol production

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Resveratrol is known to have antioxidant, anti-inflammatory, and antiviral activity. It is also a strong antitumoral agent effective against many types of cancer (Aggarwal et al., 2004). Stilbenes, including resveratrol, are synthesized via the phenylpropanoid pathway. Stilbene synthase (STS, EC 2.3.1.95) condenses three molecules of malonyl-CoA and one molecule of cumaryl-CoA to form resveratrol. *STS* exists as a multigene family. In *Vitis vinifera*, closely related to *V. amurensis*, 25 *STS* genes per haploid genome were predicted. The large number of *STS* genes has aroused considerable interest. We suggested *STS* genes of *V. amurensis* encoding protein products with different activity in resveratrol biosynthesis. We analyzed *STS* gene expression in callus cultures of *V. amurensis* with different levels of resveratrol production: 0.30–3.15% dry wt. in *rolB* transgenic cell cultures (Kiselev et al., 2007); 0.10–0.15% dry wt. in *rolC* transgenic cell cultures (not published); 0.05–0.10% dry wt. in nontransgenic cell cultures treated with 50–300 μM of salicylic acid (not published); and 0.02–0.03% dry wt. in nontransgenic cell cultures used as control (Kiselev et al., 2009). It is known integration of individual *rol* genes of *Agrobacterium rhizogenes* into the plant genome may enhance biosynthesis of certain groups of secondary metabolites. The total expression of *STS* genes was approximately on the same level in the *V. amurensis* cultures. Then, we analyzed the quantity of clones of individual *STS* genes. We sequenced more than 350 clones of different *STS* genes obtained from cDNA probes of the explored *V. amurensis* cell cultures. We detected 10 *STS* genes. The sequenced fragments of seven *STS* gens were deposited in GeneBank: *VaSTS1* (EU659862), *VaSTS2* (EU659863), *VaSTS3* (EU659864), *VaSTS4* (EU659865), *VaSTS5* (EU659866), *VaSTS6* (EU659867), *VaSTS7* (EU659868). We divided the *STS* genes on four groups:

a) *STS* gene which was being constantly expressed on a high level in all cell cultures. The expression of the gene slightly increased in cells with high resveratrol content (*VaSTS1*).
b) *STS* genes which expression was significantly increased in the cell cultures with high resveratrol content (*VaSTS2, VaSTS3, VaSTS4, VaSTS5, VaSTS7*).
c) *STS* genes which expression was strongly activated by SA (*VaSTS6*) or by *rolC* gene (*VaSTS9, VaSTS10*). However, these genes were weakly expressed in the *rolB* transgenic cell culture (highest resveratrol content).
d) *STS* gene (*VaSTS8*) which transcripts were extremely rare in cDNA probes obtained from the cultures with high resveratrol content.

We propose that an increase in the expression of the second group of *STS* genes is required to a high level of resveratrol production in a *V. amurensis* cell culture. Further study is needed to confirm this hypothesis.

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5.13.

Chromosomal analysis of *Secale cereale* × *Dasypyrum villosum* amphiploid and its parents by use of cytogenetic methods

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The wild species from *Triticeae* tribe has played an important role in the processes of increasing the genetic variation of rye (*Secale cereale* L.). The genus *Dasypyrum* comprises many agronomically important traits including disease resistance, drought and freezing tolerance, high protein content and quality, and therefore might be used as a valuable source for crops improvement. Our research was carried out on *Secale cerale* × *Dasypyrum villosum* tetraploid (2n=4x=28; RV-genome) which resulted from the intergeneric crosses between *S. cereale* (2n=2x=14; R-genome) and *D. villosum* (L.) P. Candargy (2n=2x=14; V-genome), and then obtained from colchicine treatment. In the present study, we attempted to investigate the genome structure of this amphiploid and its parents, and the analysis was conducted on somatic metaphase chromosomes using fluorescence and genomic in situ hybridization (FISH/GISH). The FISH provided valuable chromosomal landmarks to detect chromosome variations, and was able to identify rDNA-bearing chromosomes.

This approach revealed chromosomal rearrangements and allowed identification the chromosomes implied in chromosome rearrangements. For more detailed amphiploid genome analysis, a silver staining method (Ag-NOR) was applied, but no nucleolar dominance was occurred. The interspecific and intergeneric hybrids between rye cultivars and wild diploid species *D. villosum* are important for introgression breeding programs, and can be used, for example, to transfer of abiotic and biotic stress resistance traits from *Dasypyrum* species into *Secale species*.

5.14.

Nodulation *SymRK*-gene region in the narrow leafed lupin genome: localization, structure and annotation

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SymRK (Symbiotic Receptor Kinase) is one of the key genes controlling the nodulation (and mycorhization) process in legumes. In this work we established the localization and the structure of the SymRK region in the genome of *Lupinus angustifolius*.

A fragment of *SymRK* sequence from the narrow-leafed lupin was amplified by the PCR on a genomic DNA template and applied as a probe for hybridization with the *L. angustifolius* nuclear genome BAC library. The screening of the library showed positive hybridization signals corresponding to six BAC clones. The results were verified by PCR using DNA of clone inserts and two sets of primers, designed on the basis of the *SymRK* sequence. To determine the arrangement of clones in the genome region, restriction fingerprinting was carried out. Five clones containing the *SymRK* fragment were localized in one contig. Considering that the genome coverage in the lupin BAC library is estimated as 6× , the number of 5 positively verified BAC clones assembling into a single contig is strong evidence that only one region with *SymRK* sequence is present in *L. angustifolius* genome.

We confirmed the molecular data regarding *SymRK* genome representation, by cytogenetic analysis using fluorescence *in situ* hybridization (BAC-FISH). Four of five selected BAC clones were subjected to BAC-FISH on mitotic metaphase chromosomes of *L. angustifolius*. The BAC DNA was labeled by standard nick-translation. Two BAC clones hybridized to single loci and were co-localized on one pair of chromosomes. Two other BACs, however, were mapped in multiple loci on different chromosomes, most likely due to repetitive elements. Close localization of two single-locus BAC signals on the same chromosome pair confirms the presence of a unique SymRK region in the *L. angustifolius* genome.

Furthermore, sequencing of the largest BAC clone yielded a sequence of 116,636 bp from the assemblage of 1,536 tiles (average cover of 12.72×). Sequence annotation was performed using a combination of various bioinformatic tools. *SymRK* belongs to a large conserved gene rich region, which is flanked by a remarkable transposable elements rich region (25% of the BAC sequence).

5.15.

New loci controlling preharvest sprouting (PHS) in rye (*Secale cereale* L.) revealed by bidirectional selective genotyping (BSG)

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A method of bidirectional selective genotyping within the two groups of recombinant inbred lines (RILs) representing opposite extreme phenotypes in respect to PHS revealed 12 loci controlling preharvest sprouting in rye. Six loci giving strong bidirectional effect on two RILs grups were recognized as PHS directional loci (PHSD). They were mapped on chromosomes 1RL (pr310/320_380) and 3RL (pr224/289, pr181B, pr419/455, pr884/pm74, pm4/200).

Six loci giving segregation deviated from the expected 1:1 ratio only within the susceptible RILs group were recognized as PHS enhancing loci (PHSE). PHSE loci were mapped on chromosomes 2RS (pr310/320_650), 3RL (pm9/200, pm21/194, pm2/194) and 7RS (pm1/pr910_750).

New loci controlling PHS in rye found in this study substancially increase the complexity of a genomic architecture for PHS detected in earlier report (Masojć et al., 2009).

This study was supported by grant PBZ-MNiSW-2/3/2006 from the National Centre of Scientific Researcg And Development (NCBiR).

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5.16.

Development and localization of arbitrarily amplified DNA fragments on the carrot (*Daucus carota* L.) chromosomes using fluorescent in situ hybridization

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Carrot is a species with small and morphologically similar chromosomes (2n=18). To date, only the most popular chromosome-specific cytogenetic DNA markers, i.e. 5S and 45S rRNA genes, were located on carrot chromosomes using fluorescent in situ hybridization (FISH). The objective of the present study was to develop new cytogenetic markers for carrot chromosomes, using arbitrary PCR amplicons as probes for FISH. Amplification products were obtained for nine carrot accessions and *Daucus capillifolius* – the closest relative to *D. carota*, using 63 RAPD decamer primers, 16 combinations of two primers, and 8 combinations of three primers. We searched for abundantly amplified products monomorphic in all accessions, putatively indicating a high level of sequence conservation of the amplified fragment. As a result, 16 amplicons that met the above requirements were obtained and sequenced, with sizes ranging from 500 to 2200 bp. Sequence

analysis showed that six products were similar to DNA transposons or retrotransposons, ubiquitously distributed in plant genomes, while other fragments showed no sequence similarity. All selected amplicons were labeled and used as probes in FISH experiments. FISH analysis showed that four of six transposon-related probes produced multiple FISH signals dispersed throughout the genome and present on all chromosomes. Only one probe (similar to the *gypsy1/PTR1* retrotransposon) was localized at several intensive fluorescent sites on some chromosomes and thus it might be a new cytological marker for the identification of carrot chromosomes.

5.17.

Extension of the genetic map of rye (*Secale cereale* L.) and searching for loci controlling preharvest sprouting and alpha-amylase activity

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Cultivated rye is a sprouting sensitive crop, so there is important to rich the knowledge about genetic background of precocious germination of seeds (preharvest sprouting – PHS) and to recognize genetic sources of sprouting resistance. Genetic system controlling alpha-amylase (α-Amy) activity is partially connected with this responsible for PHS. Until now genetic control of both traits was studied by using two genetic maps of F_2 populations (Masojć and Milczarski, 2008). Since the mechanisms of studied traits proved to be very complex and the results didn't fully coincide, there was the necessity for identification and verification of QTLs for another accession of sprouting resistance and low α-Amy activity genes. For this reason third F_2 mapping population was generated by crossing rye inbred lines, S120 and S76, represented varied phenotypes in respect to the interesting traits, developed during a breeding program. Analysis of sprouting and α-Amy activity was provided in two years, for plants of F_2 and F_3 progenies.

The base for searching QTLs for PHS and α-Amy activity was the genetic map of S120×S76 intercross constructed by use of different PCR-based markers with JoinMap 3.0 package. The extended map has 25 new markers with known chromosomal localization (7 SCARs, 7 SSRs, 11 STSes) which allow to reduce number of linkage groups from 16 to 11 and to confirm the identification of chromosomes. The map is composed of 140 markers, single linkage groups consist of 2 to 37 loci. The longest group containing 37 loci spans the distance of 82cM; the whole map length is 435cM. Ten groups are fragments of all chromosomes (1R-7R), one group remain unidentified.

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5.18.

Colocalization of 5S and 45S rRNA genes revealed by FISH in liverworts genus *Pellia*

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The origin of land plant was probably the most important event in plant evolution. There is evidence that the first plant group that possessed land environment was liverworts. However, there is still a little information about its genome structure. Most of cytological data concerns the number and basic morphology of mosses chromosomes. Among liverworts the best analysed species is *Marchantia polymorpha*. Cytological analysis of its genome revealed colocalization of 45S and 5S rDNA that is unique in Telomophyta. Molecular research proved insertion of 5S rDNA into 45S rDNA repeat unit. Such insertion was also found in moss species *Funaria hygrometrica* (Sone et al., 1999). Analyse of 45S and 5S rDNA localization in more bryophyte species can help to understand evolution of land plants and answer phylogenetic question – is the colocalization a transitional state from Procariota to the higher Eucariota or 5S rDNA was it inserted into 45S rDNA repeat unit by transposable elements.

Karyotypes of three haploid and one allopolyploid species from *Pellia* genus was analysed. *In vitro* culture of thallus tissue was kindly provided by Prof Zofia Szweykowska, A. Mickiewicz University in Poznan, Poland. Number and localization of 45S and 5S rRNA genes was investigated by fluorescence in situ hybridization (FISH).

Colocalization of 5S rDNA and 26S rDNA hybridization signals was observed in all analysed species. In haploid species 26S rDNA loci always colocalize with 5S rDNA. In polyploid karyotype,where two 26S rDNA loci were present only one signal colocalize with 5S rDNA. Additionally single 5S rDNA loci were observed in haploid and polyploid genomes. Detailed karyotype analyses provided cytological confirmation of *P. borealis* allopolyploidy and revealed genome rearrangements.

5.19.

The chalcone isomerase genes of narrow-leafed lupin: physical and genetic mapping

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Lupins, as other leguminous plants, have a unique biosynthesis pathway of 5-deoxy-type flavonoid and isoflavonoid biosynthesis, distinct from the general flavonoid pathways. Chalcone isomerase (CHI), one of the key enzymes in this pathway, catalyzes the cyclization of chalcone into (2S)-naringenin.

Our earlier research indicated that narrow-leafed lupins have two distinct genes encoding chalcone isomerase. Sequence analyses showed that both of them are made up of 4 exons.

In this work, clones from a bacterial artificial chromosome (BAC) library of the *L. angustifolius* nuclear genome were used as probes for fluorescence in situ hybridization (FISH) on mitotic chromosomes. The BAC DNA was isolated and, after *Not*I digestion, pulsed field gel electrophoresis was done to confirm the purity and size of the isolated DNA inserts. Labeling of BAC DNA was performed by nick-translation with tetramethyl-rhodamine-5-dUTP or digoxygenin-11-dUTP. The BAC clones which include the sequence of *CHI* (*CHI1*) gene gave a single-locus signal on one

pair of homologous chromosomes while those with another sequence of *CHI* gene (*CHI2*) showed two distinct signals localized on two different pairs of chromosomes.

Two sequence-defined molecular markers for each *CHI* gene were therefore designed based on exon sequences. Both gene mapped in different groups of gene-based linkage map of *Lupinus angustifolius*.

5.20.

Control of replication of prematurely condensed chromosomes

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Nuclear rearrangements that make up structural basis for the core processes of the cell cycle comprise two opposite functional states of chromatin, one adjusted to precisely replicate DNA, and the other adapted to allocate sister chromosomes into two daughter cells. The temporal order of S phase is largely connected with various positions occupied by distinct genomic sequences, their structural features, AT/GC content, and histone modifications.

Although evident differences between the dispersed and compact forms of nuclear structures may serve as a main explanation for the mutual exclusion of DNA replication and M-phase condensation, mitotic chromatin containing pre-RCs was shown capable to undergo a complete round of DNA synthesis upon replication system derived from *Xenopus* eggs (Prokhorova et al., 2003).

Apart from a number of problems concerning functional similarities of factors triggering S and M phase, the results derived from experiments with nucleus-free extracts suggest that origins which fail to commence DNA replication in interphase could still undertake their functioning in mitosis. This work focuses on the model in which S-phase-blocked (hydroxyurea-treated) cells in primary root meristems of *Vicia faba* were stimulated to override "intra-S-phase checkpoint" and, after caffeine-mediated premature chromosome condensation (PCC), were released from the block and assayed for the ability to synthesize DNA.

It is documented, by using ^{3}H-thymidine autoradiography and BrdUrd-labelling, that DNA replication may start out in prematurely condensed chromosomes of live cells, if these have passed the metaphase/anaphase transition.

Electron microscopic examination revealed that replicating areas of chromosomes are significantly less condensed than those formed during normal mitosis and, consequently, more accessible the replication machinery.

The ability of prematurely condensed chromosomes to synthesize DNA has been further supported by observations made using anti-PCNA antibodies. These observations have also been supported by experiments using phospho-Rb and anti-ORC labeling.

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5.21.

Testing of the advanced backcross population as material for fine mapping the restorer gene in rye with the CMS-C

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The production of rye hybrid cultivars is based on the cytoplasmic-genic male sterility (CMS). The Pampa sterility-inducing cytoplasm is widely used for breeding purposes. The CMS-C source discovered over 35 years ago in the old Polish cultivar Smolickie can be applied as an alternative for the Pampa cytoplasm. Restoration of male fertility in rye with CMS-C is controlled by at least three independent loci. Between them, *Rfc1* gene localised on the long arm of 4R chromosome is considered as the most important one for phenotypic expression of male fertility. Precise localisation of this gene has been difficult to determine, because in the formerly analysed mapping populations oligogenic control of the trait was observed. In order to develop a new mapping population a male sterile 544CMS-C inbred line was crossed with the moderate restorer 51527LM. Male fertile plants from F1 hybrid and next four backcross generations were used as pollinators for the 544CMS-C (the maternal line used as a recurrent parent). In the BC4 generation DNA was isolated and molecular markers (SSR-s and STS-s) were used for determination of genetic similarity of analysed individuals to the inbred line 544CMS-C. Extensive analyses presently done allowed for detection of polymorphic regions only on the long arms of 1R and 4R chromosomes. Association of male fertility/sterility phenotypes with the genetic polymorphism was stated on only for the 4RL genomic region. Segregation for male fertile and male sterile plants in BC4 population fits well with the monogenic model of inheritance. It seems that analysed backcross generation is a valuable source material for development of population for fine mapping of the *Rfc1* gene.

This work was financially supported by Polish Ministry of Agriculture and Rural Development.

5.22.

Crystal structures of the *Arabidopsis thaliana* proliferating cell nuclear antigen 1 and 2 proteins complexed with the human p21 C-terminal segment

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The proliferating cell nuclear antigen (PCNA) is one of the key proteins of the DNA replication machinery among all studied eukaryotic organisms. Despite its crucial role, very little structural and biochemical information particularly about plant PCNAs is available, in contrast to the knowledge obtained from other eukaryotic organisms.

Using X-ray diffraction method we have determined the crystal structures of the two *Arabidopsis thaliana* PCNA proteins

(*At*PCNA1 and 2), both complexed with the C-terminal segment of human p21. Both *At*PCNAs form homo-trimeric ring structures, which are essentially identical to each other, including the major contacts with the p21 peptide. The structure of the amino-terminal half of the p21 peptide, containing the typical PIP box sequence, is remarkably similar to those observed in the previously reported crystal structures of the human and archaeal PCNA-PIP box complexes. Meanwhile, the carboxy-terminal halves of the p21 peptide in the plant PCNA complexes are bound to the protein in a unique manner, most probably due to crystal packing effects. A surface plasmon resonance analysis revealed strikingly high affinity between each *At*PCNA and the C-terminal fragment of human p21. This result strongly suggests that the interaction is functionally significant, although no plant homologs of p21 have been identified yet. We also discovered that *At*PCNA1 and *At*PCNA2 indeed form hetero-trimers, implying that hetero PCNA rings may play critical roles in cellular signal transduction, particularly in DNA repair.

5.23.

Identification of self-incompatibility (S)-haplotypes in Polish breeding lines of white cabbage

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Self-incompatibility (SI) is an outcrossing-promoting mechanism by which self-incompatible plants reject their own and genetically similar pollen. In self-incompatible reaction pollen does not germinate and/or pollen tube growth is inhibited before it reaches the ovule. The specificity of pollen-stigma interaction is controlled by multiple alleles of the S locus with over 80 alleles known in *Brassica oleracea* (Ruffio-Chable et al., 2001). Herewith, we report an attempt to identify the S alleles present in Polish breeding material of white cabbage. For this purpose, fragments of the S locus glycoprotein gene and S locus receptor kinase gene were amplified and the resulting PCR products were digested with tetra-cutting restriction enzymes. These procedures were performed for both the 32 analyzed and 24 tester lines carrying the following S-alleles: S^1, S^2, S^4, S^8, S^9, S^{11}, S^{13}, S^{14}, S^{15}, S^{17}, S^{18}, S^{20}, S^{23}, S^{28}, S^{32}, S^{39}, S^{45}, S^{46}, S^{50}, S^{53}, S^{55}, S^{57}, S^{60}, S^{61}. Comparison of the PCR-RFLP profiles allowed S-allele identification in 22 of the analyzed lines. Their profiles were identical to those obtained for testers carrying S^2, S^{14}, S^{15}, S^{28}, S^{50} and S^{55}. Moreover, self-incompatibility phenotype of the analyzed lines was tested using pollination analysis based on both seed counting and pollen germination visualized by UV fluorescence microscopy. These observations confirmed that the analyzed lines are self-incompatible and that the line with the S^2 allele had less stable SI phenotype than others.

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5.24.

Genome recombination in triploid hybrids of meadow fescue (*Festuca pratensis* huds.) with perennial ryegrass (*Lolium perenne* L.)

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Meadow fescue (*F. pratensis* Huds.) and perennial ryegrass (*L. perenne* L.) can hybridize readily at different ploidy levels, producing diploid, triploid and tetraploid hybrids. Although diploid hybrids *F. pratensis* (2n = 2x = 14) × *L. perenne* (2n = 2x = 14) exhibit full chromosome pairing, they are nearly completely sterile. In contrast to diploid hybrids, triploid hybrids, which resulted from crosses of diploid *F. pratensis* and synthetic tetraploid

L. perenne, show partially male and female fertility. These triploid hybrids *F. pratensis* (2x) × *L. perenne* (4x) can be used in backcrossing breeding programs. In this study, we present the analysis of genome structure and homoeologous recombination in the first backcross generation (using genomic in situ hybridization), obtained from crosses of triploid hybrids

F. pratensis × *L. perenne* (used as the male parent) into diploid and tetraploid *L. perenne* cultivars 'Arka' and 'Solen', respectively. Cytological analyses in both backcross progenies showed a big variability in respect of somatic chromosome number, genomic structure and recombination. The number of complete chromosomes of *F. pratensis* per recovered male gamete ranged from 0 to 7, and it was much lower in the backcross to the diploid (2.2/gamete) than in that to the tetraploid (5.5/gamete). The frequency of *Lolium*/*Festuca* recombinant chromosomes was higher in the gametes recovered in the backcross to the tetraploid (2.0/gamete) than in the backcross to the diploid (1.4/gamete). The homoeologous recombination breakpoints were distributed along almost entire length of chromosome arms. In both combinations most of recombinant chromosomes had single recombination breakpoints, however, double breakpoints and sporadically triple and quadruple breakpoints were also observed.

Reciprocal and extensive intergeneric recombination observed between the parental genomes of these triploid hybrids are important for introgression breeding programs, and can be used, for example, to transfer of abiotic and biotic stress resistance traits from *Festuca* species into *Lolium* species.

This work was partially supported by the Polish Ministry of Science and Higher Education (grant no. N N310 090736)

5.25.

Utilization of chloroplast and mitochondrial DNA sequences in analysis of organelle inheritance in *Gentiana* somatic hybrids

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Somatic hybridization offers the possibility of manipulating not only nuclear but also chloroplast and mitochondrial genomes. In 38 experiments of *Gentiana* protoplast fusion numerous calli and plants were produced. About 170 regenerants obtained after electrofusion between *G. kurroo* Royle and *G. cruciata* L. as well as between *G. cruciata* and *G. tibetica* King protoplasts were characterized by flow cytometry and AFLP analysis, which con-

firmed their nuclear hybridity. In order to investigate the inheritance of organelle genomes in these somatic hybrids, utilization of selected chloroplast (cp) and mitochondrial (mt) DNA non-coding regions (Dumolin-Lapegue et al., 1997) was tested. Four cpDNA and four mtDNA primer pairs were applied for amplification of desired cpDNA and mtDNA regions from total genomic DNA of *G. kurroo, G. cruciata and G. tibetica*. After purification obtained PCR fragments of 500–1000 bp length were sequenced to determine differences between chloroplast and mitochondrial genomes of parental species. Usability of these sequences for further CAPS or SNP analysis of somatic hybrids organelle DNA is discussed.

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5.26.

Comparison of two H^+ATPase isoforms from cucumber plants

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In cells of higher plants, P-type H^+ATPase plays a central role in active transport across the plasma membrane and thus is involved in many physiological functions such as nutrient uptake, growth of roots hairs, acid growth, osmoregulation, maintenance of cytoplasmic pH and abiotic stress resistance. Because of its important role in mentioned processes, the activity of H^+ATPase is expected to be controlled by different environmental and metabolic changes. Furthermore, plasma membrane (PM) proton pumps have been characterized in various plant species and is encoded by a multigene family: 12 genes have been described in *Arabidopsis thaliana* (*AHA 1-AHA12*), 10 genes in *Oryza sativa* (*OSA 1-OSA 10*), 9 genes in tabaco (*PMA1-PMA9*), 8 genes in tomato (http://www.ncbi.nlm.nih.gov/). It is thus imaginable that this multiplicity of isoforms makes possible an individual adjustment of the enzyme to all type of plant cell. So far, tree genes encoding PM H^+ATPase have been identified in *Cucumis sativus* (NCBI accession number AJ703810, AJ703811 and AF289025). Among them only one, *CsHA3* (NCBI EF375892), has been fully sequenced (Młodzińska et al., 2007). Here, we report the identification and characterization of a complete sequence of a new isogene, *CsHA2* (NCBI EU735752), and the determination of organ-specific expression pattern for *CsHA2* and *CsHA3* under a variety of conditions.

Amplification of 5' and 3' ends of *CsHA2* (AJ703811) by RACE-PCR technique enabled to obtain a full-length cDNA, which encodes a proteins of 954 amino acids. CsHA2 isoform contains five conserved regions found in others P-type ATPases, shows high sequence identity to CsHA3 and also to other members of the PM H^+ATPase family. The RT-PCR analysis of various organs revealed differential expression pattern for *CsHA2* and *CsHA3* genes suggesting that these proteins could be involved in separated physiological processes. *CsHA2* mRNA expression was detected in all organs examined in immature and mature plants whereas *CsHA3* was expressed only in the roots. Furthermore, we tried to find out whether the abundance of *CsHAs* transcripts in organs collected from seedling was dependent on abiotic stresses (salt stress, osmotic stress and heavy metal stress). This results showed that *CsHA3* and *CsHA2* expression was differentially altered under stress conditions and suggested that PM H^+ATPase can participate in plant stress resistance.

Session 6

Mutants in developmental and metabolic studies

PLENARY LECTURES

6.1.

Biosynthesis and accumulation of inositol phosphates in developing seeds

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Most of the phosphorus in the resting seed is stored inside protein storage vacuoles as phytic acid (PA). The biosynthesis and accumulation of PA can be detected beginning from a few days after anthesis and seem to continue during seed development until maturation. The first step in PA biosynthesis is the formation of inositol-3-phosphate by conversion of glucose-6-phosphate. This is then followed by a sequential and ordered phosphorylation of the remaining five positions of the inositol ring by a number of kinases resulting in PA (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate). Homologous kinases from the animal and plant kingdom have been cloned and, with the help of recombinant proteins, their biochemical characterization in terms of specificity and activity towards inositol-phosphates has been forwarded (Josefsen et al., 2007). Identification of low-phytic acid mutants in the cereals barley, rice, maize and in soybean is instrumental for the elucidation of genes in the biosynthetic pathway and identification of genes controlling the accumulation of PA. Understanding the biosynthetic pathway and genes controlling and limiting the accumulation of PA in plant seeds and how PA may balance the free phosphate is of importance for molecular breeding of crop plants in particular cereals and soybean. Therefore TILLING as a revers genetic tool is used in order to identify mutants in barley and hexaploid wheat. Phytic acid itself poses a number of challenges in husbandry fodder and for staples in human nutrition as reviewed in Bohn et al. (2008). PA is stored in globoids as salts with minerals like K > Mg > Ca > Fe in concentration order (Bohn et al., 2008). PA reduces the bioavailability of Fe and Zn, and thus contributes to the 'hinden hunger'.

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6.2.

Variation to dissect yield related traits in barley

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Direct identification of the genes underlying complex quantitative traits such as grain yield for the crop plants by means of conventional genetic analysis (positional cloning) requires several prohibitively large mapping populations. However, by using induced mutagenesis we propose that phenotypically similar, but more extreme allelic variants of the same genes that underlie quantitative trait loci (QTLs) can be generated. Consequently these extreme alleles can be used much more efficiently to isolate the causal genes by positional cloning and by exploiting conservation of synteny with model species.

In an attempt to identify the genes that underlie barley grain yield quantitative trait loci (QTLs) in the Steptoe × Morex doubled haploid recombinant population (*St/Mx*), we exploited a genotypic data set of ~1000 nearly isogenic barley lines (NILs) carrying induced mutations in genes that control various aspects of barley plant development. Yield can be decomposed into a number of component traits such as number of tillers per plant, number of grains per tiller and the size of individual grains. We focused primarily on NILs that contained introgression segments overlapping with yield QTLs mapped previously in the *St/Mx* population and which carried mutations affecting branching of the vegetative and inflorescence meristems, grain size, plant height, spike length, spikelet density, flowering time, lodging and fertility. In parallel, based on the rice physical map and recently developed barley gene map, we developed an accurate barley-rice synteny map, which enabled us to test whether known rice and maize yield related genes are likely candidates for barley yield QTLs and induced mutants.

As a result we identified isogenic lines that have introgressions mapping to the same chromosomal regions as grain yield QTLs. We will report on progress in identification and validation of the candidate genes for these QTLs and their putative mutant phenotypes.

6.3.

Mutants in Photorespiration Research

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Photorespiration represents one of the major highways of plant primary metabolism. By mass flow, excelled only by photosynthesis, it actually constitutes the second-most important process in the land-based biosphere. While this ancient ancillary metabolic process enables plants to thrive in an oxygen-containing environment, it also sacrifices a significant part of the freshly assimilated carbon to the atmosphere. Photorespiratory CO_2 losses can be very high and are further elevated by warmer temperatures and drought, hence reducing the yields of important crops.

In light of the importance of this process for plant metabolism, adaptation and productivity it is surprising that a significant share of the components of the pathway, such as metabolite transporters, regulatory proteins, and metabolic enzymes are not yet known. Similarly, our understanding of interactions with other major metabolic pathways is still very limited, and most of the accumulated knowledge is restricted to the C_3 type of land plant photosynthesis.

A large number of these questions can be answered by mutant analysis, which has always been an important tool in photorespiration research. In our modern days, insertional mutagenesis allows to much more easily pinpointing introduced genetic defects and even selectively knockout specific genes. Moreover, the genomes of an increasing number of photosynthetic organisms are known, and powerful technologies facilitate the analysis of gene expression and other metabolism. All this has revitalized photorespiration research and led to exciting discoveries about the evolution, functioning and metabolic integration of this important pathway.

ORAL PRESENTATIONS

6.4.

Toward the functions of plant mitochondrial proteases using a reverse genetic approach

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To understand function of mitochondrial proteases during *Arabidopsis* growth, development and in response to environmental stimuli we are using a reverse genetic approach. Mutants with a T-DNA insertion in genes of our interest and mutants generated by *RNA* interference (*RNAi*) procedure have been analyzed. Except for mutation in *AtFtsH4*, mutations in other genes encoding mitochondrial proteases failed to cause discernible alterations in plant morphology. The lack of easily detectable morphological abnormalities results from functional redundancy or other compensatory mechanisms. Functional redundancy was observed between two closely related ATP-dependent metalloproteases, *AtFtsH3* and *AtFtsH10*. Plants which are defective in *AtFtsH3* have a higher level of the *AtFtsH10* protein compared with wild type plants. The accumulation of the *AtFtsH10* protein is not due to a higher level of respective transcripts but it is associated with altered translational activity of these transcripts. We found that a loss of *AtFtsH4*, did not significantly affect *Arabidopsis* growth in long day conditions, however, severe morphological and developmental abnormalities occurred under short day photoperiod as well as after prolonged exposure to moderately high temperature of 30°C. We postulate that the increased level of the molecular chaperones in ftsh4 compensates for the deficiency of *AtFtsH4*, however, this compensation is fully efficient only in long day conditions. Under short photoperiod and prolonged exposure to moderately high temperature *ftsh4* mutants exhibit informative phenotypes which are investigating in aim to understand the function of the *AtFtsH4* protease. Our data indicate that the lack of *AtFtsH4* results in an impairment of organelle development and *Arabidopsis* leaf morphology under short-day conditions, in which the vegetative phase is extended. We postulate that at least some of these changes are associated with accumulation of oxidized proteins (Gibała et al., 2009)

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6.5.

Global analysis of root hair morphogenesis transcriptome using wild type/root hair mutant system in barley

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Root hairs are specialized epidermal cells that play an important role in plant nutrition. They are also an important model in studies of higher plant cell differentiation. Root hairs are not required for plant survival, thus, a range of mutants with abnormal root hair phenotypes can be created to investigate molecular mecha-

nisms of root hair development. Large collections of *Arabidopsis thaliana* root hair mutants made possible the identification of approximately 50 loci responsible for root hair formation. In recent years, most of these genes have been cloned and characterized. Contrary to *Arabidopsis*, there is very little information available on the genetic and molecular basis of root hair formation in monocots, including major cereals. The main limitation is the unavailability of analogous mutant collections. The collection of monogenic root hair mutants developed in our Department made it possible to initiate studies on genetic and molecular characterization of root hair morphogenesis in barley.

Our previous analysis with the use of high stringency transcriptome differentiation method (cDNA RDA) resulted in the discovery of *HvEXPB1*, a gene critical for root hair initiation in barley. In order to find new candidate genes involved in the root hair formation we continued characterization of the root hair formation transcriptome using Affymetrix GeneChip Barley1 Genome Array. Global root hair transcriptome differentiation carried out in the system of the root-hairless mutant *rhl1.a*/wild-type parent variety 'Karat' revealed 10 genes potentially involved in the first step of root hair formation in barley. Differential expression of these genes was confirmed by qRT-PCR analysis. Almost all identified genes were associated with membranes and cell wall. One gene encodes xyloglucan endotransglycosylases, three genes encode peroxidase enzymes and the five others encode proteins belonging to arabinogalactan proteins, extensins, leucine-rich-repeat proteins, phosphatidylinositol phosphatidylcholine transfer proteins and a RhoGTPase GDP dissociation inhibitors. The function of one gene is unknown. The expression profile of the identified genes was highly reduced in the root-hairless mutant *rhl1.a* compared to the parent variety, however all 10 genes showed the same transcription level in the mutant *rhp1.b* characterized by root hairs blocked at the primordium stage and the wild-type varieties. This fact clearly indicates that the identified genes are involved in the initiation of root hair morphogenesis in barley.

6.6.

How organ boundaries are formed: a case of *cuc2 cuc3* mutant of *Arabidopsis thaliana*

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Plants produce new organs throughout their lifetime. The formation of organs involves generation of boundaries, separating the primodium from the shoot apical meristem (SAM) or separating adjacent primordia. Boundary regions are unique in their geometry, growth, and expression of genes like *CUP-SHAPED COTYLEDON* (*CUC*) in *Arabidopsis* (Aida and Tasaka, 2006). Double mutations in *CUC* genes result in organ fusions, e.g. cotyledon fusion, leaf/stem or pedicel/stem fusion (Hibara et al., 2006).

We investigate how the fusions between pedicels and the inflorescence stems are formed in *cuc2 cuc3* double mutant. We quantify geometry and growth anisotropy (Dumais and Kwiatkowska, 2002) of the SAM surface as well as of the surface of the young pedicel and stem. Since the CUC expression is not only in the protodermis (and later epidermis) but also in the underlying cells at the boundary we analyze the cellular pattern in longitudinal sections of the inflorescence. Surprisingly, the effect of mutation in the shoot apex is rather subtle. The main difference between the mutant and wild type apices is in that the formation of the boundary between the SAM and the flower primodium is delayed by about one plastochron. Nevertheless, the geometry of boundaries in the mutant is very similar to that of the wild type. The differences become apparent during later developmental stages. In the wild type both growth and cell divisions of epidermis at the node are slowed down. Growth anisotropy and division planes in the mutant are different so that the typical nodes and internodes do not develop. There are also differences in the inner cellular pattern, in particular in parenchyma cell sizes and shapes.

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POSTERS

6.7.

Arabidopsis lysophospholipid acyltransferase catalyses the transfer of acyl groups from phosphatidylcholine to CoA

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Lysophospholipid acyltransferases (LPLATs) are ubiquitous enzymes, which catalyse the transfer of the acyl group from acyl-CoA to lysophospholipids (in the forward reaction), producing different types of phospholipids. In addition to their role in the syntheses of phospholipids, it was proposed that LPLATs (especially LPCAT – lysophosphatidylcholine acyltransferase) have a significant role in exchanging fatty acids between phospholipids and acyl-CoA pool (i.e. catalysing also the backward reaction). However, there was no hard experimental evidence for this proposed role of LPLATs. In the presented study, we provide such evidence for one of the *Arabidopsis* LPLATs encoded by *At1g63050* gene.

Yeast (BY4742 strain) with disrupted *YOR175c* gene (encoding yeast lysophospholipid acyltransferase, which accounts for most of the LPCAT activity found in yeast cells) were transformed with *At1g63050* gene (*Arabidopsis* gene encoding enzyme with LPLAT activity) or with pYES2 (the empty plasmid). Microsomes prepared from transformed yeast cells were used for *in vitro* assays.

In assays with 18:1-LPC and [14C]18:1-CoA *Arabidopsis* LPLAT very efficiently synthesised phosphatidylcholine (about 1,4 μmol [14C]PC/min/mg microsomal protein). In the presence of only [14C]18:1-CoA the synthesis of PC was not detected. The addition of BSA and CoA to the reaction mixture stimulated the production of [14C]PC (the backward reaction). Presence of DTNB (binds CoA) in the reaction mixture strongly inhibited the backward reaction but had no significant effect on the forward reaction.

To verify that observed *de novo* synthesis of PC from added [14C]18:1-CoA occurred *via* exchange of fatty acids between acyl-CoA pool and PC, microsomal preparations were incubated with sn-2-[14C]18:2-PC, BSA, CoA and 18:1-CoA (non-radioactive) at optimal concentrations. During the incubation time [14C]18:2 in acyl-CoA pool was gradually increasing and in the same time similar amount of [14C]18:2 disappeared from added PC, whereas no re-distribution of radioactivity occurred in control incubations (the empty plasmid). The obtained results confirm that LPLAT can operate in both forward and reversible mode and thus can participate in exchanging fatty acids between phosphatidylcholine and acyl CoA pool.

6.8.

From genetic analysis to identification genes responsible for root hair formation in barley

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Root hairs are the tubular outgrowths of epidermal cells called trichoblasts. They are responsible for anchoring plants in soil, the uptake of water and nutrients. They are also sites of interaction between the plant and symbiotic bacteria. The unique collection of root hair mutants developed in the Department of Genetics University of Silesia made it possible to initiate studies on genetic and molecular basis of root hair development in barley. The material for the presented studies were 18 root hair mutants representing different stages of root hair development, obtained by chemical mutagenic treatment of spring barley varieties: 'Dema', 'Rudzik', 'Karat', 'Diva' and 'Optic'. Studies have proved that each mutant was monogenic and recessive. Allelism test revealed that one locus (*rhl*) was responsible for root hair initiation, one locus (*rhp*) for transition to tip growth, four loci (*rhs*) were responsible for root hair elongation and 3 loci (*rhi*) for localization of root hairs. Genetic relationships between genes responsible for different stages of the root hair formation were revealed. Using SSR and AFLP markers the chromosomal position of two genes controlling root hair development was established. The gene responsible for tip growth was mapped on the chromosome 6H, and the gene for localization of root hairs was mapped on chromosome 1H. Using reverse genetic approach the identification of new genes responsible for root hair development in barley have been undertaken. On the basis of a bioinformatics analysis, the genes of Arabidopsis thaliana, maize and rice, controlling different stages of root hair development were selected to search for barley EST sequences, which can be used for isolation and characterization the homologous barley genes.

6.9.

Abiotic stress response of *Arabidopsis thaliana* mutants displaying suppression of hypersensitivity to ABA

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After chemical mutagenesis of *abh1* (*ABA hypersensitive 1*) insertion line, mutants that suppress *abh1* hypersensitive phenotype during seed germination on medium containing 0,4 μM ABA were obtained. Three lines A18, A21 and A36 were chosen for further analysis. The aim of the presented study was to investigate mutants response to selected abiotic stresses: drought and salt. Parental line, *abh1*, is drought tolerant, therefore water stress assay was performed. After 26 days of drought treatment, mutants and *abh1* displayed slightly wilty phenotype while Columbia (Col-0) did not survived. During next three days of

assay plants were rewatered. Mutants and abh1 recovered normal phenotype while Col-0 did not. RWC (Relative Water Content) in leaves and WL (water loss) in leaves were calculated. The assay revealed that mutants and *abh1* showed higher rate of RWC than Col-0. Water loss rate value for Col-0 was much higher after 200 minutes than for mutants and *abh1*. The stomatal density and distribution was studied to explain the different rates of WL and RWC. It was shown that mutants displayed changed stomatal distribution and density when compared to Col-0 and *abh1*. A36 mutant has similar stomata pattern to *sdd1* (*stomatal density and distribution 1*). Because of strong link between response to drought and high salinity stress, salt tolerance was investigated in mutants and parental lines. Seed germination on medium containing different doses of NaCl was estimated. It was shown that low concentration of NaCl did not cause any changes in seed germination. The most tolerant to salt stress proved to be A18 mutant, able to germinate on medium containing the highest concentration of NaCl (200 mM) We believe that the presented approach combined with molecular analyses can shed a light on ABA and stress signaling pathway in *Arabidopsis*.

6.10.

How the ploidy level affects size and growth rates of leaf epidermal cells in triple *cyclin D3* mutant of *Arabidopsis thaliana*?

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Cyclins D3 (CYCD3) are involved in the regulation of mitotic cycle and endoreduplication. Therefore, *cycD3* mutants provide a valuable material to study the relationships between cell size, growth rate and ploidy level. Moreover, a putative compensation of defects in cell proliferation by an increased cell expansion can be investigated.

We study the epidermis of young leaves of *Arabidopsis* triple *cycD3* mutant. We use the sequential replica method coupled with epi-fluorescence microscopy and quantify size, growth and division rates, as well as the nucleus size for individual cells. An index combining the surface area of a cell and the waviness of its anticlinal walls is used to estimate a degree of the cell differentiation. As published by DeWitte and collaborators (2007) the average epidermal cell surface areas are bigger in mutant than in wild type, and the average ploidy level of all the leaf cells in the mutant is higher. We confirm these observations and show further that in general, in case of so-called puzzle epidermal cells the bigger cell surface area is related to the bigger surface area of its nucleus (measured in the optical paradermal section). In the mutant the areal growth rates of puzzle cells do not seem to be affected by the ploidy level. The same is true for the wild type. However, there is a tendency that areal growth rates in the mutant are higher than in wild type. The analysis of cell lineages and degree of differentiation in individual cells shows that also the meristemoid function is affected by the *cycD3* mutations.

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6.11.

Identification of genes involved in somatic embryogenesis induction in *Arabidopsis* with the use of cDNA RDA method and *tan1* mutant

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Somatic embryogenesis is a process in which a bipolar structure resembling a zygotic embryo is formed from somatic plant cells. Although this phenomenon is of great practical value for plant micropropagation and genetic transformation, little is known about its molecular and genetic determination. In the presented studies we used cDNA RDA method to identify genes involved in somatic embryogenesis (SE) process. The process was induced under *in vitro* culture of immature zygotic embryo explants of *Arabidopsis* on auxin medium. To identify genes differentially expressed during SE two genotypes were applied – the control ecotype (Col-0) of high capacity for SE and *tanmei/emb2757* mutant, disabled in production of somatic embryos. The transcriptoms of analysed genotypes in cultures upon 2–8 days of SE induction were compared. Following cDNA RDA procedure including three rounds of substractive hybridization, three differentially expressed genes were identify: *At1g04660, At3g27660, At5g44120. At1g04660* encodes a glycine-rich protein, which function is still unknown, while *At3g27660* and *At5g44120* are responsible for synthesis of OLEO4 and CRA1 proteins, respectively. Both proteins are involved in the maturation phase of zygotic embryogenesis. The Real-Time qPCR technique was used to verify significant difference in expression level of the identified genes in Col-0 than in *tanmei/emb2757* culture. Additionally, the identified genes were found to be highly activated in embryogenic Col-0 cultures but not in callus tissue of this genotype. Moreover, the knock-out mutants in *OLEO4* and *CRA1* genes show significantly reduced embryogenic capacity. All three identified genes are specifically expressed during zygotic embryogenesis (www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) and their precise function in SE needs further experiments.

6.12.

Mutation in a C-terminal strand of the large subunit enhances specificity factor for rubisco from *Thermosynechococcus elongatus*

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco, EC 4.1.1.39) is the key enzyme in photosynthesis. In cyanobacteria, green algae and plants, rubisco exists as a holoenzyme composed of eight large (RbcL) and eight small (RbcS) subunits (L_8S_8 complex). Rubisco catalyzes the addition of CO_2 to ribulose-1,5-bisphosphate (RuBP). Rubisco also catalyzes an additional reaction involving molecular oxygen (O_2) in the oxygenation of RuBP and, therefore, it is also a key enzyme for the photorespiration pathway.

The preference of the enzyme for CO_2 versus O_2 is represented by specificity factor (SF). This factor is a constant that characterizes the ability of the enzyme to distribute RuBP between the

two reactions in terms of the concentrations of the gaseous substrates (CO_2 and O_2). The values of SF vary considerably among species. A high SF value means more efficient photosynthesis in an oxygenic atmosphere.

Recently we have found that the rubisco from the termophilic cyanobacterium *Thermosynechococcus elongatus* has an almost 2-fold higher SF compared with the rubisco of mesophilic cyanobacteria, reaching the value of higher plants. It seems possible to connect the enhanced specificity with differences in the primary structure of the mesophilic and thermophilic enzyme.

Comparison of the sequence of rubisco from all species where SF is known has revealed the C-terminal strand (mobile segment; residue number > 460) of the RbcL subunit as a hotspot for specificity-enhancing mutations. SeqCat algoritm based on the "time-window" hypothesis – which describes a mechanism which produces enhanced specificity – pointed aminoacid substitutions in the sequence of the RbcL of rubisco which could enhance enzyme specificity. One of those substitution for RbcL from *T. elongatus* is E470P mutation.

In this study the expression plasmid pUC18rbcL_pXST.el. containing genes for: mutated RbcL subunit (E470P), RbcS subunit and the X protein from *T. elongatus* was constructed. The recombinant enzyme was expressed in *Escherichia coli* cells, the protein was purified and SF was measured. The SF value for mutated rubisco was 95.83 ± 2.56 compare to the wild type of 83.97 ± 1.91. The results presented here are the first experimental data confirming the "time-window" hypothesis. However to verify hypothesis completely, successive RbcL C-terminal mutants will be overexpressed and characterized in our group.

6.13.

Molecular mapping of genes responsible for root architecture in barley

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Barley (*Hordeum vulgare* L.) is one of the most important crop species in the world, and also quite extensively exploited in research, for the identification of genes responsible for resistance to different diseases, yield or malting quality characters. However not much has been done to identify and isolate qualitative and quantitative trait loci (QTL) responsible for root architecture in barley. According to the data previously published, genes responsible for semidwarfness often express a pleiotropic effect on the length of barley root system. Our previous studies on the root length using semidwarf barley mutants indicated that the length of root and shoot mutated independently, and dwarf recombinants with long roots were obtained. We report on progress in molecular mapping of qualitative and quantitative trait loci underlying the root architecture and root hair morphology in a semidwarf mutant line 225DV from 'Diva' cultivar. In an attempt to map the genes of interest, doubled haploid populations were developed from F_1 of the crosses 225DV × 'Steptoe' (225DVS) and 225DV × 'Morex' (225DVM). The mapping procedure included several steps starting from the construction of a consensus molecular map using SSR markers together with AFLP and SNP markers. The analysis of root architecture and root hair morphology was performed in two week old plants after their growth in plexiglass tubes filled with the vermiculite. In the analysis of the root system, the following traits were measured with the use of the winRhizo program: the length of seminal and lateral roots, root surface area, root volume and root diameter. The Axiovision LE program was applied to estimate the root hair morphology. A final mapping analysis was performed using the MapQTL program, and combined the phenotypic data set with the molecular mapping information. In future, fine mapping of loci responsible for the root architecture might enable the isolation and transfer of desirable alleles into barley germplasm, and the construction of a new plant type with semidwarf shoot and long, fast penetrating roots, especially important for plants grown under drought and poor nutrient uptake stresses.

6.14.

Changes in phenylpropanoid metabolism in the leaves and roots of cucumber wild-type and *MSC16* mutant subjected to chilling or phosphate deficiency

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In the tissues of cucumber (*Cucumis sativus* L.) *MSC16* mutant the concentrations and antioxidant capacity of phenylpropanoids, the activities of peroxidases (POX) and polyphenol oxidases (PPO) were initially higher than in WT plants and did not significantly change during chilling and/or phosphate (Pi) deficiency. However, stress conditions changed the level and metabolism of phenylpropanoid compounds in the leaves and roots of cucumber wild-type (WT) plants. Total phenolics, free or cell wall-bound phenolic compounds and flavonoid concentrations were always higher in the tissues of WT plants subjected to low temperatures and Pi starvation. Higher antioxidant capacity (as measured by $ABTS^{\bullet+}$ test), higher activity of POX and leaf PPO were also observed in WT plants grown under stress conditions. The activity of the only one enzyme of phenylpropanoid metabolism, L-phenylalanine ammonia lyase (PAL), was almost doubled, similarly in WT and *MSC16* plants as a respond to low temperature.

The mitochondrial genome rearrangements in *MSC16* plants resulted in modifications of phenylpropanoid metabolism similar to those observed following abiotic stress conditions (chilling and/or Pi deficiency) in WT plants. Mitochondrial mutation in cucumber plants affects the overall antioxidant defense system and respiratory metabolism to the same extent as low temperature stress (Szal et al., 2009). Mitochondria functioning directly coordinate the redox, energy status and reactive oxygen species turnover in the plant cell *via* the signals transmitted to the nucleus in mitochondrial retrograde regulation (Rhoads and Subbaiah, 2007), but indirectly might also modify phenylpropanoid metabolism. Similar changes in the level of phenolic compounds in cucumber caused by mitochondrial genome rearrangement and abiotic stresses indicate that variety of environmental and/or developmental cues might induce a common physiological symptoms of plant acclimation.

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6.15.

Phosphatidylglycerol: an important phospholipid for the maintenance of thylakoid structure and function during cold acclimation of *Arabidopsis thaliana*

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Phosphatidylglycerol (PG) biogenesis, specific occurrence and high abundance in thylakoid membranes designate its important role in chloroplast development and the integrity of the photosynthetic apparatus. In this study, PG deficient mutant (*pgp1*) created by mutation of the phosphatidylglycerolphosphate synthase isoform encoded by a gene on chromosome 2 was used for assessing the role of PG in formation and functioning of the photosynthetic apparatus during cold acclimation of *Arabidopsis* plants. In contrast to wild type (WT) *Arabidopsis* plants, a distinct yellow-white phenotype was produced during cold acclimation of *pgp1* mutant, which was not present under optimal growth temperature of 20°C. This was accompanied by a drastic reduction of total chlorophyll content and a sharp increase of Chl *a/b* ratio (about 5), thus suggesting lower amount of LHCII proteins in cold acclimated *pgp1* mutant. Indeed, immunoblot analysis indicated much lower abundance of Lhcb1 and Lhcb2 polypeptides under the same conditions. Transmission electron microscopy revealed a reduced number of thylakoids per grana stack in *pgp1* mutant grown at 20°C compared to WT, while the number of stromal thylakoids remained the same. In the cold acclimated *pgp1* mutant, the formation of grana stacks was completely inhibited and the number of stromal thylakoids per chloroplast was significantly reduced compared to WT. Concomitant with this, low temperature (77K) chlorophyll fluorescence measurements demonstrated significant redistribution of excitation light energy in favor to PSI in *pgp1* mutant regardless of the growth conditions.

6.16.

FISH in analysis of gamma ray-induced aberrations in *Hordeum vulgare* cells

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Fluorescent *in situ* hybridization (FISH) is not widely applied in plant mutagenesis for the detection and precise localization of chromosome aberrations, as DNA probes required for chromosomes of particular plant species are limited. In contrast, the combination of multiple DNA probes labeled with different fluorochromes makes FISH a powerful tool in human radiation cytogenetics. Chromosomal aberrations can be as well detected with classical cytogenetic methods, however FISH provide new tool for their analysis. Unquestionable advantage of FISH technique is the detection of small chromosome rearrangements, as well as their detailed characterization in dividing cells. Additionally, FISH with specific DNA probes can improve existing micronucleus test providing information on the mechanisms underlying the formation of chromosome aberrations.

In present study fluorescent *in situ* hybridization (FISH) using telomere/centromere and rDNA probes was used in detailed analysis of the cytogenetic effects of gamma ray in root tip meristem cells of *Hordeum vulgare* (2n=14). The micronucleus test (MN) combined with FISH allowed quantitative analysis of the involvement of chromosome fragments in micronuclei formation and thus enabled explanation the origin of gamma ray-induced aberrations.

The most frequently gamma ray has caused terminal deletions, as micronuclei with signals of telomeric DNA were the most often observed. No micronuclei with centromere specific signal were observed. Thanks to specific localization of rDNA loci on barley chromosomes the results of FISH with rDNA as probes specified the size of telomeric fragments. The chromosome breaks in barley occurred in heterochromatin regions near the centromere. Additionally it has been showed that 5S rDNA bearing chromosomes were more often involved in formation of chromosome aberrations than chromosomes with 25S rDNA loci.

As the quantification of micronuclei in plant cells is not very popular, presented results proved that FISH with centromere/telomere-specific and rDNA probes can be promising technique in the evaluation of the origin of chromosome aberrations.

6.17.

The effects of phosphorus deficit on growth and metabolism of *Arabidopsis* hormonal mutants

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Arabidopsis thaliana mutants are great tools to investigate almost all physiological and biochemical processes (Gazzarrini and McCourt, 2003). In this work we used hormonal mutants as a model system to characterize the physiological basis by which phosphorus availability regulates plant growth. Plant responses to hormones and nutrient deficit are often unique, depending on the system, stage of development or the specific tissue involved (Gibson, 2004). Phosphorus is an essential nutrient for plant growth and metabolism but in many soils, phosphorus is the major limiting nutrient for agriculture (Lopez-Bucio et al., 2002). The influence of inorganic phosphate (Pi) deficit on growth and development of different *A. thaliana* mutants was studied. Mutants with changed hormonal metabolism: *aba2–3* (abscisic acid deficit), *abi4–101* (insensitive to abscisic acid), *aux1–7* (insensitive to auxin and ethylene), *eto1–1* (with overproduction of ethylene) and *etr1–1* (insensitive to ethylene) were cultured for 2–4 weeks on medium without Pi. Generally, phosphate starvation decreased Pi content in tissues and reduced plant growth, like fresh mass of rosette leaves or roots. Plants cultured without Pi had longer roots than control (for about 30%), but *eto1–1* had shorter roots. Mutants *abi4–101*, *aux1–7*, *etr1–1* had reduced level of Pi compared to wild type plants, even during culture on full nutrient medium. Pi deficiency decreased level of chlorophyll and carotenoids in leaves of studied plants. Phosphate limitation decreased soluble carbohydrates content in leaves but starch content increased both in wild type and mutants of *A. thaliana*. Pi deficiency reduced proline content in leaves of wild type, *abi4–101, aux1–7, eto1–1*, had no influence on proline level in *aba2–3*, but in leaves of *etr1–1* mutant increased content of proline compared to control. These results indicated that phosphorus availability and changes in hormones biosynthesis (sensitivity to) have significant influence on growth of *A. thaliana* and metabolite content in tissues at the early stages of low-Pi stress.

We wish to thank TAIR for kindly providing us with seeds of mutants.

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6.18.

Altered growth as the effect of changed ROS/antioxidants equilibrium in the apoplast of cucumber mitochondrial mutant

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Reactive oxygen species (ROS) are generally regarded as harmful products of oxygenic metabolism causing oxidative stress and cell damage. However, it is now accepted that ROS such as H_2O_2 can also serve as the regulators of the redox state of the cell allowing controlled oxidation of cell components. ROS production initiated by the plasma membrane NAD(P)H oxidase can be used for controlled polymer breakdown leading to cell wall loosening during extension growth. In recent years it has become clear that redox signals are also integral to the transduction sequences by which changes in the environment modify metabolism and gene transcription. ROS perception, signaling and ROS-mediated protein modifications are controlled and modulated by array of the antioxidants. Here we propose, that mitochondrial genome mutation influences ROS/antioxidants metabolism and the signal originating from the dysfunctional mitochondria is transmitted to the nucleus by H_2O_2, formed as a result of increased superoxide production by the respiratory chain (Szal et al., 2009). Altered growth and development of *MSC16* cucumber mutant may result from disturbed ROS/antioxidants equilibrium, mainly in the apoplast. The concentration of H_2O_2 in leaf extracts of MSC16 plants was by about 45% lower than in the extracts from the WT. Cytochemical localization of H_2O_2 in leaf cells showed that in the WT H_2O_2 was present mostly in the apoplast whereas very little of it was localized in the apoplast of MSC16 leaf cells. The activity of plasma membrane NAD(P)H oxidase was about 30% lower in the plasmalemma vesicles isolated from MSC16 leaf tissue as compared to WT. About 3% of the ascorbate content of the whole leaf ascorbate was localized in the apoplast but in the MSC16 it was considerably more reduced. We conclude that lower content of apoplastic ROS caused by decreased activity of plasma membrane NAD(P)H oxidase and elevated ascorbate pool may result in altered growth and development of *MSC16* cucumber mutant.

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6.19.

The research on autonomous endosperm development in *Arabidopsis thaliana* cultured *in vitro*

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Autonomous endosperm (AE) is a very rare phenomenon in natural sexual plant but exist commonly in apomicts. In *fis* mutant seeds *in situ* AE is partly developed and overproliferated; only combination: *fie* mutation and hypomethylation let to full endosperm development. The main issue of our research was to induce and investigate the development of endosperm arose in the absence of fertilization. We cultured *in vitro* unpollinated ovaries of wild-type (Col-0) and mutants (*fie* and hypomethylated *met1* mutants) of *Arabidopsis*.

The embryological and molecular analysis showed that: (1) Induction of AE development occurred in all of genotypes, on the all media used and in the all repeats; (2) The average frequency of AE was a quite high (35% ovaries and 4% ovules) as AE is a very rare phenomenon; (3) The genotype but not the kind of medium used was essential importance; (4) AE nuclei originated from the secondary nucleus as indicated by their nuclear size and structure; (5) *in vitro*, AE development is delayed in comparison with normal developmental pattern *in vivo*; (6) Multinuclear AE represented several developmental patterns, but no regular cellularization was noted. Although some symptoms prior to cellularization were observed: regional specification, AE nuclei surrounded by RMS and arranged the nuclear network, AE-like tissue in some cases; (7) A very high frequency and advanced developmental stages of AE in *met1*/*met1* genotype is probably the effect of concentration of hypomethylation caused by both mutation and *in vitro* condition; (8) In unpollinated ovules of FIE/fie mutants cultured *in vitro* AE development progressed far compared with *in situ*. Thus, hypomethylation caused *in vitro* may be promote more advanced AE development than in hypomethylated *fie* mutants *in situ*; (9) AE induction in genotypes without mutation may be the effect of general stress *in vitro*, involves changes in DNA methylation (e.g. in *FIE* and other genes required for correct fertilization and seed formation). To sum up, if AE induced *in vitro* occurred (a) in mutationless genotypes of *Arabidopsis* and changes in DNA methylation influence genes expression, (b) in hypomethylated *met1* mutants, (c) in *fie* mutants *in vitro*, in which AE represented more advanced developmental stages than *in situ*, there is (more than) likely that changes in one or some genes are the issue of AE phenomenon. Additionally, the occurrence of AE *in situ* show, that natural ability to AE induction is unique to some species.

6.20.

Glucosinolate profiling in *CORI-2* T-DNA insertion mutants of *Arabidopsis thaliana*

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CORI-2 (CORI = coronatine induced) is one of octadecanoid-inducible genes expressed in coronatine-treated plants of *Arabidopsis thaliana* which in TAIR database has been assigned as At4g04840 and annotated as a putative methionine sulfoxide reductase (MSR). Based on the classification proposed by Rouhier et al. (2006), the *CORI-2* gene encodes the AtMSRB6 protein. In different biological systems, enzymes performing MSR B activity are responsible for the reduction of one of stereoisomeric forms of methionine sulfoxide (Met-(R)-SO) back to methionine. Data available from higher plants reveal that MSRs fulfill an essential physiological role in protein repair and in protection against oxidative damage although some evidence may suggest other functions of these enzymes.

Glucosinolate (GS) profiles were assessed in seeds and leaves of *A. thaliana*, ecotype Wassilewskija, comparing knock-out *CORI-2* homozygous lines with wild type lines. The separation and quantification of different GSs was conducted according to Piotrowski et al. (2004) with glucotropaeolin as an internal standard. Two main classes of desulfoglucosinolates were detected – indolic and aliphatic, the latter appearing as oxidized or reduced forms. The concentration of oxidized aliphatic GSs was several times higher than that of reduced forms in leaves. The opposite was detected in seeds in which the reduced forms predominated. There was no difference in non-aliphatic GS contents between wild-type and mutant seeds. Also the levels of oxidized and reduced short-chain aliphatic GS were not altered by the mutation in *CORI-2* gene. However, significantly higher content of long chain oxidized aliphatic GSs (7MSOH, 8MSOO) accompanied by lower levels of their reduced counterparts (7MTH, 8MTO) in homozygous mutants in comparison with wild type were detected. These results may indicate the involvement of *CORI-2* gene in secondary modifications during GS biosynthesis.

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Session 7

Secondary metabolites as pharmaceutics and nutraceutics

PLENARY LECTURES

7.1.

Functional characterization of new P450s involved in plant furanocoumarins biosynthesis, and possible applications in a metabolic engineering approach

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Furanocoumarins are plant phytoalexins synthesized by 4 higher plant families (Apiaceae, Rutaceae, Leguminosae and Moraceae). These molecules act as plant protectors against bacteria and insect larvae. Linear furanocoumarins (psoralens) constitute the main form and can be found in all four families whereas angular furanocoumarins (angelicins) are exclusively synthesized in some Apiaceae and Leguminosae which could reflect an evolutionary process driven by plant/insect competition. Biosynthetic pathways of linear and angular furanocoumarins are still poorly understood at the molecular level although several enzymatic steps were demonstrated to be P450-dependant.

We recently reported the isolation and functional characterization of 4 enzymes acting as psoralen synthase and angelicin synthase, which constitute the first cytochromes P450 described in the furanocoumarin pathways (Larbat et al., 2007; Larbat et al., 2009). CYP71AJ1, CYP71AJ2 and CYP71AJ3 were respectively isolated from *Ammi majus, Apium graveolens* and *Pastinaca sativa*. Functional expression was accomplished in yeast cells and the recombinant enzymes were identified as psoralen synthase with narrow substrate specificity for (+)-marmesin. In parallel, CYP71AJ4, isolated from *Pastinaca sativa*, catalysed the conversion of columbianetin into angelicin and an hydroxycolumbianetin compound in a lower extent consequently, CYP71AJ4 was assigned as an angelicin synthase and constitutes the first P450 monooxygenase identified at the molecular level and involved in the angular furanocoumarin pathway.

In parallel to the functional characterization of various P450s involved in the furanocoumarin pathway, we decided to genetically transform the medicinal plant *Ruta graveolens* with the objective to modify the furanocoumarin concentration in plant tissues. Genetic transformations were achieved following the method of Lièvre et al., 2005. A 4 fold increase in the furanocoumarin (psoralen, 5-methoxypsoralen, 8-methxypsoralen) content was recorded in plant leaves bearing a *35S::CYP71AJ1* construction. This demonstrates that the furanocoumarin content of higher plants can be highly modified by a metabolic engineering approach.

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7.2.

Genistein – a natural isoflavone with a potential for treatment of genetic diseases

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Isoflavones are heterocyclic polyphenols that naturally occur in plants (soy is especially rich in these compounds). Due to their multiple biological activities, they became a subject of intensive studies as potential phytotherapeutic compounds. Genistein (4', 5, 7-trihydroxyisoflavone or 5, 7-dihydroxy-3- (4-hydroxyphenyl)-4H-1-benzopyran-4-one) appears to be one of the most abundant isoflavones, and because of its phytoestrogenic, antioxidant, tyrosine kinase inibiotor and other activities, as well as its relatively low toxicity, it is being tested as a potential protective and/or therapeutic agent in variety of disorders, including cancer, cardiovascular diseases, menopausal and postmenopausal symptoms. Perhaps surprisingly, recent studies indicated that genistein can be also considered as a potential drug for treatment of some genetic diseases. The best examples are cystic fibrosis (CF) and mucopolysaccharidosis (MPS) (particularly MPS type III, called also Sanfilippo disease), though molecular mechanisms by which this isoflavone may be beneficial for patients suffering from these diseases are different. In CF, dysfunction of the *CFTR* gene causes a defect in the function of the transmembrane chloride pump, leading to a lack of movement of chloride in nose, sinuses, lungs, intestines, pancreas and sweat glands, and thus, to clinical manifestations of the disease. One the most common mutations found in CF patients is ΔF508, and it appears that the function of the mutated gene product may be partially restored by genistein, especially in combination with phenylbutyrate. Phase I/II of a clinical trial with such a therapy is being finished (1). MPS is a group of inherited metabolic disorders, caused by mutations leading to dysfunction of one of enzymes involved in degradation of glycosaminoglycans (GAGs) in lysosomes. Due to their impaired degradation, GAGs accumulate in cells of patients, which results in dysfunction of tissues and organs, including the heart, respiratory system, bones, joints and central nervous system. Genistein has been shown to act as an inhibitor of GAG synthesis (2) due to its function of the inhibitor of kinase activity of epidermal growth factor receptor (3). Results of pilot clinical studies indicated that treatment of patients suffering from MPS III with a genistein-rich isoflavone extract resulted in statistically important improvement of all tested parameters, including cognitive functions (4), and the double-blinded placebo-controlled clinical trail is being started. Therefore, these results indicate that natural plant metabolites, like genistein, can be considered as therapeutics for as complicated diseases as inherited metabolic disorders.

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ORAL PRESENTATIONS

7.3.

New isoflavone glycoconjugates identified in Mexican lupine species

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Isoflavones are a group of plant secondary metabolites raising increased interest due to their important role in plant physiology, biochemistry and wide range of biological activities. Searching for new plant sources of this class natural products is of the highest importance due to perspectives of application of biotechnological methods for industrial synthesis of new compounds important in different fields of human activities.

Two different LC/MS techniques were used during profiling of the targeted secondary metabolites. The application of a CID MS^n (ion trap) allowed the structural elucidation of the analyzed compounds, on the other hand utilization of a high resolution analyzer in LC/ESI/q-ToF system permitted to confirm elemental composition of ions originating from studied compounds.

Profiles of flavone conjugates in leaves and roots of seven wild Mexican lupine species *(Lupinus exaltatus, L. madrensis, L. mexicanus, L. montanus, L. reflexus, L. rotinduflorus, L. stipulatus*) differ considerably from these recorded previously for the European lupine species (*L. albus, L. angustifolius, L. luteus*). The differences are related to the presence of glycosides of various isoflavones (biochanin, formononetin, wighteone and luteone), additionally to genistein and 2'-hydroksygenistein observed in earlier studied European species. In green parts flavane (naringenin) and flavone (tricin) not detected previously in the Old World lupines were identified. Some differences in the glycosylation and sugar acylation pattern of these compounds are also observed in samples obtained from several Mexican species (both root and leaf). Sugar moieties of these conjugates are substituted with succinic acid molecules in addition to malonic acid. The former type of substitution was not observed in samples obtained from in European lupine species.

Information obtained from the flavonoid conjugates profiles registered with LC/MS systems will be used as a tool for chemotaxonomic analysis of different lupine species.

7.4.

Agrobacterium – mediated genetic transformation of barely (*Hordeum vulgare*) by human lactoferrin gene

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Lactoferrin (LF) is an 80kDa iron binding protein, a member of the transferrin family, which was found in high concentration in human milk, tears, nasal exudates, saliva, bronchial mucus, gastrointestinal fluids, cervico-vaginal mucus, and seminal fluids (Hayes, 2005). LF is a multifunctional protein with different biological activities including antimicrobial properties (Nandi, 2005), immune system modulation (Nandi, 2005), etc. Recombinant human lactoferrin (*hLF*) has been already produced in microbial, animal and plant system (Nandi, 2005). In plant system LF has been produced in tobacco, potato and rice (Nandi, 2005). Here we describe a system for introduction of *hLF* gene in mature embryos and callus cultures of commercial Ukrainian barley cultivars by *Agrobacterium*-mediated genetic transformation. For this purpose, plasmid pBiLF carrying *hLF* was created. This gene was driven by barley glutelin promoter (tissue-specific) and terminated by GluB-1 terminator. It also included selectable marker gene *hpt* for hygromycin resistance under the control of PUbi promoter. The binary vector pBiLF was transferred to *A. tumefaciens* strain AGL1 for transformation. AGL1 inoculum was prepared on shaker at 28°C in LB medium containing appropriated antibiotics. Cells were immersed into liquid LB medium containing *Agrobacterium* cells and subjected to sonication (1–3s) and vacuum infiltration for 20 min. Mature embryos were treated with tungsten M17 for plant cells damaging, immediately after that embryos were immersed into bacterial suspension and subjected to vacuum infiltration for 20 min. Transformed plant material was transferred to the co-culture medium, and incubated at 28°C in the dark for 2 days. Transformed cells, were transferred than to the resting period on the medium supplemented with 250 mg/l cefotaxime for bacteria elimination. After 3–4 weeks, growing tissues were transferred to selection medium containing 5 mg/l hygromycin and 250 mg/l cefotaxime. Later transformed embryos were placed on the selection medium containing 25 mg/l hygromycin and 250 mg/l cefotaxime.

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7.5.

Natural variation in *Arabidopsis thaliana* as a tool for identifying differentially expressed genes involved in the elicitation process of pharmacologically active plant secondary metabolites

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Plants produce a wide variety of secondary metabolites that have a range of important functions and biological activities valuable for both plants and humans. They are produced in plants as a defence mechanism against pathogen attack and other stress events, and serve as key signalling compounds in mutualistic interactions and plant development. Numerous plant secondary metabolites have significant biological, pharmacological and therapeutic activities in humans, and have found medical application in the treatment of various diseases. Even though plants are a good biotechnological source of biologically active compounds,

the commercial production of secondary metabolites using plant culture is normally limited by their low yield. Therefore better understanding of the elicitation process, which is an extensively used tool for enhancing secondary metabolite yields, is of great importance. In spite of the fact that all medicinal plants currently studied are non-model organisms, *Arabidopsis thaliana* with its extensive genetic natural variation, and its tools of molecular biology, biochemistry, and functional genomics provides an excellent model to study plant secondary metabolism and elicitation process. The aim of this work is to identify differentially expressed genes that determinate the phenotypic variation in secondary metabolite accumulation between *Arabidopsis* accessions treated with various elicitors. Sets of *Arabidopsis* natural accessions originating from a wide range of habitats were grown together in control conditions and were subsequently treated with different biotic and abiotic elicitors. The harvested samples are being used for the parallel analysis of transcript profiling using a fluorescent differential display technique and metabolic profiling of secondary metabolites. The long-term aim of the project is to expand molecular understanding of the secondary metabolite biosynthesis at an ecological level in order to get insight into the elicitation process.

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POSTERS

7.6.

Determination of the toxicity of secondary metabolites produced by *Woronichinia naegeliana* Elenkin

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The cyanobacterial blooms are undesirable as equally as a result of ecological as well as health reasons – often they discharge into the water compounds produced by them that are toxic for animals and people. Amongst the cyanobacteria which are appearing increasingly frequently in fresh water areas, *Woronichinia naegeliana* is noted by its number. In connection with this feature of it, research has been undertaken with the aim of a determination of the degrees of toxicity of secondary metabolites. The evaluation of toxicity is conducted through the application of commercially available acute toxicity tests based on the crustaceans *Thamnocephalus platyurus* and *Daphnia pulex*, which are particularly sensitive to the cyanobacterial toxins. The greatest biological sensitivity on the effect of the water extract obtained from the *W. naegeliana* cells was displayed by *T. platyurus* – the denoted lethal concentration, 50% (LC_{50}) after 24 hours of exposure constituted 1.03 mg of dry weight (d.w.)/ml. A lower sensitivity was obtained through the conducting of tests with the use of *D. pulex*, though with the passing of time toxicity grew. Upon a comparison of the results obtained for the various species and strains of cyanobacteria it resulted that the toxicity of the extract from the *W. naegeliana* cells were located in a value range from those classified as average to low and were less toxic than that obtained from the *Microcystis aeruginosa* cells. In order to determine the toxic values of particular products synthesized by *W. naegeliana* experiments were conducted using tests of acute toxicity utilising *T. platyurus*. The authors of the few publications that describe the structures of secondary metabolites identified in the cells of *W. naegeliana* were not in agreement as to their toxicity. The results here presented show that two from amongst the eighteen aquatic fractions extracted from this cyanobacteria display biological activeness. The value LC_{50} was determined for the fraction containing the microginin FR3 (MG-FR3) constituting 5.88 mg d.w./ml as well as the fraction containing aeruginopeptin 917S-C, microginin 51A and cyanopeptolin D representing 0.42 mg eqw.MG-FR3 d.w./ml. The remaining products synthesized by *W. naegeliana* displayed an absence or a low level of toxicity making it impossible to determine theLC_{50} value.

7.7.

Identification of phenolic compounds in *Lolium multiflorum*

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Italian ryegrass (*Lolium multiflorum* Lam.) is a fodder grass which due to strong seedling vigor and high level of carbohydrates is a desirable species for livestock pasture. Unfortunately, it does not withstand hot, dry summers or severe winters and extensive

breeding work is needed to widen stress tolerance. Nothing is known about the composition of secondary metabolites such as polyphenols in this species. These compounds play a crucial role in response to unfriendly environmental conditions and their presence influences the feeding quality of plant material. We report results of studies on the variation in phenolic compounds, especially flavonoid glycoconjugates and chlorogenic acids, that are present in leaves of three Polish *L. multiflorum* cultivars Atos, Lotos and Tur. The analyses have been performed using two different chromatographic systems: high performance liquid chromatograph (HPLC) coupled to ultraviolet UV detector and electrospray ionization (ESI) ion trap mass spectrometer (IT MS) and ultraperformance liquid chromatograph (UPLC) with UV detector. Both systems provided opportunities to identification of 56 different compounds. Among them tri-, di- and monoglucosides of flavons: apigenin, chrysoeriol and tricin as well as flavonols: quercetin, kaempferol and isorhamnetin have been found. The identified sugar derivatives include mainly glucosides, rutinosides and in a lesser proportion glucuronates. The identified compounds are mainly *O*-glycosides, but some *C*-glycosides have also been detected. Some glucosides are esterified with malonic acid. In addition three isomeric chlorogenic acids occur in the studied plants in relatively high concentrations. The qualitative and guantitative differences in flavonoid profiles observed among cultivars will serve to a further determination of the role of poliphenols in plant response to abiotic stresses.

7.8.

Establishment of hairy root clones of *Nigella arvensis* and antimicrobial activity of their methanol extracts

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Transgenic hairy root cultures of medicinal plants offer an alternative and promising system for secondary metabolite production. Though transformed roots of many plant species have been obtained and widely studied, to the best of our knowledge, there is no report on the application of this approach to the genus *Nigella* (Ranunculaceae). Some species of this genus are known for their pharmacological properties.

Genetic transformation protocol, based on vacuum-infiltration of explants with *Agrobacterium rhizogenes A4* strain, has been developed for induction and establishment of hairy root cultures of *Nigella arvensis*. Petiole segments of aseptic *N. arvensis* plants were precultivated on basal medium SH (Schenk and Hilderbrandt, 1972) supplemented with 2 mg/l 2,4-D and 0,5 mg/l BA for a week prior to transformation. Cocultivation of petiole explants with the agrobacteria resulted in the emergence of hairy roots on hormone-free SH medium at 5% relative transformation frequency. Five of the rapidly growing hairy root clones were analysed by PCR for the presence of *rol*B gene. Transformed nature of three of them has been confirmed.

The hairy root biomass was lyophilized and extracted either with chloroform or methanol. The extracts were tested for their antimicrobial activity using modified disk diffusion method (Poulev et al., 2003) for qualitative assessment and broth microdilution method (Setzer and Vogler, 2006) for quantitative assessment of bacterial growth. The inhibitory effect on a number of bacteria was detected for the methanol extracts of all clones. The chloroform extracts were significantly less active than the methanol ones.

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7.9.

Influence of *Dionaea muscipula* extract on viability and pectinolytic enzyme production by *Pectobacterium carotovorum* subsp. *artrosepticum*

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Extracts from carnivorous plants exhibit strong antibacterial activity against plant pathogenic bacteria from genus *Dickeya* and *Pectobacterium in vitro*. However, *in vivo* the effect is weaker probably due to the presence of plant metabolites modulating bacterial viability and pathogenicity (Ravirala, 2007). The influence of *Dionaea muscipula* (Droseraceae) chloroform extract, its component: naphthoquinone – plumbagin and synthetic peptide CAMEL on viability and pectinolytic enzyme production in two strains of *Pectobacterium carotovorum* subsp. *artrospecticum* (Pca1043 – laboratory strain and Pca5A/1 strain isolated from potato in Poland) was examined. Bacteria were grown in minimal (M63) or rich (LB) medium supplemented or not with 20% potato tissue filtrate (PF). *D. muscipula* extract, plumbagin or CAMEL were added up to 0.25 times of minimal bactericidal concentrations (MBC) according to the previously established values. On the basis of optical density the generation time (GT) was calculated for each culture variant. Cell-free supernatants from 24 h cultures were used for determination of extracellular pectate lyase (PL) activity. Plumbagin has shown higher bacteriostatic activity than *D. muscipula* extract while CAMEL exhibited no antibacterial activity in tested concentrations. The addition of PF significantly increased resistance of both strains to tested compounds. GT for Pca5A/1 grown in LB medium or LB with plumbagin was 94.1±3.5 min and 282.5±3.8 min, respectively, while in media with 20% PF GT value was 72.8±2.2 and 91.3±4.8, respectively. Supplementation of media with PF as well as addition of extract and plumbagin inhibited production and/or secretion of PL. The highest PL activity was observed in the supernatants from cultures grown on M63 medium: 5.1±0.1 and 8.7±0.6 U·g DM of bacteria^{-1}·ml^{-1} for control cultures of Pca1043 and Pca5A/1 respectively. Addition of extract or plumbagin decreased the Pca1043 PL activity to 0.5±0.01 and 0.4±0.1 U·g DM of bacteria^{-1} ·ml^{-1} respectively and in case of Pca5A/1 to 6.5±0.2 and 5.1±0.5 respectively.

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7.10.

Gentiopicroside content in *in vitro* plants, cell suspension and genetically modified tissues of selected species from *Gentiana* genus

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Secoiridoid glucosides are the main pharmacological active *Gentiana* species compounds. Acyl derivatives of glucoside – amarogentin and amaroswerin are the most bitter substances among natural products. But the main secoiridoid glucoside of the family *Gentianaceae* is gentiopicroside. It was found in 17 species of the *Gentiana* genera and *Gentianella* while the other iridoids were present occasionally. Its bitter coefficient is the lowest however the content in the drug is higher than other iridoids. Enzymatic hydrolysis causes release of its aglucone – gentiogenal (gentiopicral) which shows antibacterial and anticancer properties.

Most of gentians growing wild is protected by law so harvest of plants from natural conditions is excluded. Field cultivation is often difficult and unprofitable that's why *in vitro* cultures became an alternative which enables to propagate big amount of plant material in short period of time.

Four gentians species were used in our experiment: *G. cruciata*, *G. kurroo*, *G. lutea* and *G. tibetica*. Gentiopicroside content in *in vitro* plants of all investigated species was determined with the use of HPLC method. Aggregates of three different cell suspension cultures were examined in relation to its amount: *G. cruciata* cotyledon-derived, *G. kurroo* hypocotyl-derived and *G. lutea* root-derived. Gentiopicroside concentration was also determined in genetically modified *G. tibetica* plants regenerated after *Agrobacterium*-mediated transformation. The differences in secoirydoid glucoside amount between species, tissues and type of culture were investigated.

Session 8

Plant membranes

PLENARY LECTURES

8.1.

Stomatal control of plant water status

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The plant hormone abscisic acid (ABA) is involved in the transmission of environmental changes like drought-, saline-, and cold-periods into stress adaptation processes. Based on the timescale of the individual ABA evoked responses they have been subdivided into fast (membrane transport) and slow (transcription) signalling. In contrast to the latter process the fast ABA response – exemplified by half times of stomatal closure around 5–10 min – is believed to not involve gene activation. Instead stomatal closure is accomplished by the release of potassium ions and chloride as well as the metabolic degradation of the major organic anion malate.

In search for ABA signalling intermediates the response of ion channels of guard cells in epidermal peels as well as guard cell protoplasts and vacuoles have been challenged with well-characterized modulators effective in signal transduction pathways of animal cells. Isolated, experimentally well controlled guard cell preparations, however, often lack communication with neighbouring cells, turgor or cytosolic components. In addition potential signalling components derived from mutants altered in ABA-induced stomatal closure. To online record changes in ion fluxes across the plasma membrane of guard cells in intact plants, we have developed a method, based on multi-barrelled microelectrodes introduced into the cytoplasm of these sensory motor cells. Using this online, in planta approach we have been able to identify signalling elements required for fast ABA-induced stomatal closure.

Recently mutants were shown to lack a gene encoding a putative guard cell anion transporter named SLAC1. SLAC1 function and stomatal closure-related signaling components leading to anion channel activation, however, remained still unknown. Using protein-protein interaction assays we identified a protein kinase and -phosphatase within the ABA transduction pathway as regulators of SLAC1. Our studies demonstrate that SLAC1 represents the slow inactivating, weak voltage-dependent anion channel of guard cells controlled by phosphorylation- dephosphorylation. A model on the ABA-based regulation of guard cell ion transport will be presented at the meeting.

8.2.

Regulation of channels by phosphoinositides in the plasma membrane

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A wide range of ion channels in animals are controlled by phosphoinositides (PIs), particularly by phosphatidylinositol (4,5) bisphosphate ($PtdInsP_2$). In Planta such information is scarce and – for water channels (aquaporins) – non existent. We examined these questions in a plant model, tobacco cultured cells in suspension, NT1 (*Nicotiana tabacum*, var BY2), some of which had their PIs levels – the plasma-membrane $PtdInsP_2$ and cellular inositol (1,4,5) trisphosphate – genetically modified: elevated in "High PIs" and depressed in the "Low PIs". We assayed the activity of their water channels, via the osmotic water permeability (P_f) of protoplasts isolated from the NT1 cells, using a hypotonic-challenge paradigm. The higher absolute P_f values and its constancy during cell swelling (as opposed to its decrease in other cells), as well as the lower Arrhenius energy of P_f activation in the High PIs, indicated aquaporins-dominated water permeability in the High PIs, consistent with activation of plasma membrane aquaporins by membrane PIs.

In this model system we examined also the effect of $PtdInsP_2$ on the tobacco K^+-efflux channel, NtORK, using patch-clamp, with fixed "cytosolic" $[Ca^{2+}]$ and pH. In all these cells, K channel activity, reflected in the net, steady-state outward K^+ currents, I_K, was inversely related to the $PtdInsP_2$ level, whether established genetically or by short-term pharmacological manipulations. In all cases, I_K decreases stemmed largely from decreased maximum-attainable NtORK channels conductance and partly from shifted voltage dependence of channel gating to more positive potentials, making it more difficult to activate the channels. These results are consistent with NtORK inhibition by the negatively-charged $PtdInsP_2$ in the internal plasma membrane leaflet. Similar regulation of ion and water channels is likely to underlie PIs signaling in intact cells of whole plants.

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8.3.

Light-driven molecular regulation mechanisms in LHCII

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Plants have developed multiple regulatory mechanisms, at all their organization levels, to adjust a number of excitations in the photosynthetic antenna, generated by light absorption, to current capability of the photosynthetic apparatus. Such a regulation involves leave movement and chloroplast phototranslocation, on the one hand, and quenching of excessive excitations in the photosynthetic pigment networks, on the other hand. The reported recently, light-driven molecular mechanisms which operate in the largest photosynthetic antenna pigment-protein complex LHCII, active to protect photosynthetic apparatus against overexcitation-related damage, will be overviewed and discussed. Among those mechanisms are: the light-induced molecular configuration change of the LHCII-bound neoxanthin [1], the light-induced molecular configuration change of the LHCII-bound violaxanthin [2] and the molecular organization-related formation of the low-energy excitation traps [3].

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ORAL PRESENTATIONS

8.4.

Influence of thylakoid lipids on the de-epoxidation of violaxanthin associated with the photosystem II light-harvesting complex (LHCII)

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In higher plants, the major part of the xanthophyll cycle pigment violaxanthin (Vx) is non-covalently bound to the main light-harvesting complex of PSII (LHCII). Under saturating light conditions Vx has to be released from its binding site into the surrounding lipid phase, where it is converted to the energy-dissipating pigment zeaxanthin (Zx) by the enzyme Vx de-epoxidase (VDE). In the present study we investigated the influence of thylakoid lipids on the de-epoxidation of Vx which is still associated with the LHCII. We isolated LHCII with different concentrations of natively bound lipids and Vx by sucrose gradient centrifugation or successive cation precipitation. Analysis of the different LHCII preparations showed that decreases in the content of the main thylakoid lipid monogalactosyldiacylglycerol (MGDG) were accompanied by a diminished concentration of LHCII associated Vx, indicating that part of the Vx was located in the MGDG phase, whereas another part was bound to the LHCII apoproteins. We further studied the convertibility of LHCII-associated Vx by addition of isolated VDE. We observed an efficient and almost complete Vx conversion in LHCII containing high amounts of associated lipids. LHCII preparations with low concentrations of MGDG exhibited a strongly reduced Vx de-epoxidation which, however, could be increased by addition of external MGDG. It is of further interest that the de-epoxidation of LHCII-associated Vx was saturated at much lower concentrations of natively bound MGDG

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8.5.

Spatiotemporal changes of membrane potential during pollen activation and tube growth

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Mature pollen from most plant species is metabolically quiescent and highly desiccated. Upon pollination, pollen grain undergoes rapid activation and grows a pollen tube. It elongates by a tip growth process and transports sperm cells to the embryo sac for fertilization. Besides its reproductive importance, pollen germination and tube growth have been considered unique developmental processes for studying cell polarity establishment, cell differentiation and polar growth. It is known that ion currents and fluxes play a key role in establishing and maintaining polarity of pollen germination and tube growth. But crucial question about membrane potential changes remains undecided.

We studied transmembrane potentials in cells (vegetative cell of pollen grain during activation and growing pollen tube), protoplasts from pollen grains and isolated pollen mitochondria. In our study we applied noninvasive fluorescent methods devised for animal cells. Three voltage-sensitive dyes were used: $DiBAC_4(3)$ allowed to determine the membrane potentials of cells and protoplasts on the absolute (mV) scale, Di-4-ANEPPS gave an opportunity to map spatial differences in membrane potential, $DiOC_3(5)$ was used for isolated mitochondria.

We have shown here that pollen grain activation and germination are accompanied by hyperpolarization of the vegetative cell plasmalemma. However, protoplast isolation also caused significant hyperpolarization, probably due to metabolic activities concerned with wall regeneration. At the same time we found out an uneven spatial distribution of membrane potential along the surface of pollen protoplast, presumably connected with cell polarization. The mapping of growing pollen tube revealed a longitudinal gradient of transmembrane potential. The involvement of plasma membrane H^+-ATPase in the gradient maintenance was shown using orthovanadate and fusicoccin that inhibited or activated the enzyme, respectively. These treatments changed the pattern of potential distribution, but didn't destroy the gradient. Dissipating effect was observed only when pollen tubes were treated with NPPB (5-nitro-2-(3-phenylpropylamino) benzoic acid) – specific anion channel blocker. Using another blocker of transmembrane anion transport – DIDS (4,4'-diiso-thiocyano-2,2'-disulfonic acid stilbene) we showed that DIDS-sensitive anion channels control membrane potential in isolated mitochondria. The data suggest an important role of anion channels of plasmalemma and mitochondria in the regulation of transmembrane potential during pollen germination.

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8.6.

A possible role of CsNAR2 in nitrate uptake in cucumber (*Cucumis sativus*)

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Nitrate (NO_3^-) is the major source of nitrogen for most crop plants of aerable soils. The uptake of these ions by root cells is the first and therefore the most important step in nitrogen acquisition and assimilation. Although physiology of nitrate transport across plasma membrane seems to be well established, the molecular mechanisms governing this process remain unclear. Two classes of genes encoding nitrate transporters have been identified in plants, *NRT1* and *NRT2*, involved in low (LATS) and high (HATS) affinity transport systems, respectively. Recently it was discovered that NRT2.1 in *Arabidopsis thaliana* and *Hordeum vulgare* requires a second protein to mediate NO_3^- transport, NAR2. The data obtained from the heterologous expression systems using *Xenopus* oocytes have shown that NRT2.1/NAR2 is a two-component high-affinity nitrate transport system. However it has also been demonstrated that expression of *NAR2* is not essential for transcription of *NRT2.1*. The components interact rather at the

protein level, with NAR2 being probably involved in proper targeting of NRT2s to the plasma membrane.

NAR2 is a small protein with predicted one membrane-spanning domain. The sequences of genes belonging to the NAR2 family have been identified in many plant species, but it is not known whether they share common function in nitrate transport. Based on the presence of conserved regions in the amino acid sequences of NAR2s, the homologous nucleotide sequence of the *CsNAR2* gene was found in cucumber. The predicted protein is in ~60% identical to the AtNAR2.1 and HvNAR2.3. Analyses of gene expression have shown that *CsNAR2* is nitrate-responsive. We've analyzed in detail the *CsNAR2* expression level in different cucumber organs and under different N regimes in comparison with the expression of high-affinity nitrate transporter gene, *CsNRT2*. We've also measured the nitrate uptake rate by cucumber roots under variable nitrate nutrition (nitrate induction, starvation and re-induction). The obtained results are discussed in the context of possible role of *CsNAR2* in regulation of nitrate uptake in cucumber.

POSTERS

8.7.

Local motions of protein matrix influenced by the spin state of the non-heme iron in bacterial reaction centers

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Non-heme Fe is a highly conservative cofactor of photosynthetic reactions centers of type Q but its role remains unclear.

We show that the non-heme iron can be stabilized either in a high and in a low spin ferrous state is in the isolated *Rhodobacter sphaeroides* reaction centers whereas it was found mainly in a low spin ferrous state in the *Rhodospirillum rubrum* reaction centers. The temperature dependence studies of the iron mean square displacement using Mössbauer spectroscopy show that the high spin ferrous non-heme iron has more flexible surrounding (θ_D ~165 K) that the low spin ferrous one (207 ± 17 K). We suggest that the spin state of the non-heme iron may regulate the strength of coupling between the two quinone acceptors and indeed we observe that it influences the stabilization of charge separation and the rate of charge recombination between the quinone acceptors and the special pair of bacteriochlorophylls. The nuclear inelastic scattering measurements of the collective motions in these reaction centers show that the density of vibrational states originating from the non-heme iron bonds to the protein matrix has well separated modes in the *Rb. sphaeroides* BRCs at low (around 4–17 meV) and higher (around 17–25 meV) energies whereas in *Rs. rubrum* BRCs the vibrations have more uniform distribution with much lower contribution of vibrations at around 6 meV.

These results show that the activation of electron transfer between the two quinone molecules on the acceptor side of the bacterial reaction centers via the motion of the protein matrix is related to the spin state of the non-heme iron.

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8.8.

Photosynthetic electron transfer in bacterial reaction centers

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The conversion of light energy into useful chemical energy in photosynthetic organisms takes place in specialized pigment-protein complexes called reaction centers. The mechanisms of primary function of reaction centers, i.e. charge separation across the photosynthetic membrane, are still not fully understood. One of the unsolved questions concerns the role of a very conservative non-heme iron localized between the two quinones in type II reaction centers. In the present study, we applied UV-VIS absorption and thermoluminescence spectroscopies in order to investigate the correlation between the stability of the ubiquinone-iron complex and the efficiency of the charge recombination within the reaction centers from two strains of purple photosynthetic bacteria, *Rs. rubrum* and *Rb. sphaeroides*. The main thermoluminescence component from the reaction center from *Rs. rubrum* is shifted by 12°C towards lower temperatures with respect to the one from *Rb. sphaeroides*. The measurements of ground state recovery in these two reaction centers show that the rates of charge recombination between the Q_A and Q_B quinone and the special pair of bacteriochlorophylls are also very different. Both observations point to a large effect of the non-heme iron state on electron transfer on the acceptor side of the reaction center.

8.9.

Cadmium caused Indian mustard (*Brassica juncea*) photosynthetic apparatus changes

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Heavy metal contamination of soils is a serious environmental problem with potentially harmful results to agriculture and human health. It is proved that cadmium ions cause significant changes in photosynthetic activity by disrupting chloroplast ultrastructure, inhibiting chlorophyll, carotenoid and plastoquinone synthesis, suppressing electron transport and also decreasing Calvin cycle ferment activity in plants sensitive to heavy metal exposure. This can suppress metabolic rates and also restrict plant survival. Indian mustard (*Brassica juncea*) is considered to be one of the prospective cadmium hyperaccumulative species. Such property has practical application in bioremediation and in efforts to colonize polluted locality.

In our research Indian mustard shoots were treated by 0.2 mM cadmium nitrate solution. The first test modification was carried out with cadmium solution unchanged and roots remaining unwashed (with mucus) during exposure period. Another modification included changing the pollutant solution every 24 hours and rinsing the roots off. We have investigated the effect of Cd on the photosynthetic pigments and the lipids membrane composition. Our results show a slight decrease in total Chl content during the exposition. The reduction in Chl *b* content was extremely sharp in 72 h which resulted in higher Chl *a*:*b* ratio as the concentration of Cd increased. A decrease of DGDG and MGDG content was noticed. Particularly DGDG reduced the most essential in 48 h and MGDG in 72 h. Furthermore, the MGDG/DGDG ratio have been risen in 72 h comparison with the control plants. Changes in lipid-pigment composition might have stabilizing effects on photosynthetic membranes during cadmium stress in general adaptation mechanism.

8.10.

Inhibitory action of copper and cadmium ions on electron transfer in photosystem II

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The most sensitive target of heavy metal toxic action is photosystem II (PSII). Copper and cadmium ions are known to be strong inhibitors of the photosynthetic process but their binding sites are unknown. We performed measurements of electron and energy transfer in photosystem II isolated from tobacco in the presence of various concentrations of copper and cadmium salts using double modulated fluorescence spectroscopy. We studied kinetics of variable fluorescence and Q_A reoxidation. Obtained results allowed us to distinguish at least four binding sites of copper and cadmium ions within PSII. Basing on other biochemical studies we could indicate the non-heme iron and cytochrome b559 as the most susceptible components of PSII to these heavy metals toxicity. Copper caused inhibition of the photosynthetic process already at 100 times lower concentrations than cadmium

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8.11.

Effect of trimethyllead chloride (Met_3PbCl) on SV channels and volume changes in the red beet vacuoles

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Two major ion currents in the vacuolar membrane of higher plants are mediated by non-selective cation channels: SV channel (slowly activating) and FV channel (fast activating). The FV chan-

nel conducts various monovalent cations with poor selectivity among them, whereas the SV channel conducts K^+, Na^+, Mg^{2+}, Ba^{2+} and Ca^{2+}. Under conditions usually applied to patch-clamp experiments on vacuoles (symmetrical K^+, milimolar luminal Ca^{2+}) SV channels activate at positive potentials.

The aim of the present study was to determine the effect of Met_3PbCl on SV channel and volume changes in the red beet vacuoles. Vacuoles were isolated by using of microsurgery method from red beet (*Beta vulgaris* L.). The SV channels were studied by the patch-clamp technique in the whole-vacuole or the (cytoplasmic side-out) excised-patch configuration using EPC-7 amplifier (List-Medical-Electronic, Darmstadt, Germany). Obtained data were analysed using software PatchMaster and FitMaster (HEKA Electronic, Lambrecht, Germany). Patch pipettes were prepared from Kimax-51 glass capilaris and coated with Sylgard. The diameter of vacuoles was measuremed by means of microscope Ax70 (Olympus Provis) connected with Hammamatsu camera.

It was found that under symmetrical K^+ concentrations (100 mM K^+ on both sides of the vacuolar membrane) SV channels mediate outward currents only, which probably correspond to K^+ uptake into the vacuole. Macroscopic whole-vacuole SV currents elicited by a series of positive voltages were in the range of a few hundred pA to 10 nA. The single channel records display a slow activating channel with a conductance in the order 70 pS.

It was found that the cytoplasmic Met_3PbCl at both concentrations (10^{-5} and 10^{-4} M) studied inhibited SV channels in the red beet vacuolar membrane. We have also showed that the incubation of vacuoles in the presence of 100 μM Met_3PbCl caused destruction of the tonoplast which was preceded by a rapid increase in vacuole volume.

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8.12.

Ion channels in the tonoplast of the moss *Physcomitrella patens*

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Patch-clamp measurements were performed with excised tonoplast patches. Single-channel recordings with vacuolar-side-out patches showed SV channels, which seems to be ubiquitous in the tonoplast of higher plants (Pottosin and Schönknecht, 2007). The probability of opening of single channels increased at positive potentials and current-voltage relationship displayed outward rectification. Measurements in KCl gradient confirmed selectivity of these channels for K^+.

SV were observed even in nominal absence of Ca^{2+} on the cytoplasmic side of the membrane. In addition to the currents carried by SV channels, small currents were recorded with unitary conductance approx. 25 pS. Both SV and the other channels were selective for K^+. There is high probability that K^+ channels with low unitary conductance are FV type. FV like SV are well-known (Allen and Sanders, 1997) and their features are similar to these described above.

In the tonoplast of the liverwort *Conocephalum conicum*, high concentration of Mg^{2+} activates anion channels (Trebacz et al., 2007). Anion channels in *Conocephalum* carried anions from the cytoplasm to the vacuole and were active at negative potentials. *Conocephalum* and *Physcomitrella* are closely related (both belong to Bryophyta) thus, we examined if Mg^{2+} can activate anion channels in the tonoplast of *Physcomitrella*. Unfortunately, we have found no currents carried by Cl^-. The currents we recorded in the gradient of $MgCl_2$ indicated that the observed currents were carried by Mg^{2+}, but not Cl^-.

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8.13.

Lipid peroxidation and change in monogalactosyldiacylglycerol level by heavy metals in wheat

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Heavy metals occupy one of dominating places among different groups of industrial emission. Increase of heavy metal concentration lead to the changes of plant physiological/biochemical reactions directed to activation of protection and adaptation mechanisms. One of the first reactions in development of the general adaptive syndrome is an activation of lipid peroxidation processes. Exposure of plants to heavy metals action also induces oxidative stress developed as lipid peroxidation and an oxidative burst generating free radicals and active oxygen species. These species react with lipids, proteins, pigments, and nucleic acids and cause lipid peroxidation, membrane damage, inactivation of enzymes, thus affecting cell viability.

Our data show that activation of lipid peroxidation process in leaves under lead and cadmium action depends on concentration, duration and terms of treatment. Wheat shoots treated by the high dose of cadmium revealed tetra-n-butylammonium perruthenate (TBAP) increase in 6 hours. However, after 24h in variant treated with the same toxicant dose we observed a smaller increase of this parameter. The lipid peroxidation level in all variants of treated plants decrease in 72 hours.

In plants treated by lead induced TBAP level increase proportionally to stress prolongation. Glycolipid content changes depend upon lead and cadmium concentration applied as root treatment.

We observed lipid content change being depended upon prolongated stressors influence. Data obtained showed that MGDG content changed in time-dependent manner and these changes was similar during the first period of time in both experimental variants, but Cd action was more severe. Action of both heavy metals induced an increase of MGDG level at 6 hour. In variant treated Pb MGDG increased by 23% and in variant treated Cd MGDG increased by 32% comparing to control. But in 24 hours MGDG content decrease in both experimental variants. In 72 hours MGDG increase in variant with Pb. Second sampling period (24 h) in plants treated with Cd was characterized by falling MGDG level which could be caused by peroxidation processes.

8.14.

On the mode of integration of the thylakoid membrane protein cytochrome b_6 into cytoplasmic membrane of *Escherichia coli*

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In the stroma compartment, several pathways are used for integration/translocation of chloroplast nuclear-encoded proteins into the thylakoid membrane (Robinson et al., 2001). We have investigated this aspect for chloroplast encoded cytochrome b_6, which operates with an uncleaved signal for insertion into the thylakoid membrane by expressing the mature cytochrome b_6 or constructed deletion mutants of this cytochrome in *Escherichia coli* as a fusion to a maltose-binding protein or to the bacterial SSpelB. In search of potential signal sequences and signal anchor sequences within the apocytochrome b_6 sequence, we decided to carry out computational analysis. In our case, for the prediction of signal sequence (SS) and/or anchor signal sequence (STS), we chose the program SignalP. SignalP uses two HMMs, one that models the signal peptide and a second that models a signal anchor (Nielsen and Krogh.,1998) The results presented in this paper show that the cytochrome b_6 forced to use the Sec translocase pathway, has a native structure in the bacterial cytoplasmic membrane, but also has an opposite orientation compared to that in the thylakoid. The insertion of this cytochrome into the cytoplasmic bacterial membrane is absolutely dependent on the presence of N-terminal presequence artificially added, the proper folding and anchoring depends of it's N-terminal part (first ~110 aminoacid). Helices A and B are responsible for the anchoring of cytochrome b_6 and both (A and B) or one (B) are necessary for insertion and proper folding of helices C and D.

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8.15.

Relationships between changes of electrical potential and organ movements in *Helianthus annuus* and *Phaseolus vulgaris*

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Circumnutations in many plant species have been described in literature. However, the mechanism of these phenomena is not clearly established. The existing theories suggest that circumnutations result from unequal growth and are influenced by gravity or turgor changes in moving parts of plants (Mungai et al., 2007). To check the relationship between circumnutations and electrical potential changes in the bean leaflet pulvinus and sunflower stem, recordings of movement and extracellular electrical potential measurements were made. The experiments revealed occurrence of oscillations in the electrical potential correlated with oscillations of organ movements in the sunflower and in the bean. The similarity between the recordings suggests that the mechanism of movement in the bean leaflet pulvinus (Mayer and Hamp, 1995) consisting of turgor changes can have common features with the mechanism of circumnutations in the sunflower stem.

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8.16.

Effect of selected physicochemical parameters of inverted micelles on xanthophyll de-epoxidases activity

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The presence of inverted micelles is necessary for de-epoxidation in the xanthophyl cycle, one of the most important photoprotective mechanisms in plants. Two kinds of de-epoxiadses were tested in presented studies: violaxanthin de-epoxidase (VDE) existing in higher plants, green and brown algae and diadinoxanthin de-epoxidase (DDE) which can be found in diatoms, dinophytes and haptophytes.

The effect of molecular dynamics of hydrophobic fraction of aggregates of inverted micelles composed with one of the following lipids: monogalactosyldiacylglycerol (MGDG), phosphatidylethanolamine from eggs (PE_{egg}), 1,2-dioleoyl-snglycero-3 phosphatidylethanolamine (PE1) or 1,2-dilinoleoyl-sn-glycero-3-phosphatidylethanolamine (PE2) on de-epoxidases activity was tested. The highest activity was observed in PE1 and MGDG for VDE and DDE respectively, although molecular dynamics of tested structures in case of these lipids was not the highest. Thickness of the hydrophobic fraction of the aggregates was the second tested parameter. In this experiment two types of PE esterified with fatty acids having one double bond but differing in length were used. One of them was PE1 (18°C) and the second was 1,2-dipalmitoyloleoyl-sn-glycero-3-phosphatidylethanolamine (PE-C16). DDE activity was about 25 % lower in PE-C16 than in PE1 micelles, whereas activity of VDE was 50 % lower in system composed with PE-C16. Size of the inverted micelles, suggested by mathematical description of the structures, was the last tested parameter. Diameters of the micelles varied from 7 to 9 nm, when they were created by MGDG and from 20 to 21nm when mixture of MGDG and digalactosyldiacylglycerol (DGDG) (60:40, MGDG:DGDG) was used. Total concentrations of MGDG, violaxanthin (Vx) or diadinoxanthin (Ddx) were constant and set to 0.33 μmol/dm^3 for Vx or Ddx and 12.9 μmol/dm^3 for MGDG. The bigger micelles were tested the lower activity of VDE and DDE was observed. Obtained results show that the activity of de-epoxidation is strongly dependent on physicochemical parameters of inverted micelles such as thickness, molecular dynamics of hydrophobic core and their diameter. Mutual orientation of enzymes and substrates or dilution of pigments by lipids are postulated as main mechanisms to explain the results.

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8.17.

Effect of trimethyllead chloride (Met_3PbCl) on SV channels probability distribution function of ion current

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Two major ion currents in the vacuolar membrane of higher plants are mediated by non-selective cation channels: SV channel (slowly activating) and the FV channel (fast activating). Under conditions usually applied to patch-clamp experiments on vacuoles (symmetrical K^+, milimolar luminal Ca^{2+}) SV channels activate at positive potentials.

The aim of the present study was to determine the effect of Met_3PbCl on distribution of ion current of SV channel in the red beet vacuoles.

Vacuoles were isolated by using of microsurgery method from red beet (*Beta vulgaris* L.). The SV channels were studied by the patch-clamp technique in the whole-vacuole or the (cytoplasmic side-out) excised-patch configuration using EPC-7 amplifier (List-Medical-Electronic, Darmstadt, Germany). Patch pipettes were prepared from Kimax-51 glass capilaris and coated with Sylgard.

The recordings of ion current were examined and measurements with more than one active channel rejected. The statistical parameters i.e. mean value, standard deviation, skewness and kurtosis of measured ion channels calculated. In order to approximate interval of confidence the bootstrap technique was applied. From the recorded time series a sample of the length 10^4 data points was chosen and the considered statistical parameters. The procedure was repeated 10^3 times. Finally the estimators of mean value, standard deviation, skewness and kurtosis were calculated with its confidence intervals. The results were analyzed as a function of membrane potentials. It has been shown that the presence of Met_3PbCl influence strongly the probability distribution function of ion current while the open channel was active. In the next step the currents of open and close stated were separated and properties of its distribution functions analyzed. It has been showed that the Met_3PbCl strongly influence the standard deviation of open channel current distribution.

The presented shows that the Met_3PbCl influence the characteristics of open channel current, moreover it shows that other than mean value of ion current should be taken into account in the analysis of patch clump recordings.

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8.18.

Alternative biosynthetic pathways of isoprenoid quinones in microorganisms

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Isoprenoid quinones are a group of compounds occurring in membranes of all living cells. These compounds function mainly as electron carriers, but have also other roles such as antioxidant. Due to their chemical structure they were divided into two main groups: naphtoquinones and benzoquinones. Benzoquinones comprise ubiquinones, plastoquinones (PQs) and tocopherolquinones (TQs). TQs regarded as oxidation products of tocopherols, were reported to be found also in a wide range of microorganisms unable to synthesize tocopherols (Hughes and Tove 1982), what raised the question about alternative biosynthetic pathway and special functions of these compounds. We checked the presence and abundance of TQ (and its reduced form tocopherolquinol, TQH_2) in selected species of microorganisms. Among fungi, we chose three yeast species: *Saccharomyces cerevisiae*, *Candida utilis* and *Pichia Pastoris*. Among bacteria we analyzed *Escherichia coli*, anaerobic *Butyryvibrio fibrisolvens* and four cyanobacteria species: *Synechocystis* sp. PCC6803, *Synechococcus* sp. *7002*, *Synechococcus elongatus* sp. PCC7942 (formerly known as *Anacystis nidulans* R2) and themophilic *Phormidium laminosum*. In most cyanobacteria, tocopherol biosynthetic pathway is present, but in some of them (i.e. *Synechococcus elongatus* sp. PCC7942) there is no tocopherol and homologues of enzymes participating in tocopherol biosynthesis cannot also be found. What is even more interesting, there are many questions concerning PQ biosynthesis in cyanobacteria. In higher plants, PQ and tocopherol biosynthetic pathways share initial steps. In species or mutants of cyanobacteria lacking tocopherol, PQ is found at level similar to tocopherol-containing species which suggests additional, unknown pathway of PQ biosynthesis. Occurrence of TQ/TQH_2 was confirmed for *Butyryvibrio fibrisolvens* (although there were about ten times less TQ comparing to results published by Hughes and Tove) and *Synechocystis* sp. PCC6803. For the other of the examined species we have not detected TQ/TQH_2. We observed similar levels of PQ in all the examined cyanobacteria species. The isoprenoid quinone profile is discussed in light of the occurrence of known isoprenoid quinone biosynthetic pathways in the examined microorganisms.

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8.19.

Antioxidant activity of different polarity flavonoids by modified ABTS cation radical decolorization assay and EPR technique

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Antioxidants act as protective agents against harmful oxygen molecules and free radicals and can prevent cell damage and many civilization diseases such as a cancer, cardiovascular, neurodegenerative and age related.

The main natural antioxidants: flavonoids and carotenoids are present in the human diet rich in fruit and vegetables, so their consumption is associated with numerous health benefits.

We investigated the antioxidant properties of the following three flavonoids with different polarity: the polar tricine 7-*O*- β-[2-

O-feruloyl-β-glucuronopyranosyl (1→2) glucuronopyranoside], less polar 4'-methoxy 5,7-dihydroxyflavone 6-*C*-*β*-glucopyranoside (isocytisoside) and the most nonpolar flawonoid in this study I 2 II 8 biapigenine (amentoflavone) from *Axyris amaranthoides* L., *Aquilegia vulgaris* L., and *Viburnum lantana* L., respectively, and also well known antioxidant β-carotene (Vitamin A precursor), as a standard. β-Carotene is a very powerfully scavenger of the singlet oxygen.

For the screening of antioxidant activity an modified ABTS cation radical decolorization assay and EPR technique have been introduced. Modification of ABTS method based on applying DMF as a solvent applicable to compounds with different polarity. Measurements of flavonoids, β-carotene and Trolox were performed in the concentration range from 0.01 to 10 μM. Monitoring of measurement data was done in long period of time, up to eight days after sample preparation.

Summing-up the studied flavonoids exhibited antioxidant activity higher than that of β-carotene and they can more rapidly scavenge free radicals.

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8.20.

Effects of apigenin, genistein and quercetin on membrane potential in the liverwort *Conocephalum conicum*

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Apigenin, quercetin and genistein are members of the family of plant flavonoids suspected to prevent a number of human diseases, for instance cancer development. They display a lot of activities and part of their beneficial effects could come from its affinity to the cellular membranes. At present study we used *Conocephalum conicum*, a well elaborated model of liverwort. Intracellular microelectrode measurements were carried out to examine the effects of flavonoids in combination with neomycin on the resting and action potentials.

Neomycin triggered gradual decline of AP amplitudes, an MP decrease (MP became less negative) and the peak value of the depolarization decrease. Duration of action amplitudes measured at half of the amplitude increased in neomycin treated plants. However, the membrane did show hyperpolarization in reaction to quercetin and neomycin combine perfusion. Duration of action amplitudes measured at half of the amplitude decreased in quercetin or apigenin plus neomycin treated plants in respect to control. Hence, if quercetin or genistein were used simultaneously with neomycin, they hindered neomycin-specific actions. Because combine use of apigenin and neomycin did not, again a decrease of AP amplitudes accompanied by depolarization of MP was observed.

It may be concluded that application of at least some flavonoids (namely quercetin and genistein) exert strong influence on electrical membrane responses in *Conocephalum conicum*.

8.21.

Mechanisms for the insertion of cytochrome b_6 proteins into the thylakoid membrane

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In the stroma compartment, several pathways are used for integration/translocation of chloroplast nuclear-encoded proteins into the thylakoid membrane Thylakoid membrane proteins are inserted by different mechanisms: the SRP (signal recognition particle) pathway or the apparently "spontaneous" pathway. The SRP pathway appears to be a specialized mechanism for the insertion of many members of the light-harvesting complex superfamily and requires binding of SRP to the substrate, GTP hydrolysis, and the assistance of proteins FtsY and Alb3. By contrast, the "spontaneous" insertion pathway operates without detectable assistance from other proteins and in the absence of both the thylakoidal ΔpH or nucleoside triphosphate hydrolysis (Robinson et al., 2001).

We have investigated which pathway from described above is proper for the transport and insertion of the chloroplast encoded cytochrome b_6 into the thylakoid membrane *in vivo* (in higher plants). In order to do so, we are planning the isolation of RNC (ribosome nascent protein chain) complex from chloroplasts of several organisms (*Spinacia oleracea, Pisum sativum, Arabidopsis thaliana*). From the whole pool of RNC using immunoprecipitation we will isolate the complex containing cytochrome b_6 nascent chain. The usage of crosslinking factors and antibodies together with 2D electrophoresis and Western Blot analyses will allow us to identify the translocon proteins or others participating proteins in the integration of the cytochrome b_6 into thylakoid membrane.

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8.22.

Investigation of the interconnection between the structure of inner etioplast membranes, organization of the subcomplexes POR-Chl synthase and their function

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The influence of the carotinoid biosynthesis inhibitors norflurason and amitrol on the photosynthetic pigments contents, pigments native state, 5-aminolevulinic acid accumulation, protochlorophyllide (Pchlide) resynthesis and chlorophyllide (Chlide) estherification in etiolated and shortly irradiated postetiolated barley (*Hordeum vulgare* L.) leaves has been studied. The increase of the nonphotoactive Pchlide content on the 15% after norflurason and on the 200% after amitrol treatment has been demonstrated. The content of photoactive Pchlide has been not affected after norflurason treatment and it has been diminished about 50% after amitrol treatment. The absence of the rapid and the lag phase (Rassadina et al., 2004) of the Chlide esterification in norflurason treated leaves were observed. The velocities of the

Chlide esterification in the main reaction phase in norflurason treated leaves and in the control leaves were similar. In leaves treated with amitrol the rapid phase of Chlide esterification process was expressed accurately, the lag phase was increased in comparison with the control and the velocity of Chlide esterification in the main phase was slowed down. The velocities of Pchlide resynthesis in the leaves lacking carotenoids and in the control leaves were close, however the final level of Pchlide accumulation was higher in the norflurason or amitrol treated leaves compared to the control. The 2–4 times increase in the 5-aminolevulinic acid accumulation after herbicides treatment was observed. It is concluded that change in carotenoid composition of inner plastid membranes leads to modification of the hypothetical complex consisting of 8 Pchlide650-POR molecules and 1 molecule of Chl-synthase, and to disturbance in coordinating of the initial and final stages of Pchlide biosynthesis process.

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8.23.

Influence of thylakoid lipids on the macrodomain-structure of photosystem II light-harvesting complex (LHCII)

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The influence of thylakoid membrane lipids on the macrodomain-structure of the main light-harvesting complex of PSII (LHCII) was studied by means of low temperature fluorescence, circular dichroism (CD) and photon correlation spectroscopy (PCS). Measurements were carried out with a lipid-depleted LHCII isolated by successive cation precipitation, and a more lipid-containing LHCII which was isolated by dodecyl maltoside solubilization and sucrose gradient centrifugation (DM-LHCII). PCS measurements revealed that the lipid-depleted LHCII formed aggregates with varying, but generally high particle sizes, in contrast to the detergent treated DM-LHCII whose domain structures were very small and more homogenously distributed. The addition of thylakoid lipids to lipid-depleted LHCII led to an increase of the fluorescence signal at 680 nm accompanied by a significant or even complete loss of the 700 nm shoulder. The decrease of the 700 nm shoulder was observed concurrently with a diminishment of psi-type CD bands which can be interpreted as a disaggregation of the LHCII macrodomain-structure. Addition of thylakoid lipids to the DM-LHCII led to a decrease of the strong fluorescence signal at 680 nm and the PCS measurements indicated the formation of lipid-LHCII structures of higher size. The amount of LHCII disaggregation depended both on the lipid concentration and the nature of the lipid. The anionic lipids sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) led to a significantly higher disturbance of the LHCII macrodomain-structure than the neutral galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). MGDG and DGDG exhibited the most pronounced effects at low pH-values, whereas SQDG and PG had the strongest influence on the LHCII macrodomain-structure at neutral or slightly basic pH-values. This finding indicates that the negative charge of the anionic lipids, which is only present at neutral and basic pH values, is responsible for the strong interaction between these lipids and the LHCII. This assumption is supported by the observation that addition of cations to the medium which shields the negative lipid charge partly suppressed the LHCII disaggregation by the anionic lipids.

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8.24.

Ferredoxin:NADP$^+$ oxidoreductase bound to the cytochrome b_6f complex is active in plastoquinone reduction. Implications for cyclic electron transport and chlororespiration

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In the present studies, we have applied for the isolation of the cytochrome b_6f complex from spinach (*Spinacia oleracea*) three methods differing in the preservation of the cytochrome b_6f-associated ferredoxin:NADP$^+$ oxidoreductase (FNR). Although the complexes isolated by all the methods showed the presence of the FNR peptide(s), when incorporated into liposome membranes, the NADPH-PQ (plastoquinone) oxidoreductase activity was not detected for the cytochrome b_6f complex isolated with the classical method including NaBr wash. The partial activity in was found for the complex isolated with the omission of the wash, but the highest activity was detected for the complex isolated with the use of digitonin. The reaction rate of PQ reduction of the investigated complexes in liposomes was not significantly influenced by the addition of free FNR or ferredoxin. Moreover, the reaction was also not affected by the presence of inhibitors either of the cytochrome b_6f complex at the Q_i site or the recently identified heme *x*. The obtained data indicate that FNR associated with the cytochrome b_6f complex can participate in the cyclic electron transport or chlororepiration as PSI-PQ or NADPH-PQ oxidoreductase, respectively. Moreover, we have shown that PQ in liposomes can be non-enzymatically reduced by ascorbate and this reaction might be involved in dark-reduction pathways of PQ-pool in chloroplasts.

Session 9

Integrating plant functions *via* signaling molecules-molecular mechanisms

PLENARY LECTURES

9.1.

Long range signalling in the control of shoot branching

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Plants continuously adjust their body plan to suit the environmental conditions in which they are growing. A good example of this is in the regulation of shoot branching. Axillary meristems, which are established in each leaf formed from the primary shoot apical meristem, can remain dormant or activate to produce a branch. The decision whether to activate an axillary meristem involves assessment of a wide range of external environmental, internal physiological and developmental factors. Much of this information is transmitted via a network of interacting hormonal signals that can integrate multiple inputs to generate a rich source of systemically transmitted information. We are studying this network, combining molecular biological, physiological and quantitative genetic approaches with computational simulation to try to understand how the component parts of the system are able to deliver environmentally responsive shoot branching patterns. Our recent progress will be reported.

9.2.

Signalling in epidermal growth control

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Co-ordination of cell division and expansion between different cell types is critical for the efficient functioning of plant organs, and is likely to be effected by multiple interlinked mechanisms allowing adaptation to both exogenous and endogenous cues. Increasingly, cell fate specification and maintenance is being functionally linked to plant growth control at the molecular level. Classical studies have indicated that epidermal identity is maintained by intercellular signalling throughout plant development, and that loss of epidermal integrity can therefore lead to an irreversible loss of epidermal cell fate. Decreased accumulation of PHYTOCALPAIN leads to severe epidermal abnormalities, including loss of epidermal identity. Here we present data indicating that PHYTOCALPAIN (also known as DEK1), a unique plant-specific protein, plays a fundamental role in regulating both cell division and cell expansion in *Arabidopsis*. Phenotypes generated by over-expression of an over-active version of DEK1 show that it does not directly regulate epidermal cell fate. We therefore propose that PHYTOCALPAIN controls aspects of growth co-ordination which are critical for maintenance of epidermal integrity during development. Ongoing research into understanding the mechanisms underlying PHYTOCALPAIN-mediated growth control will be discussed.

9.3.

Small GTPases effectors in plant cell morphogenesis

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Eukaryotic cell morphogenesis is an outcome of life processes encompassing all cellular components, yet it became obvious that small GTPases (especially those from Rho and Rab families) play central co-ordination roles.

Exocytosis is the major mechanism for morphogenesis in plant cells; yet we know so little about some aspects of this process, starting with exocytotic vesicle sources (TGN, endosomes) and mechanisms of their formation. The Rho/Rop GTPase regulatory module is central for initiating exocytotically active domains in plant cell cortex (ACD). Most plant cells exhibit several distinct plasma membrane domains, established and maintained by endocytosis-driven membrane constituent recycling. We propose the concept of a "recycling domain" (RD), uniting the ACD and the connected endosomal compartment, as a dynamic spatiotemporal entity. We have recently described Rab and Rho GTPases efector exocyst tethering complex in plant cells. Especially due to the multiplicity of its Exo70 subunits, this complex along with many RabA GTPases may belong to core regulators of RD organization.

Structural phospholipids are important constituents of cell as well as signals and phospholipid modifying enzymes are well known effectors of small GTPases. We are studying phospholipases D (PLD) and its product, phosphatidic acid, as regulators of secretory pathway in plants. We show that antisense-mediated knock-down of PLDβ1 and or PLDδ lead to time- and concentration-dependent inhibition of pollen tube growth. PLDβ1 is implicated in the regulation of actin and microtubular cytoskeleton, while PLDδ signaling possibly operates downstream of reactive oxygen species. We have also shown that pollen specific NADPH oxidases (another putative Rop GTPases effectors) are involved pollen tube growth. Also in plant cells small GTPases are orchestrating morphogenetic signalling pathways.

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9.4.

Phytochrome 3D structures and functions

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The complete 3D sensory module structures of the Pr ground state of *Synechocystis* phytochrome Cph1 (2VEA: Essen et al., 2008, PNAS) and the unusual Pfr ground state of the bacteriophytochrome PaBph1 (3C2W: Yang et al., 2008, PNAS) have now been solved, revealing an dumbbell-like form, with the PAS and GAF domains forming the major, chromophore-bearing lobe and the PHY domain the minor lobe. The PHY domain is structurally related to the GAF family but carries an intriguing tongue-like structure which contacts the major lobe.

On the basis of these data and studies of site-directed mutants it is possible to peer dimly into the mechanism of phytochrome photoactivation and photochromicity at the atomic level. Our 2VEA structure additionally shows the structural relationships for phytochromes in which the chromophore is covalently linked to the GAF domain, as in plant phytochromes, instead of the N-terminus as in the more primitive bacteriophytochromes. The Pr and Pfr states are characterised by *ZZZssa* and *ZZEssa* chromophore conformation, respectively. These D-ring isomers are associated with tautomeric differences in several nearby tyrosine residues in the two 3D structures. Two salt bridges on opposite sides of the chromophore seen in 2VEA are differently organised in 3C2W, as are the associations of the C-ring propionate. The extreme N-terminus and the tongue are also quite different in the two structures. It is still unclear, however, which of these structural differences are associated with bacteriophytochromes vs. Cph1 and plant type phytochromes, the unusual 3C2W Pfr ground state functionality vs. the Pr ground state and the Pr vs. Pfr photoisomerism. New solid-phase NMR data for Cph1 in the Pr and Pfr states as well for as the *lumi*FR and *meta*Rc intermediates (Rohmer et al., submitted) reveal unexpected changes in the chromophore during Pfr→Pr photoconversion, quite different from those expected for Pr→Pfr. The current state of these studies will be presented and discussed.

9.5.

Nitric oxide and hydrogen peroxide signalling

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Nitric oxide (NO) and hydrogen peroxide (H_2O_2) are now well-established as endogenous signalling molecules in plants. They are generated and removed by a variety of mechanisms, some of which can be activated by various stimuli, including several plant hormones, abiotic stresses and biotic interactions. Such stimuli often appear to modulate the levels of both NO and H_2O_2 concurrently; in some cases NO generation is activated by H_2O_2; and NO and H_2O_2 can react with each other. High concentrations of NO and H_2O_2 can be damaging, but at lower levels they modulate a range of intracellular processes with physiological and developmental effects. They can react directly with proteins via oxidation and nitrosylation, thereby modifying the activities and functions of target proteins. They interact with a range of intracellular signalling pathways including reversible protein phosphorylation, calcium signalling and gene expression. They have the potential to move within and between cells and even over longer distances. Thus, they are involved in signal cross-talk between various stimuli and can serve to integrate various functions in plants. In my presentation I will outline the current models of NO and H_2O_2 turnover in plant cells and focus on the molecular mechanisms of NO and H_2O_2 perception and action that underpin their biological effects.

ORAL PRESENTATIONS

9.6.

SnRK2's cellular partners: identification and characterization

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Plants respond to environmental stresses by induction of various defense mechanisms. Stress signals are recognized and transmitted to different cellular compartments by specialized signaling pathways, in which protein kinases and phosphatases are the main signaling components. The SnRK2 family members are plant specific kinases considered as important positive regulators of plant response to drought and salinity. There is also strong evidence indicating that SnRK2s are involved not only in environmental stress signaling but also in plant development. However, information concerning the mechanism(s) regulating their activity is still limited. Results presented by several groups provide proof that phosphorylation/dephosphorylation is responsible for SnRK2 activation/inactivation, respectively. Therefore, we applied several independent approaches (two-hybrid system, co-immunoprecitation, *in vitro* pull down assay, as well as various biochemical methods) to identify specific SnRK2 regulators, upstream protein kinase(s), as potential SnRK2 activator(s) and phosphatase(s) – SnRK2 inhibitors. *Nicotiana tabacum* Osmotic Stress Activated protein Kinase (NtOSAK) and its two closest homologues from *Arabidopsis thaliana* (SnRK2.4 and SnRK2.10) were used as bait. So far, we have identified two protein kinases phosphorylating NtOSAK and at least two different protein phosphatases, which potentially can be involved in SnRK2 inactivation. Moreover, during our search a plant specific calcium sensor, which interacts with the SnRK2 family members and can act as a negative regulator of their activity in plant cells, was identified. The studies of identified proteins on SnRK2s activity and their localization inside the cell are currently being characterized.

9.7.

COP1 complex and COP9 signalosome (CSN) regulate the chlorophyll biosynthesis in *Arabidopsis*

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In plants, COP1 and CSN complexes serve as central components of signalling pathways mediating the overall response of gene expression to light (Strickland et al., 2006).

In angiosperms, the light-triggered photoreduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) has a key role in the regulation of chlorophyll (Chl) biosynthesis. The Pchlide pool with a fluorescence maximum at 655 nm, accumulated preferentially in etiolated *Arabidopsis* seedlings, is denoted as photoactive Pchlide and referred to as $Pchlide_{655}$. This Pchlide forms an aggregate built of at least two ternary complexes: Pchlide:LPOR: NADPH (LPOR: Light-dependent Pchlide oxidoreductase) and it can be immediately reduced to Chlide with short (~ ms) illumination. The pool of Pchlide, which is not bound to the active site of LPOR has a fluorescence maximum at 628-633 nm, and it is called non-photoactive form or short-wavelength form ($Pchlide_{633}$) (Masuda, 2008).

Using low-temperature fluorescence spectroscopy (at 77K) and HPLC, we have examined mutants of constitutive photomorphogenic phenotype (5-days-old etiolated seedlings), bearing lesions in COP1 (*cop1-4*) and some of CSN subunits: CSN8 (*cop9*), CSN7 (*fusca5*) and CSN1 (*fusca6*), with respect to Pchlide forms and their photoconversions in situ.

In all analyzed mutants the dominant Pchlide form was $Pchlide_{633}$. Also, both the lack of $Pchlide_{655}$ and no Chlide formation after flash illumination indicated that functional Pchlide:LPOR: NADPH complexes are absent in these mutants. Upon illumination with continuous light, a subsequent degradation of $Pchlide_{633}$ was observed in CSN mutants, whereas *cop1-4* was able to form Chlide normally. The formation of both Chl *a* and Chl *b* was observed in *cop1-4* mutant. In contrast, CSN mutants overaccumulated Chl a in dim light (5 μmol m^{-2} s^{-1}). Our results indicate that both COP1 and CSN complexes are essential for greening of *Arabidopsis* seedlings, playing partially overlapping role in the control of chlorophyll biosynthesis.

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POSTERS

9.8.

Real-time observation of light-induced movement of nuclei in mature leaves of transgenic tobacco

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Plant nuclei move not only during cell division and growth but also in response to light. Intracellular position of nucleus was reported to depend on blue light (BL) in *Arabidopsis thaliana* leaves (Iwabuchi et al., 2007)). In darkness, the nuclei were located at the center of the mesophyll cell bottom. After irradiation with BL (100 μmol m^{-2} s^{-1}) they migrated towards light position, along the anticlinal walls. Microfilaments cooperating with myosins were earlier postulated in the dynamics of nuclei in *Arabidopsis* roots (Chytilova et al., 2000). We studied light-induced nuclei movement in transgenic *Nicotiana tabacum* with the actin cytoskeleton labelled with GFP fused to truncated plastin, an actin bundling protein. These plants were previously used to study organisation of the actin cytoskeleton in light-induced chloroplast responses (Anielska-MAzur and Gabryś, 2009). The movements of nuclei in tobacco are based on the actin cytoskeleton. The nuclei are enclosed within fine-structured actin baskets connected with thicker actin bundles. Live observations of nuclear migration were performed in dark-adapted and blue- or red-light-treated mesophyll. In the dark-adapted leaves nuclei were located at the bottom of mesophyll cells. The same location of nuclei was observed also in the cells irradiated with weak BL (1–2 μmol $m^{-2}s^{-1}$). Upon irradiation with strong BL of 40 μmol $m^{-2}s^{-1}$ the nuclei migrated to the anticlinal walls. Dynamic interactions between nuclei and the actin bundles were conspicuous during the relocation. In red light the location of nuclei changed a little, but they did not attain the characteristic light position. Also in this case, the connection between nuclei and actin was maintained. Tracks for nuclei redistribution are formed by the actin filaments similar to chloroplasts.

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9.9.

Expression of *AtCLH1* and *AtCLH2* is up-regulated by light

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Chlorophyllase (EC 3.1.1.14, Chlase) is a key enzyme involved in chlorophyll catabolism. Its expression is influenced by several phytohormones: ethylene, gibberelins and cytokinins, but up to date, the involvement of light in the regulation of chlase amount and/or activity has not been shown.

Two chlorophyllase-coding genes: *AtCLH1* and *AtCLH2* have been identified in *Arabidopsis* genome. In order to investigate the influence of light on the level of mRNA of these two genes real-time PCR was performed. We used leaves from 6 week-old wild type *Arabidopsis* dark-adapted overnight and then illuminated for 3 h. The expression of both genes was very low in the dark-adapted plants. The (steady state) level of *AtCLH1* mRNA increased significantly after illumination with blue (40 $\mu molm^{-2}s^{-1}$), red (40 $\mu molm^{-2}s^{-1}$), or white (120 $\mu molm^{-2}s^{-1}$) light. In contrast, the expression of *AtCLH2* was up-regulated only by blue light.

To further identify photoreceptor(s) involved in regulation of chlase genes by light we investigated leaves from 6 week-old photoreceptor mutants (*phot1, phot2, phot1/phot2, cry1, cry2, cry1/cry2, phyA/phyB*). The dark level of *AtCLH1* mRNA was very low and comparable in WT and the mutants. Up-regulation by red light was found in all tested plants, while blue light acted similarly in all but the *phot2* mutant. This result points to the involvement of phototropin2 in the up-regulation of *AtCLH1* expression by blue light. The up-regulation observed in the *phot1/phot2* double mutant suggests that cryptochromes substitute for phototropin in the mutant.

The dark level of AtCLH2 *mRNA* was comparable in all, but the *cry1* and *cry2* plants in which it was significantly lower. The expression of *AtCLH2* was up-regulated only by blue light in WT, *cry1* and *phyA/phyB* plants. Illumination with red light lead to the increase of *AtCLH2* mRNA in *phot1, phot2, phot1/phot2* and *cry2* plants. Neither blue, nor red light was effective in the *cry1/cry2* double mutant. Thus, both cryptochromes seem to bee necessary for up-regulation of *AtCLH2* expression by blue and red light.

These results show the key role of light in control of proper expression of chlases and a complex network of photoreceptors cooperating in the regulation of *AtCLH1* and *AtCLH2* expression.

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9.10.

Antioxidant system in apple seedlings developed from dormant embryos pre-treated by ROS or RNS

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Reactive oxygen species (ROS) and nitric oxide (NO), one of reactive nitrogen species (RNS), play a regulatory role in the control of plant growth and development, as well as plant response to different stresses. ROS (in particular H_2O_2) and NO are considered as signalling molecules. There are several data that both of them may function as dormancy braking agents in seeds, since they are produced endogenously during early phase of seed germination. Moreover, it was suggested that H_2O_2 and NO are necessary for the control of the post-germination seedling growth by their interaction with classical phytohormones.

Mature seeds of apple (*Mallus domestica* Borkh.) are dormant and do not germinate unless their dormancy is removed by several weeks of moist-cold stratification. Short term pre-treatment of dormant apple embryo with H_2O_2 and NO donors resulted in enhanced germination and removed morphological abnor-

malities of young seedlings (e.g. asymmetric growth and greening of cotyledons) (Gniazdowska et al., 2007).

As the maintenance of cellular redox homeostasis requires a balance between ROS/RNS production and scavenging, we investigated activity of cellular antioxidant system in seedlings developed from dormant embryos shortly pre-treated by NO donors and ROS (H_2O_2).

ROS and RNS pre-treatment of the embryos enhance activities of ROS processing enzymes: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) in 10 days old seedlings. However, seedlings developed from dormant (untreated) embryos were characterized by slightly higher glutathione reductase (GR) activity. ROS and NO pre-treatment enhanced also ascorbic acid concentration and the ratio of GSH/GSSG in both cotyledons of young seedlings.

The presented results allow us to create a comprehensive view of the mechanism of ROS-and RNS-dependent dormancy alleviation on development and growth of the young seedlings. The interaction between ROS, RNS, and other signalling molecules e.g. HCN in regulation of these processes will be discussed.

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9.11.

Abscisic acid inhibits flowering of *Pharbitis nil* through stimulation of ethylene production

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Although, recent results showed an important role of the FT protein in the photoperiodic flower induction, many experiments indicate that plant hormones may also be involved in the generation, transport or activity of the floral stimulus. ABA, widely considered as a plant growth inhibitor, can both promote and inhibit flowering in short day plant *Pharbitis nil*, depending on the time and place of its application. Inhibition of flowering is observed when ABA is applied at the beginning of a full inductive 16-h long dark period. In this study, we have shown that application of ABA is associated with the increase in both ethylene production, and stimulation of *ACSs* and *ACOs*, the genes involved in biosynthesis of ethylene, which is considered as another flowering inhibitor in *Pharbitis nil*. The increase in ethylene production observed between hours 3 (45%) and 8 (181%) after ABA treatment is correlated with an increase in *PnACS1* and *PnACS2*, as well as *PnACO1* and *PnACO3* transcriptional activity. The raise of the *PnACS1* and *PnACS2* mRNA levels was observed between hours 2 and 4 after ABA application. Next, *PnACS1* transcript level gradually decreased and reached its minimum at hour 24, while *PnACS2* mRNA lowered at hour 6 and then increased again at hours 16-18. Treating *Pharbitis nil* seedlings with ABA also caused an increase in the mRNA level of *PnACO1* and *PnACO3* genes but their maximal values were shifted in relation to *ACSs*'. In the subsequent hours of 24-h cycle, their transcripts accumulation decreased gradually to reach the value comparable to the level observed in untreated plants.

On the basis of the data obtained here we suggest that abscisic acid inhibition of *Pharbitis nil* flowering occur through a stimulation of ethylene production.

9.12.

miR172 and its target gene *InAPETALA2-like* are putative involved in the flower development of *Pharbitis nil* (*Ipomoea nil*)

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miRNAs are 21 to 24 nt long single-stranded RNAs that act to negatively regulate the expression of their target genes. The miR172 is involved in the regulation of flowering time and floral organ identity in *Arabidopsis thaliana* by regulation of *APETALA2-like* genes' activity (Aukerman and Sakai, 2003; Jung et al., 2007). In our study a full-length cDNA encoding *InAPETALA2-like* transcription factor was isolated from cotyledons of morning glory (*Ipomoea nil* named also *Pharbitis nil*), a model short day plant. The identified sequence shows a significant similarity to the cDNA of *TOE1* from *A. thaliana* and contains nucleotides complementary to miR172. A direct role for miR172 in reducing *InAP2-like* mRNA level was demonstrated by the identification of *InAP2-like* cDNA fragments whose 5' termini are consistent with products expected from miR172-directed mRNA cleavage (Glazińska et al., in publish). The aim of this work was to investigate the involvement of miR172 and its target gene indentified here *InAP2-like* during flower organs development of *Pharbitis nil*. We indicated that miR172 is expressed in all the plant organs examined with the highest accumulation in flower tissues. At the same time *InAP2-like* mRNA was detected in these tissues in much lower level. Additionally, microRNA and *InAP2-like* transcripts are presented during all stamen and pistil development of *P. nil*. In situ hybridization in flower tissues showed the presence of *InAP2-like* mRNA especially in the cytoplasm of anthers and stigma papillae cells. Our results suggest the potential involvement of miR172 and *InAP2-like* in the regulation of flower development in *P. nil*.

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9.13.

Recombinant expression and purification of novel cystatin from *Triticale*

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Phytocystatins are cysteine proteinase inhibitors from plants implicated in endogenous regulation of protein turnover, pro-

grammed cell death and in defense mechanisms against pathogens. These inhibitors may protect developing seeds of *Triticale* from pre-harvest sprouting since some of them are synthesized before accumulation of storage proteins.

The full-length cDNA encoding novel cystatin named Trc4 was received by 5' 3' RACE method. The ORF of Trc4 was subcloned and expressed in *Escherichia coli* using pET 28 expression vector. The pure recombinant protein was obtained by affinity chromatography in a single step of purification. The recombinant protein of Trc4 significantly decreased activity of papain. These results encourage to study activity of Trc4 against endogenous cysteine proteases responsible for pre-harvest sprouting during maturation of *Triticale* seeds.

9.14.

Interaction between HCN and ROS in the alleviation of apple embryo dormancy

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Mature apple (*Malus domestica* Borkh.) seeds are dormant and they do not germinate. Their dormancy may be overcome by 3 month long cold stratification or by short term application of exogenous hydrogen cyanide (HCN) to isolated embryos. Apple seeds contain a great amount of cyanogenic compounds (amygdalin, prunasin) realizing free cyanide in the early phase (10–14 days) of cold stratification (Dziewanowska et al., 1979).

The aim of this work was to compare the mode of action of ROS in dormancy removal by cold stratification and HCN pretreatment. The stimulatory effect of cyanide on germination of dormant embryos is associated with marked increase in hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) concentration mainly in the embryo axes, and is also mimicked by short pretreatment by H_2O_2 or ROS generating compounds. We observed changes in ROS (H_2O_2 and O_2^-) concentration in the embryos during stratification. It was relatively low at the beginning of cold stratification but increased markedly after 30 days of seeds imbibitions in 5°C. After additional two months of cold stratification ROS concentration in the embryos was detected at the level observed for HCN pre-treated embryos.

We suggest that oxidative signaling by ROS, as second messengers of cyanide, may explain the cellular mode of action of HCN in apple seed dormancy alleviation.

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9.15.

Primary assessment of α-amylase gene expression dependence upon somatic embryos vigor in *Medicago* sp.

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Somatic embryo quality is an important factor decisive for the efficiency of somatic embryogenesis. In our previous experiments we suggested that α-amylase activity can be considered a good vigor marker of *Medicago sativa* somatic embryos (1). In this study we would like to check α-amylase gene expression during germination of somatic embryos in presence of gibberellic acid (GA_3) and ancymidol (inhibitor of gibberellins biosynthesis). At first we performed an alignment of known plant α-amylase amino acid sequences and utilizing highly conserved regions designed degenerate primers. Subsequently PCR products after sequencing and *in silico* verification were basis for new set of primers. As a positive control of primer specificity we used RNA extracted from *Medicago sativa* seeds 24 hours after imbibitions. For analysis we used somatic embryos obtained by using modificated McKersie method (2). RNA was isolated from embryos germinated on solid MS medium with GA_3 and ancymidol and after cDNA synthesis and PCR, products were visualized on 1% agarose gel. All data were standardized to Ubiquitin and Actin2 genes. The obtained results serve estimation of α-amylase activity based assay usefulness to somatic embryos vigor assessment.

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9.16.

The release of primary dormancy in *Avena fatua* L. caryopses

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Smoke and aqueous smoke extracts stimulate seed germination and improve seedling vigor in a wide variety of plants. The butenolide, 3-methyl-2H-furo[2,3-c]pyran-2-one has been isolated from plant derived-smoke. This compound has a stimulatory effect on seed germination and seedling growth similar to that of smoke or aqueous extracts of smoke.

The present study was undertaken to elucidate the role of butenolide in releasing primary dormancy in *Avena fatua* caryopses. Caryopses of *Avena fatua* germinated partially at 15°C but almost not at 20 and 25°C. In the presence of smoke-water or butenolide caryopses germinated at all temperatures. Seedling growth was also affected. Ethephon, ethylene and ACC did not affect caryopses germination at 20°C. Butenolide stimulated ethylene production and enhanced ACC oxidase activity *in vivo* before radicle protrusion.

2,5-norbornadiene, a competitive inhibitor of ethylene binding, completely prevented the action of butenolide over 3 days of incubation. The inhibition was partially or completely relieved, depending on concentration of norbornadiene, when these seeds were transferred to air. Ethephon, ethylene or ACC overcame the inhibitory effect of 2,5-norbornadiene in the presence of butenolide. Butenolide increased α-amylase activity before radicle protrusion. Likewise, this compound induced an increase in the proportion of cells with 4C amounts of DNA and increased the ratio of G_2/G_1 after 30 hours of imbibition at 20°C. The presented results indicate that butenolide is a very active dormancy break-

ing factor involved in mobilization of stored reserve materials and in induction of cell cycle activities. The release of dormancy by butenolide appears to involve ethylene action.

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9.17.

Effect of stratification, ethephon, gibberellin A3, nitric oxide donors and cyanide donors on germination of primary dormant *Amaranthus retroflexus* L. seeds

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Seeds of *Amaranthus retroflexus* L. after harvest are in deep primary dormancy. Dry storage or exogenous plant growth regulators break primary dormancy in these seeds.

The aim of this study was to check the response of *Amaranthus retroflexus* L. to stratification, ethephon, gibberellin A_3 and nitric oxide and/or cyanide donor as dormancy breaking reagent. As nitric oxide and cyanide donor sodium nitroprusside (SNP) was used. S-nitroso-N-acetyl-DL-penicillamine (SNAP) and nitroglycerin (NTG) were used as nitric oxide donors and potassium ferrocyanide as cyanide donor. These donors need light to release proper compounds. Germination of stratified seeds, and dormant seeds after ethephon or gibberellin treatment was tested in the darkness at 25°C and 35°C. Germination test with donors was conducted under light (intensity 120 $\mu Em^{-2}s^{-1}$, photoperiod 16h day/8h night) and 25°C.

Dormant seeds after harvest did not germinate at 25°C and 35°C in the dark. Cold stratification for 16 weeks partially released dormancy at 25°C and only 20% of seeds germinated. At 35°C primary dormancy was almost completely released and ca. 70% of seeds were able to germinate. Ethephon slightly increased germination at 25°C and at 35°C to 20 and 40% of seeds respectively. Gibberellin A_3 did not break dormancy at 25°C and at 35°C only 30% seeds germinated.

Dormant seeds incubated on the light in presence of water germinated from 5 to 18%. Sodium nitroprusside caused more than 40% of seeds to germinate after one day of treatment. Prolonged treatment (three days) resulted in 60% germination. Potassium ferrocyanide had similar effect to SNP in dormancy breaking. S-nitroso-N-acetyl-DL-penicillamine had no effect on dormancy release irrespective of time of treatment because germination was similar to dormant seeds. Nitroglycerin caused partial dormancy release, only 35% of seeds were germinated. DL-dithiothreitol used as inhibitor of nitric oxide receptor and N-(omega)-Nitro-L-Arginine (L-NAME), which is inhibitor of nitric oxide synthase, has no effect on seed germination in the presence of nitric oxide and cyanide donors.

9.18.

Involvement of ROS and RNS in photosynthetic activity of young apple seedlings developed from dormant embryos

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are bioactive molecules that play important roles in diverse processes in plants. Both of them are involved in regulation of plant growth and development, stomata movement, responses against a variety of stresses. Moreover ROS and RNS are identified as endogenous metabolite in biological system and are proposed to act in similar signal transduction pathways.

Dormant apple (*Malus domestica* Borkh.) embryos were shortly pre-treated by HCN, H_2O_2 and different donors of NO (SNP, acidified nitrite). It was reported before that ROS and RNS act as signaling molecules leading to dormancy alleviation, expressed as stimulation of germination and elimination of morphological anomalies typical for the seedling developed from dormant embryos (asymmetric growth and greening of cotyledons) (Bogatek and Gniazdowska, 2006).

The aim of this study was to investigate some aspects of photosynthetic metabolism in young, 10 days old seedlings developed from dormant embryos shortly pre-treated by ROS and RNS.

Photosynthetic activity (measured as O_2 evolution) of the seedlings grown from embryos pre-treated by ROS and RNS was higher than in control once. The beneficial effect of ROS and RNS on photosynthetic activity of young seedlings was associated with increased chlorophyll concentration as well as reducing sugar and starch concentration in both (upper and lower) cotyledons. Treatment by HCN and NO did not induce significant changes in Rubisco content detected by SDS-PAGE, while H_2O_2 slightly decreased Rubisco concentration, probably by extent protein oxidation. The chlorophyll *a* fluorescence measurement indicated that short treatment of the embryos by ROS and RNS did not produce a severe effect on photochemical efficiency of PSII.

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9.19.

The GFP-FABD2 fusion protein is unsuitable for visualizing the actin cytoskeleton in *Arabidopsis thaliana* mature leaves

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One of the most difficult technical problems in cell biology is the visualization of the actin cytoskeleton in cells. The widely used GFP-FABD2 fusion protein, derived from the C-terminal part of *Arabidopsis* fimbrin 1 [1,2,3] is considered as one of the best tools for studying actin architecture in living plants. Stable trans-

formation of *Arabidopsis* with this construct does not cause any phenotypic changes or alterations in phototropic and gravitropic responses [3]. Moreover, GFP-FABD2 enables visualization of very detailed F-actin structures in practically all cell types of *Arabidopsis* seedlings [3]. Our results show however that this system is inadequate for studying F-actin networks in mature leaves of *Arabidopsis thaliana*. A characteristic time-course of loss of GFP fluorescence was observed under the confocal microscope for all plants carrying this fusion protein. This was an age-dependent effect. Detailed studies at the mRNA (semi-quantitative PCR) and protein (Western Blots) level revealed a sudden decrease of GFP-FABD2 expression in leaves after the third week of plants' growth. Moreover, a similar decline in mRNA level was observed also for the natural fimbrin 1 in the transgenic plants. This phenomenon was not observed in wild type *Arabidopsis*. A plausible explanation is the occurrence of RNA silencing triggered by the introduction of the GFP-FABD2 construct. Therefore, transgenic *Arabidopsis*, which carries GFP-FABD2, is not a good model system for the visualization of actin cytoskeleton in leaves of mature plants.

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9.20.

Cloning of PnCDPK1 into bacterial expression vector and purification of the recombinant protein

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Ca^{2+}-dependent protein kinases (CDPKs) are vital Ca^{2+}-signaling proteins in plants and protists which have both a kinase domain and a self-contained calcium regulatory calmodulin-like domain. Previous reports show isolation and molecular characterization of *PnCDPK1* gene from *Pharbitis nil* seedlings. Here, we present cloning and purification of a recombinant protein kinase PnCDPK1. To obtain purified recombinant protein the pGEX-PnCDPK1 plasmid construct contained the glutathione S-transferase (GST) sequence joined to 5' coding region of the *PnCDPK1* cDNA was made. ORF region of *PnCDPK1* gene was subcloned, transformed and expressed in *Escherichia coli*. GST-PnCDPK1 fusion protein was purified by affinity chromatography using glutathione immobilized to a Sepharose matrix. Analysis of recombinant proteins by SDS-PAGE show the 85 kDa size of the purified fusion protein corresponded to the molecular weight of GST (27 kDa) and PnCDPK1 (58 kDa). Presence of GST tag was proved using monoclonal anti-GST antibodies. Cleavage of PnCDPK1 protein from GST was conducted on-column using a site-specific protease. Immunoblot analysis with polyclonal antibodies made against the calmodulin-like domain of soybean CDPK confirm that the studied PnCDPK1 kinase isolated from *Pharbitis nil* is a typical CDPK.

9.21.

The expression of somatic embryogenesis receptor-like kinase 1 (*TnSERK1*) during somatic embryogenesis in *Trifolium nigrescens* Viv

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Somatic embryogenesis (SE) is the development of embryos from somatic cells. In recent years several genes involved in somatic embryogenesis were identified. One of them is *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*), encoding a receptor-like protein kinase, containing leucine-rich repeat. This gene was initially identified in carrot embryogenic tissue culture. An enhanced *SERK1* expression was detected in somatic *D. carota* embryos (from induction phase to globular stage) as well as during zygotic embryogenesis (Schmidt et al., 1997). *SERK1* gene is considered as a molecular marker of embryogenic competent cells although its function during somatic embryogenesis is still not known (Ikeda et al., 2006). The analysis of *SERK1* gene expression can help to distinguish between somatic embryos and shoots at early stages of development.

To study the relationship between somatic embryogenesis in *Trifolium nigrescens* Viv. and the expression of *TnSERK1* the in situ hybridization was performed. SE was induced by the culturing of *T. nigrescens* zygotic embryos on solid medium supplemented with growth regulators (auxin and cytokinin). The 322 bp sequence fragment of *TnSERK1*, amplified from *T. nigrescens* genomic DNA has been cloned into pJET1.2/blunt plasmid (Fermentas, LT). This construct was used to synthesise both sense and antisense DIG-labeled RNA probes. The *TnSERK1* transcripts were localized in sections of explants with somatic embryos at different stages of development formed after 7 days of culture. *TnSERK1* transcripts were detectable in cytoplasm and nuclei of small meristematic cells, the emerging and fully developed somatic embryos but also in embryo-like structures. These results suggest the possible involvement of *SERK1* gene in induction and maintenance of embryogenic competence in *T. nigrescens*.

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9.22.

Effect of C and N metabolites on nitrate uptake and expression of genes encoded nitrate transporters in *Arabidopsis thaliana hxk1* mutant

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Uptake of nitrate by plant roots is a key step of nitrogen metabolism and has been widely studied at the physiological and molecular level. So far two distinct NO_3^- transport systems with high (HATS) or low (LATS) affinity to the substrate encoded by *NRT2* and *NRT1*, respectively, were identified in plants. The activities of those systems are tightly controlled by different endogenous molecules such as sugars and amino acids. It is well known that incorporation of inorganic N into organic compounds requires carbon skeletons derived from photosynthesis. Feeding of plants with sugars stimulates nitrogen assimilation, but the transduction of sugar signal is still not clear. Also the final products of N assimilation affect nitrate uptake to prevents of both the excessive utilization of storage carbohydrates and accumulation of toxic products of N assimilation.

The aim of this study was to characterize the HATS nitrate transporters regulation under selected photosynthates and N metabolites treatment. The experiments were done on 6 weeks old wild *Arabidopsis thaliana* and *hxk1* mutant disordered in glucose signaling. Plants were grown for 5 weeks on nitrate nutrient solution and than one week in N-free solution. Nitrate uptake as well as expression of two *NRT2.1* and *NRT2.2* genes encoding HATS nitrate transporters were measured during 8 hours exposition of starved plants to 0.5 mM NO_3^- with or without sugars (glucose and sucrose) or amino acids (glutamine and glutamic acid).

Addition of sugars into the nitrate solution significantly increased the NO_3^- uptake by wild as well as *hxk1 Arabidopsis* plants. Stimulation was maximal after first 2 hours of sugar treatment and then slightly dropped. The changes in the NO_3^- uptake rate were correlated with the level of *NRT2.1* and *NRT2.2* gene expression. Opposite effects were observed in plants treated with glutamine or glutamic acid. The NO_3^- uptake decreased in WT as well as in *hxk1* plants. Concluding, presented data suggest, that nitrate uptake by roots in high affinity range as well as the expression of genes encoding nitrate transporters with high affinity to the substrate are regulated by C and N metabolites. Similar effect of sugar observed in wild type and *hxk1* mutant plants suggests that transduction of sugar signal is not mediated via AtHXK1 sensing-dependent pathway.

9.23.

What do we know about sink effect of cytokinins in plants?

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Sink-source relationship is the main integrating factor in plants. Cytokinins play an important role in sink-source regulation. In particular they increase sink capacity of plant tissues, inducing re-orientation of assimilate transport and partitioning of towards cytokinin-riched tissues and enhancing of accumulation or metabolisation of organic substances in sinks.

In order to study the mechanism of sink effect of cytokinins we used model systems (detached leaves, locally treated by cytokinin benzyladenine) and intact plants. Our results let us to conclude that sink effect of cytokinins is connected with the activation of growth and metabolism in sinks → reducing low-molecular-weight substances → removing of assimilates from a phloem and increasing of an osmotic potential in sinks → declining pressure at the end of transport system → stimulating assimilate inflow to sinks. The same processes provide integration of different organs and transform a plant into well-balances sink-source system.

9.24.

Tyrosine phosphorylation modulates organization of cortical microtubules in *Arabidopsis* root cells

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Protein kinases and protein phosphatases regulate a number of processes in eukaryotic cells through controlling of protein phosphorylation level on serine/threonine residues as well as tyrosine residues. Recently it was demonstrated that both α- and β-subunits of plant tubulin, the main structural component of microtubules (MTs), undergo phosphorylation on tyrosine residue (Blume et al., 2008). However, a possible functional role of this post-translational tubulin modification with impact on MTs organization and stability in different types of plant cells remains an open issue to date. To investigate the role of tyrosine phosphorylation/dephosphorylation processes in plant cells the morphology of *Arabidopsis thaliana* primary roots and MTs organization in different types root cells were studied after inhibition of protein tyrosine kinases (PTKs) and tyrosine phosphatases (PTPs). Effects of inhibitors were examined *in vivo* on *A. thaliana* line expressing GFP-MAP4 using confocal laser scanning microscopy (LSM 510 META, Carl Zeiss, Germany).

It was found that all tested types of PTKs inhibitors (herbimycin A, genistein and tyrphostin AG 18) altered root hair growth and development, probably as a result of their significant influences on MTs organization in root hairs. The treatment also led to MTs reorientation and disruption in epidermis and cortex cells of both elongation and differentiation zones of primary roots. Enhanced tyrosine phosphorylation after treatment with a PTPs inhibitor (sodium orthovanadate) resulted in intense induction of root hair development and growth and caused a significant shortening of the elongation zone. It also led to changes of MTs orientation from transverse to longitudinal in epidermis and cortex cells of the elongation and differentiation zones of the root. We can summarize that our results suggest the involvement of tyrosine phosphorylation/dephosphorylation processes in the regulation of growth and development of *A. thaliana* roots as well as in the regulation of overall MTs organization in different cell types.

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9.25.

Inhibitors of protein phosphatases block chloroplast relocations in *Arabidopsis thaliana*

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Chloroplasts move in response to incident light in numerous plant species including *Arabidopsis thaliana*. Upon weak irradiation they gather at the cell walls perpendicular to the light direction (accumulation response), thereby maximizing light absorption. Upon strong light chloroplasts relocate to the cell walls parallel to the light direction (avoidance response). This response is considered a photoprotective mechanism. In *Arabidopsis*, chloroplast responses are elicited only by blue light and the sensor proteins involved are phototropins. Phototropin 2 alone controls the avoidance response, whereas both phototropins control the accumulation response. So far, little is known about subsequent signaling pathways leading to activation of the movement.

Protein phosphatase inhibitors were found to affect two other phototropin-controlled responses: phototropism and blue light-induced opening of stomata. For phototropism, they were found to block de-phosphorylation of NPH3, the scaffold protein known to be involved in the phototropism signaling (Pedmale and Liscum, 2007). High inhibitor concentrations required suggest the involvement of protein phosphatase 1 (PP1).

In this study we tested the influence of two protein phosphatase inhibitors, endothall and cantharidin, on chloroplast movement. Infiltration of leaves with 50 μM cantharidin blocked chloroplast relocations completely. Both velocity and amplitude of chloroplast responses were reduced in leaves treated with 5 μM cantharidin. Prolonging the preincubation time resulted in a more severe effect. Similar effects were observed for 60 μM endothall. Our results suggest that a signaling mechanism similar to NPH3 de-phosphorylation can be responsible for chloroplast movement. A group of homologous genes, present in *Arabidopsis* genome encodes the NPH3/RPT2 family of proteins, mostly of unknown function. We speculate that some of these proteins may be targets of protein phosphatase in the signaling pathway leading to chloroplast relocations.

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9.26.

Interactions between abscisic acid and gibberelline GA_3 in the regulation of flowering of *Pharbitis nil*

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Flowering of plants is controlled by hormones among which both stimulators and inhibitors are present. Natural balance between these substances decides about direction of plant differentiation. There are only few papers concerning the role of hormone interactions in flowering. Data presented here concerns the interaction between abscisic acid (ABA) and gibberellin (GA_3) in the regulation of flowering of short day plant *Pharbitis nil*. In many physiological processes interaction between ABA and GAs is governed by the same molecular mechanism, which relies upon opposite effect both of the hormones on stability of DELLA proteins, which are the repressors of GAs responses (Weiss and Ori, 2007). Thus, interesting question was: Does interaction between ABA and GAs really take place during the transition from vegetative to reproductive phase in *Pharbits nil*?

Exogenous ABA applied on the cotyledons just before or during the first half of the inductive 16h-long night inhibited flowering. In this conditions the seedlings produced about 80% flower buds less than the control plants. GA_3 applied during the inductive night period did not influenced on flowering ability in *Pharbitis nil*, however the increased number of flower buds per plant was observed when plants were cultivated in subinductive conditions (12 h of darkness). Application of GA_3 just before and during the inductive night reversed the inhibitory effect of abscisic acid on flower bud formation. These results suggest that interactions between abscisic acid and GAs may play a significant role in photoperiodic flower induction in short day plant *Pharbitis nil*.

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9.27.

Involvement of cortical microtubules in nitric oxide donor and scavenger influence on *Arabidopsis thaliana* root development

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Various experimental data indicate signaling roles of nitric oxide (NO) in plant cell by mediating growth, flowering, root formation, seed germination, stomatal closure, gravitropic responses as well as disease and abiotic stress tolerance. However, it still remains unclear how NO signal is transduced, particularly directly through posttranslational modifications of downstream effectors of NO. Among candidates for NO-signal transduction are microtubules because of their involvement in regulation of different physiological processes in plants. α-Tubulin tyrosine nitration could realize direct NO signalling via microtubules in plants because in animal cells α-tubulin is one of crucial targets for C-nitration of tyrosine residues. Thus, the presence and functional role of plant tubulin tyrosine nitration have to be elucidated.

Effects of SNP (NO donor) and c-PTIO (specific NO scavenger) on primary root morphology and cortical microtubules organization in root cells of *Arabidopsis thaliana* line expressing (GFP-MAP4) were studied. Short-time treatments (1–6 h) with donor and scavenger separately in different concentrations revealed no appreciable effects. Root hair initiation in differentiation zone and further hair growth were activated after SNP treatment at concentrations 250 and 500 μM during 24 h, After 48–72 h of treatment, lateral and adventitious roots formation was triggered. Alterations of primary root morphology correlated with reorganization of cortical microtubules in epidermal cells of different root zones, where they became randomized, oblique or longitudinal. As distinct from SNP influence, after 12–24 h of treatment with c-PTIO (50 μM – 1 mM) cortical microtubules randomization and

fragmentation in swelled epidermal cells of transition and elongation zones were observed. That was accompanied with excessive short root hair formation in differentiation zone. Finally, c-PTIO treatment during 48 h caused die-off of meristematic, transition and elongation zones. However, differentiation zone was pronouncedly extended and abundant root hairs with ceased growth were observed.

We suppose that microtubules appear to be regulated by NO, because both NO donor and scavenger affect cortical microtubules organization. Hence, NO could play as microtubule-regulating agent and also as one of intracellular triggers of plant cell differentiation.

9.28.

Nitric oxide donor and scavenger influence on *Arabidopsis thaliana* root development *via* cortical microtubules reorganization

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NO in plant cell mediates growth, root formation, stomatal closure, gravitropic responses as well as disease and abiotic stress tolerance (Besson-Bard et al., 2008). However, components of NO-signalling cascade remain unclear. Among candidates to for NO-downstream effectors are microtubules because of their involvement in plenty of processes in plants regulated by NO. α-Tubulin nitrotyrosination could realize direct NO-signalling via microtubules in plants because in animal cells α-tubulin is one of C-nitration targets of tyrosine residues (Yemets et al., 2009). Thus, the presence and functional role of plant α-tubulin nitrotyrosination have to be elucidated.

Effects of NO donor SNP and NO scavenger c-PTIO on root morphology and microtubules organization in root cells of *Arabidopsis thaliana* line expressing *gfp-map4* were studied *in vivo*. Treatments during 1-6 h separately with donor and scavenger revealed no appreciable effects. Root hairs initiation in differentiation zone and their further growth were activated after 250 and 500 μM SNP treatment during 24 h, and, after 48–72 h of treatment, lateral and adventitious roots formation was triggered. Alterations of root morphology were correlated with reorganization of cortical microtubules in epidermal cells of different root zones. As distinct from SNP influence, after 12–24 h of treatment by 50 μM – 1 mM c-PTIO cortical microtubules randomization and fragmentation in swelled epidermal cells of transition and elongation zones were observed. That was accompanied with excessive short root hair formation in differentiation zone. Finally, 48 h of c-PTIO treatment caused die-off of meristematic, transition and elongation zones. However, differentiation zone was pronouncedly extended and abundant root hairs with ceased growth were formed.

We suppose that microtubules appear to be regulated by NO, because both NO donor and scavenger affect cortical microtubules organization. Hence, NO could play as microtubule-alterating agent and also as one of intracellular triggers of plant cell differentiation depending on its concentration in plant cell.

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9.29.

Unusual ligand specificity of cytokinin-specific binding protein from mung bean analyzed by fluorescence correlation spectroscopy

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Cytokinins are essential plant hormones involved in cell division, shoot initiation, leaf and root differentiation, photomorphogenesis and senescence. Cytokinin binding proteins (CBPs) have been identified in many species, from mosses to higher plants. Unfortunately, the biological function of these proteins is still unclear, with the exception of cytokinin receptor – CRE1, and some proteins of PR-10 class. Identification and characterization of new CBPs is important to better understand the mechanism of cytokinin action.

Fluorescence correlation spectroscopy (FCS) allows the investigation of intermolecular interaction in solution. During FCS measurement, fluorophores are excited and the fluorescence intensity can be measured. The autocorrelation function of the time-dependent fluorescence intensity provides information about fast- and slow-diffusing populations which is translated into the relative amount of bound and unbound species.

For our studies we first designed and synthesized two fluorescent cytokinin derivatives. We attached two fluorophores, NBD (7-chloro-4-nitro-2,1,3-benzoxadiazole) and Rhodamine B, to one of the most active urea-type cytokinin 4PU (N-phenyl-N'-(4-pyridyl)urea). The binding behavior of fluorescent cytokinins to the proteins was investigated by FCS experiments with several proteins. First, we found that the fluorescent probe did not bind to any accidental proteins like BSA, lysozyme or proteinase K. Second, we asked if fluorescent cytokinin would bind to cytokinin-specific binding protein from *Vigna radiata* (VrCSBP). Indeed, this proteins bound fluorescent probe strongly whereas rhodamine B moiety did not interact with VrCSBP at all. A diffusion coefficient range from $2.24\times10^{-6}cm^2s^{-1}$ (free state) to $0.75x10^6cm^2s^{-1}$ (bound state). The protein binding was saturated at a concentration of about 1.7 μM, and a Kd value of 628±28 nM was obtained by scatchard analysis of the binding data. Using a competition assay we determined dissociation constants of non-fluorescent ligands. Surprisingly, strong affinity of gibberellins to VrCSBP was found. These results suggest that VrCSBP is a bifunctional protein which binds cytokinin as well as gibberellins.

Summarising, we demonstrate here fast and precise tool to investigate interaction in solution between cytokinins and any soluble protein of interest.

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