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Ministry of Science and Higher Education
Republic of Poland

The National Centre for Research and Development

UNIVERSITY OF GDANSK
Rector of the University of Gdansk

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OPENING CEREMONY

09:00 - 09:10 Welcome Speeches
EWA ŁOJKOWSKA
President of Polish Society of Experimental Plant Biology, Chair of Organizing Committee and Members of Honorary Committee

09:10 - 10:00 Keynote Lecture: **Exploring Arabidopsis natural variation**
MAARTEN KOORNNEEF
Max Planck Institute for Plant Breeding Research, Cologne, DE

SESSION 1: NATURAL VARIATION AND PHENOMICS

CHAIRS: *ANNA IHNATOWICZ, MAARTEN KOORNNEEF*

10:00 - 10:30 Plenary Lecture: **The versatile use of genome elimination in developing novel genetic mapping resources in Arabidopsis**
JOOST JB KEURENTJES
Wageningen University, Wageningen, NL

10:30 - 11:00 Plenary Lecture: **The genotype-phenotype map in *Arabidopsis thaliana***
ARTHUR KORTE
Gregor Mendel Institute, Vienna, AT

11:00 - 11:30 Coffee Break

11:30 - 12:00 Plenary Lecture: **High-throughput phenotyping to decode the complexity of growth response to the environment in Arabidopsis**
OLIVIER LOUDET
Institut Jean-Pierre Bourgin, Versailles, FR

12:00 - 12:30 Plenary Lecture: **Species migration in plants is associated to quantitative genetic changes in the circadian clock**
SETH DAVIS
University of York, York, UK

12:30 - 12:45 Selected Talk: **Brassinosteroid metabolism and its disturbances as a source of semi-dwarf barley mutants**
DAMIAN GRUSZKA
University of Silesia, Katowice, PL

12:45 - 13:00 Selected Talk: **The distribution of coumarins and furanocoumarins in Citrus species closely matches Citrus phylogeny and reflects the organization of biosynthetic pathways**
ALAIN HEHN
Université de Lorraine – INRA, Nancy, FR

13:00 - 13:15 Selected Talk: **Towards the genetic architecture of scopolin and scopoletin biosynthesis in *Arabidopsis thaliana***
JOANNA SIWIŃSKA
Intercollegiate Faculty of Biotechnology UG&MUG, Gdańsk, PL

13:15 - 13:30 Selected Talk: **ROS production and scavenging in stress resistant *Eutrema salsugineum* plants**
MARIA PILARSKA
Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences, Krakow, PL

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CHAIRS: *EWA ŁOJKOWSKA, ALESSIO MENGONI*

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DOMINIQUE LOQUE
Cell Wall Engineering, Berkeley, CA, US

15:00 - 15:30 Plenary Lecture: **Genome evolution and sociomicrobiology in the alfalfa nitrogen fixing microsymbiont *Sinorhizobium meliloti***
ALESSIO MENGONI
University of Florence, Florence, IT

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ANNA KULMA
University of Wroclaw, Wrocław, PL

16:00 - 16:30 Coffee Break

16:30 - 16:45 Selected Talk: **Global control of eukaryotic gene expression depends on chromatin remodeling by SWI/SNF complexes**
TOMASZ SARNOWSKI
Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, PL

16:45 - 17:00 Selected Talk: **EcoFactory, a multipromoter explorer expression system for *Escherichia coli***
MARCIN OSUCH
Adam Mickiewicz University, Poznań, PL

17:00 - 17:15 Selected Talk: **Chloramphenicol acetyltransferase - new selectable marker for stable nuclear transformation of the red alga *Cyanidioschyzon merolae***
ANNA DROŻAK
University of Warsaw, Warsaw, PL

Poster Session I

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Thursday, 10th September 2015

08:30 - 09:00 Registration

Session 3: Plant Development and Gene Regulation

Chairs: Małgorzata Gaj, Artur Jarmołowski

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Michael Nodine
Gregor Mendel Institute, Vienna, AT

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Kim Boutilier
Wageningen University and Research Centre Bioscience, Wageningen, NL

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Gordon Simpson
Dundee University at The James Hutton Institute, UK

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Michael Lenhard
University of Potsdam, DE

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Konrad Dełeńko
Nicolaus Copernicus University, Toruń, PL

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Anna Maria Wójcik
University of Silesia, Katowice, PL

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Aleksandra Eckstein
Jagiellonian University, Krakow, PL

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Katarzyna Knop
Adam Mickiewicz University, Poznań, PL

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MALWINA HYJEK
Nicolaus Copernicus University, Toruń, PL

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SEBASTIAN SACHAROWSKI
Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, PL

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CHAIRS: ANDRZEJ KONONOWICZ, VIOLETTA MACIOSZEK, JADWIGA ŚLIWKA,

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DIERK SCHEEL
Leibniz Institute of Plant Biochemistry, Halle, DE

14:30 - 15:00 Plenary Lecture: **Pathogenomics of Verticillium wilt diseases**
BART THOMMA
Wageningen University, Wageningen, NL

15:00 - 15:30 Plenary Lecture: **Foundations of quantitative disease resistance in Cereals and Brassicas**
CHRISTOPHER RIDOUT
John Innes Centre, Norwich, UK

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ANDREAS VON TIEDEMANN
Georg-August-University Göttingen, Göttingen, DE

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RAFAŁ HOSER
Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, PL

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AGATA MOTYKA
Intercollegiate Faculty of Biotechnology UG&MUG, Gdańsk, PL

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AGNIESZKA SZUBA
Institute of Dendrology Polish Academy of Sciences, Kórnik, PL

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MARTA BRYLIŃSKA
Plant Breeding and Acclimatization Institute – National Research Institute, Młochów, PL

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FRIDAY, 11TH SEPTEMBER 2015

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CHAIRS: IWONA CIERESZKO, STANISŁAW KARPIŃSKI

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WIM VAN DEN ENDE
Institute of Botany and Microbiology, KU Leuven, BE

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WIESŁAW GRUSZECKI
Maria Curie-Sklodowska University, Lublin, PL

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CHRISTOPHE BAILLY
Universite Pierre & Marrie Curie, FR

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KRYSTYNA ORACZ
Warsaw University of Life Sciences, PL

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11:30 - 11:45 Selected Talk: **Methylome-transcriptome relationships during drought stress in model and crop monocots**
MIROSŁAW KWAŚNIEWSKI
University of Silesia, Katowice, PL

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MARIA BARCISZEWSKA-PACAK
Adam Mickiewicz University, Poznań, PL

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WOJCIECH POKORA
University of Gdańsk, Gdańsk, PL

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GRAŻYNA BIAŁEK-BYLKA
Poznań University of Technology, Poznań, PL

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JAGNA CHMIELOWSKA-BĄK
Adam Mickiewicz University, Poznań, PL

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MICHAŁ RUREK
Adam Mickiewicz University, Poznań, PL

13:00 - 14:00 Lunch

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CHAIRS: KRZYSZTOF BIELAWSKI, ROBERT CZAJKOWSKI

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FREDERIC BOURGAUD
Université de Lorraine UMR 1121, FR

14:30 - 15:00 Plenary Lecture: **Treatment of acute and chronic inflammation by novel natural plant extracts with high biological activity**
KRZYSZTOF LEMKE
Biovico, Gdynia, PL

15:00 - 15:15 Plenary Lecture: **Plant naphthoquinone derivatives as MAPK/ERK kinase inhibitors – potential application in breast cancer treatment**
ANNA KAWIAK
Intercollegiate Faculty of Biotechnology UG&MUG, Gdansk, PL

15:15 - 15:30 Plenary Lecture: ***Iv vitro* cultured carnivorous plants as a source of secondary metabolites with high antibacterial potential in combination with silver nanoparticles**
MARTA KRYCHOWIAK
Intercollegiate Faculty of Biotechnology UG&MUG, Gdansk, PL

15:30 - 15:45 Plenary Lecture: **The use of microalgae for the production of active compounds**
MARCELINA JAROS
Svanvid, Gdańsk, PL

15:45 - 16:00 Closing Ceremony

KEYNOTE LECTURE

Exploring natural variation in Arabidopsis

Maarten Koornneef

Max Planck Institute for Plant Breeding Research, Carl von Linné Weg 10, 50829, Cologne, Germany

The genetic variation of Arabidopsis in nature provides a useful resource for the functional analysis of genes. Due to it wide distribution much variation is found, which is expected to reflect adaptation to specific environments as well as neutral variation. The genetic complexity of this type of variation requires Quantitative Trait Loci (QTL) analysis using association mapping panels, as well as in biparental mapping populations. To the latter so-called multiparent populations including the MAGIC and AMPRIL populations have been added recently. Epistasis is a complicating factor reducing the power of genetic analysis further and can lead to novel phenotypes in segregating populations derived from intercrossing Arabidopsis accessions. In addition to the analysis of gene function, natural variation studies may reveal signatures of selection in nature that may explain local adaptation. To demonstrate the power of natural variation, examples on the analysis of plant architecture (plant length and branching patterns) will be presented. For plant length we found ample functional variation for the GA 20 oxidase (*GA5*) gene of which gene mutants have been exploited to generate modern short straw varieties in barley and rice. For branching an example on how to analyse such variation up to the gene level in Arabidopsis is the finding of the *AGL6* gene to be involved in this process. The complexity of the genetic variation in nature and its consequences for evolution is also shown by genetic incompatibilities that arise in certain combinations of genotypes. An example of this is the incompatibility between many Asian accessions and genotypes from Gorzow, Poland, in which a specific cluster of resistance genes at the *RPP1* locus is causing it.

SESSION 1: NATURAL VARIATION AND PHENOMICS

Plenary Lectures

The versatile use of genome elimination in developing novel genetic mapping resources in Arabidopsis

Joost J.B. Keurentjes, Cris L. Wijnen, Ramon Botet Vaca and Erik Wijnker

Laboratory of Genetics, Wageningen University, The Netherlands

The genetic analysis of quantitative traits has always depended on the availability of proper genetic resources, such as experimental mapping populations. In *Arabidopsis thaliana*, the reference species for many plant related traits, such populations have for long been generated by classical crossing schemes. Well known are linkage mapping approaches in biparental F2 populations or more sophisticated resources, like backcross, near isogenic line (NIL) or recombinant inbred line (RIL) populations. Each of these populations suffer from their own drawbacks such as long developing times, large population sizes or low statistical detection power.

Recently, however, the detection of a centromere mediated genome elimination mutant line in Arabidopsis (CenH3) opened up the way for a whole new suite of mapping resources. Many of the disadvantages of currently used mapping resources can be alleviated by novel designs using the genome elimination mutant. For instance, double haploid formation was thus far not possible in Arabidopsis but can be accomplished within three generations using the CenH3 mutant line, greatly reducing the time and efforts needed to create a comparable RIL population. Another application results in the generation of chromosome substitution strains (CSSs), which encompasses large advantages in terms of detection power, population size and statistical analysis of genetic architecture.

These and other population types generated by the use of the CenH3 mutant line will be discussed in light of their development and performance.

The genotype-phenotype map in *Arabidopsis thaliana*

Arthur Korte

Gregor Mendel Institute

Genome-wide association mapping has become a standard tool to elucidate genotype-phenotype relationships in many different species. The idea of using natural variation to pinpoint and identify alleles that have been selected by nature is tempting and many significant associations for a plethora of different traits have been already identified. However the approach is heavily dependent on the set of different natural accessions used for the analysis. We use *Arabidopsis thaliana* as a model organism to further explore this finding. Flowering time is an important life history trait in *Arabidopsis thaliana* which is prone to local adaptation. As a consequence different association can be found in the analysis of different local subsets. Here we try to understand and explain these differences. Furthermore, we try to answer the general question, if different phenotypes in different population are mainly due to allele frequency changes of causative alleles in the population, or if the actual effect of the respective Allele is different. The latter arguing for epistatic interactions, where the effect of a given Allele is dependent on the genetic background. We developed a framework to detect these interactions in a GWAS setting and aim to clarify and better understand their nature.

High-throughput phenotyping to decode the complexity of growth response to the environment in Arabidopsis

Olivier Loudet

INRA, UMR1318, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France
The VAST Lab : www.inra.fr/vast

Following a long history of quantitative genetics in crop plants, it is now relatively popular as well to use naturally-occuring variation contained in *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map QTLs and try and resolve them at the gene level. Apart from being able to exploit –in multiple genetic backgrounds– allelic variation that cannot be easily retrieved from classical mutagenesis, the success of the QTL studies has often been because of the use of quantitative phenotyping, as opposed to the qualitative scales often used in typical mutant screens. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the abiotic environment (especially drought stress). We are using a combination of our unique high-troughput phenotyping robot (the Phenoscope), RNA-seq, fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale thanks to the Phenoscope should allow to resolve enough quantitative loci to start drawing a more general picture as to how and where in the pathways adaptation is shaping natural variation. I will present recent results obtained when trying to decipher the genetic architecture of growth response to the environment, to illustrate our strategies and research.

Species migration in plants is associated to quantitative genetic changes in the circadian clock

Seth Davis

University of York

The circadian clock provides adaptive benefits in part by coordinating a photoperiodic timer that drives growth and acts as a determinant for flowering. We recently explored natural allelic diversity of the clock in the dicot Arabidopsis and found a "memory" of the proceeding environment. Furthermore, we showed that clock variation has a large role in directing seasonal survival under field conditions. Cloning of one circadian quantitative trait locus revealed variation at a core-clock gene, and we showed the cellular basis of how a QTL-effect can occur to promote growth and flowering. This has found relevance in crops as the same genetic pathway has been co-opted for species migration in barley. This is used by breeders in crop improvement. Together it is clear that circadian-gene variation contributes to oscillator control of life history.

Selected Talks

T.1.1. Brassinosteroid metabolism and its disturbances as a source of semi-dwarf barley mutants

Gruszka D., Dockter C., Hansson M.

[1]*Department of Genetics, Faculty of Biology, University of Silesia, 40-032 Katowice, Jagiellonska 28, Poland*
[2]*Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-1799 Copenhagen V, Denmark*
[3]*Department of Biology, Lund University, Sölvegatan 35, SE-22362 Lund, Sweden*

Keywords: barley, brassinosteroids, genetics, mutant, semi-dwarf

Brassinosteroids (BRs) are polyhydroxylated plant steroid hormones extracted for the first time from pollen grains of *Brassica napus* in the late 1970's. BRs regulate a wide range of physiological processes during the plant life cycle - from seed development to the modulation of flowering and senescence. Extensive genetic and biochemical research conducted over the last two decades, mainly in *Arabidopsis thaliana*, however these aspects have been studied to much lesser extent in crop plant, including cereals. Defects in BR metabolism often result in plant height reduction. Semi-dwarfism of cereal crops has long been recognized as indispensable trait in breeding and is still of great importance for agriculture. Reduced plant height and culm robustness are quantitative characteristics important for assuring cereal crop yield and quality under adverse weather conditions, as they improve lodging resistance. In our studies, a set of phenotypic traits specific for deficiencies in BR biosynthesis and signaling in semi-dwarf mutants of barley (*Hordeum vulgare L.*) was established. The unique combination of BR-mutant characters was used to perform fast and efficient screening of near-isogenic, short-culm barley mutant lines. Applying various approaches led to identification and functional analysis of BR-related genes and assigned more than 20 mutants to three BR-biosynthesis genes (*HvBRD*, *HvCPD*, *HvDIM*) and one BR-signaling gene (*HvBRI1*). Additionally, alternatives to the widely used, but highly temperature-sensitive uzu1.a allele of *HvBRI1* have been identified.

T.1.2. The distribution of coumarins and furanocoumarins in Citrus species closely matches Citrus phylogeny and reflects the organization of biosynthetic pathways

Dugrand A., Olry A., Hehn A., Froelicher Y. and Bourgaud F.

[1]*Université de Lorraine, UMR 1121 Laboratoire Agronomie et Environnement Nancy-Colmar, 2 avenue de la forêt de Haye, TSA 40602, 54518 Vandœuvre-lès-Nancy, France*
[2]*INRA, UMR 1121 *Université de Lorraine, UMR 1121 Laboratoire Agronomie et Environnement Nancy-Colmar, 2 avenue de la forêt de Haye, TSA 40602, 54518 Vandœuvre-lès-Nancy, France*
[3]*CIRAD, UMR AGAP, F-34398 Montpellier, France*

Keywords: Coumarin, Furanocoumarin, Citrus, Secondary Metabolism

In 2011, citrus crops represented one of the most important fruit productions in the world with more than 131 million tons. Citrus belong to the Rutaceae family able to produce coumarins which constitute a class of secondary metabolites commonly found in higher plants. In these plants, 7-hydroxycoumarin can undergo a subsequent two step enzymatic modification corresponding firstly to the prenylation at C6 and secondly to the closure of a furan ring, leading to furanocoumarins. In parallel to ecological functions in plants, these molecules can be deleterious for humans. They are potential photosensitizers that can cause photophytodermatitis either after skin contact or ingestion followed by UV A exposure. This photosensitization property is a notable problem with Citrus essential oils, such as bergamot oil, since they are extensively used in perfumes.

In this study, coumarin and furanocoumarin contents found in 61 citrus varieties are compared and the chemical diversity is discussed with respect to the genetic diversity. Based on hierarchical ascendant classification, coumarins and furanocoumarins can be separated into 4 groups. Each of these 4 groups is logically organized with respect to the already described plant biosynthetic pathways and allows drawing hypotheses on the doubtful biosynthetic origin of compounds. With reference to Citrus diversity obtained from molecular markers, we highlight the genetic crossings which may have resulted in low coumarin and furanocoumarin content varieties.

Citrus varieties identified in this study with low coumarin and furanocoumarin content will constitute invaluable genetic resources to breeding programs, promoting citrus species devoid of these toxic molecules.

T.1.3. Towards the genetic architecture of scopolin and scopoletin biosynthesis in *Arabidopsis thaliana*

Siwinska J.[1], Kadzinski L.[1], Banasiuk R.[1], Gwizdek-Wisniewska A.[1], Olry A.[2,3], Banecki B.[1], Lojkowska E.[1] and Ihnatowicz A.[1]

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[2]*Université de Lorraine, UMR 1121 Laboratoire Agronomie et Environnement Nancy-Colmar, 2 avenue de la forêt de Haye, Vandeuvre-lès-Nancy 54505, France*
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Keywords: natural variation, coumarins, Arabidopsis

Scopoletin and its glucoside scopolin belong to the group of natural plant products – coumarins, which have antimicrobial and antioxidative activities. Coumarins are used in pharmaceutical and cosmetics industry for their wide range of biological properties. Up to now, the research on coumarins was performed on the medicinal plants and only on the reference genotype for Arabidopsis - accession Columbia-0 (Col-0). In recent years, coumarin compounds like scopoletin, scopolin, skimmine and esculin were identified in Arabidopsis roots and shoots. Even though, most biochemical steps involved in coumarins biosynthesis in Arabidopsis are known, its' regulation is not understood. Our study aimed at elucidating the natural variation in scopoletin and scopolin accumulation between different Arabidopsis accessions and subsequently identifying QTL regions underlying the observed variation. In this study, for the first time a presence of naturally occurring intraspecies variation in scopoletin and scopolin accumulation was detected in Arabidopsis. On the basis of these results, the mapping population derived from the cross between Col-0 and Estland (Est-1) accessions was chosen for QTL mapping study. We identified five QTLs for scopoletin and one QTL for scopolin accumulation. The obtained models explain 37.60 % and 13.86 % of the observed phenotypic variation, respectively. On the basis of *in silico* analysis, we identified in the mapped genomic intervals known and novel loci potentially involved in coumarins biosynthesis. The selected candidate genes are currently being further characterized. We proved that Arabidopsis is a good model plant for studying the genetic basis of natural variation in coumarins biosynthesis.

T.1.4. ROS production gand scavenging in stress resistant *Eutrema salsugineum* plants

Pilarska M.[1], Wiciarz M.[2], Jajic I.[2], Niewiadomska E.[1]

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Keywords: Eutrema salsugineum, chloroplasts, antioxidants, ROS, salinity

Recently, we demonstrated that thylakoids isolated from *Eutrema salsugineum*, a highly stress resistant relative of *Arabidopsis thaliana*, are capable for the enhanced production of H2O2 from PQ pool (Wiciarz et al. 2015). This points to the 'stress preparedness' of Eutrema, which might be achieved by up-regulation of the antioxidant system already in the absence of stress. To verify that, we investigated the extent of oxidative damage and components of the antioxidant system in leaves of Arabidopsis and Eutrema. A lower level of oxidative damage was detected in Eutrema concomitantly with a higher concentration of H2O2, comparing to Arabidopsis. Among the antioxidant enzymes (SOD, CAT, APX, GPX) only the activity of GPX was significantly enhanced in Eutrema and was not influenced by salinity. In contrast ascorbic acid and glutathione underwent a salinity-dependent increase only in *E.s.*. Differences in the antioxidant system between these species were related to chlo roplasts. Thylakoids of *E.s*. were equipped with a membrane-bound FeSOD and APX forms, whilst these forms were absent in *A.t.*. In agreement with that, by EPR analysis made with illuminated thylakoids we documented a lower generation of superoxide in Eutrema. Obtained results support the view of the chloroplastic origin of high H2O2 level in Eutrema, and suggests that anticipation of stress might be related to: a better antioxidant protection of photosynthetic membranes, a lower ratio of superoxide to hydrogen peroxide, and stimulation of GPX activity.

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Wiciarz M. et. al. (2015) Physiol Plant 153: 467-476

Acknowledgements:
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Poster Presentations

P.1.1. Anatomical traits correlated with needle longevity in Scots pine (*Pinus sylvestris L.)* from Scandinavia

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Keywords: Scots pine, needle anatomy

Scots pine is a species with a wide latitudinal distribution. In Europe it ranges from northern Scandinavia to Spain and the Balkans. In the Scandinavian part of its range the decreasing mean air temperature is correlated with an extended needle life-span. For example in mountains of northern Scandinavia needles are retained for up to 11 growing seasons. The aim of this study was to identify anatomical modifications enabling pine needles to achieve high longevities in the harsh climate of Northern Scandinavia.

Microscopic studies were conducted on 1-year-old needles collected from 15 sites located in Sweden between the latitudes of N 56º and N 68º. Thirty-two potentially functional micrometric traits were analyzed.

Long-living needles from the northern parts of Scandinavia were shorter, wider and thicker then short-living needles from the south. The increase in thickness of the needle resulted from greater mesophyll thickness on its abaxial side and from a thicker vascular cylinder. The thickness of xylem and phloem layers in the studied populations was, however, relatively uniform. Needle longevity was positively correlated with the thickness of epidermal cells and with the thickness of their secondary walls, indicating a higher mechanical resistance of the epidermis in long-living needles. Longevity was also associated with a larger needle resin duct diameter while populations did not differ in resin duct number. Long-living needles thus appear to possess enhanced adaptations to cope with both mechanical and biotic stresses.

Asknowledgements:
The project is funded by the National Science Centre grant 2011/02/A/NZ9/00108 to J. Oleksyn

P.1.2. Internal transporting pathways in the early land plants

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Keywords: bryophytes, lycophytes, ferns, internal transport

The plants, which were first to colonize the land, had to develop a set of structural and physiological adaptations to deal with demands of the new environment. The formation of efficient transporting pathways was crucial for the supply of the whole organism in water and nutrients. However, the transporting routes in early land plants, represented by mosses, lycophytes and ferns, are insufficiently characterized. Therefore, we focused on the transporting pathways, their regulation and development to decipher the specialization of conducting systems in these plant groups, depending on the dominant life form, gametophyte vs. sporophyte.

With the use of histological techniques, coupled with the dye-loading experiments, we demonstrated the presence of the internal transport, as well as the structural and functional adaptations of specialized conducting cells, corresponding to the symplasmic and apoplasmic transporting routes in the species examined.

Our results clearly demonstrate that the internal transport is the crucial mechanism that orchestrates the plant growth and development. Importantly, the fundamental rules of the transport characteristics are conserved in examined groups of early land plants suggesting the universality of the internal communication systems in the plant kingdom.

P.1.3. Predicting the phenotype in *Arabidopsis thaliana* from SNPs

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Keywords: arabidopsis, phenotype, SNPs, variation

A computational pipeline combining an array of bioinformatics tools allows predicting the phenotypes from SNPs in *Arabidopsis thaliana*. The steps include:
1. Functional annotation of genomic regions.
2. Assessing the magnitude of the SNP's effects.
3. Determining the affected phenotypes based on a background population.

In order to further develop and verify the method I am looking for collaborators who are willing to share phenotypic data collected from multiple accessions of *Arabidopsis thaliana* ecotypes. Additionally, new datasets may allow establishing a mapping from phenotypic effects to biological or molecular function pathways.

P.1.4. *Verbena officinalis* - a new model plant for phyllotaxis research

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Keywords: phyllotaxis, apical meristem, inflorescence,indetermined growth

In *Verbena officinalis*, during the transition from vegetative to generative phase of shoot growth, the identity and distribution of lateral organ primordia change. Phyllotaxis of the leaves proper is decussate whereas that of bracts is spiral. In a second phase of development, distinguished by an indetermined growth of the inflorescence rachis, the distribution pattern of bracts changes further, even though their identity does not. Serial ontogenetic transformations of bract phyllotaxis involve different spiral patterns such as the main Fibonacci, Lucas or bijugy but suprisingly also whorled tricussate or even tetracussate patterns. Measuring the lateral organ primordia sizes relative to the size of the apical meristem revealed that the primordia of bracts are significantly smaller than the primordia of the leaf proper. The experiments *in silico* showed that this itself cannot be held responsible for all observed phyllotactic transitions. To obtain them in computer simulation we had to apply the parameter of tolerance. Worked out protocol of easy propagation in the laboratory, both from seeds and from cuttings, added to such virtues shared with *Arabidopsis thaliana* as a small size and proliferous seed production, make *Verbena* altogether an attractive model for studying experimentally the activity of shoot apical meristem and the mechanisms of plant organogenesis.

P.1.5. Yellow lupin transcriptome sequencing towards identification of genes associated with resistance to *Fusarium* sp.

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Keywords: yellow lupin, transcriptome sequencing, fusariose

Yellow lupin (*Lupinus luteus L.*) is one of the three lupin crop species. Lupins farming gained much attention recently, due to the fact that lupin seeds are a valuable protein source. On the other hand a serious threat in yellow lupin cropping are fungal diseases e.g. fusariose and anthracnose, which may result in the total destruction of plantation. Up until now, only one gene *Fus1* underlying resistance to *Fusarium* sp. has been identified and used in the yellow lupin breeding programs, however its molecular function has not been yet determined.

The main goal of this study was the transcriptome sequencing of the four different yellow lupin genotypes: two resistant lines (94% and 90-100% plants survival rate respectively) and two susceptible lines (72% and 46% plants survival rate respectively), in order to identify differentially expressed genes potentially determining resistance to *Fusarium* sp.

Sixteen RNA-seq libraries were sequenced using the Illumina NGS platform (2x75 bp PE, HiSeq 1500). A minimum of 73 million high quality sequence reads were obtained for each of biological replicates. The short reads have been *de novo* assembled into the reference transcriptome and used later as a reference in the analysis of differentially expressed genes in the group of resistant versus susceptible genotypes to *Fusarium* sp. qPCR analyses will be further conducted in the wider group of yellow lupin lines to confirm differential expression of selected genes assumed to be involved in the resistance against this fungal disease.

SESSION 2: SYSTEM AND SYNTHETIC BIOLOGY

Plenary Lectures

Gene stacking and metabolic engineering in plants

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Engineering plants with complex metabolic pathways or multiple traits is often inhibited by the number of genes that are required to reach the final product. It also built the need of synthetic biology tools to express multiple genes with controllable expression strengths and in specific tissues. This presentation will highlight our recent progress in synthetic biology to support plant engineering. It will include *in vitro* and *in vivo* DNA assembly methods to stack multiple gene cassettes and novel devises to fine-tune gene expression in plants. During this presentation, you will hear also about some of the successes in tissues specific engineering approaches that were developed to manipulate plant cell wall recalcitrance and increase sugar quantity and quality without compromising plant biomass yield. We believe the development of these tools and approaches have the potential to support scientists and engineers who are looking at stacking multiple genes to entire regions of chromosomes and interested in manipulating endogenous metabolic pathway.

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Genome evolution and sociomicrobiology in the alfalfa nitrogen fixing microsymbiont *Sinorhizobium meliloti*

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Keywords: Bacterial genomics, Systems biology, Sinorhizobium meliloti, Symbiosis, Competition

Legume-rhizobia symbiosis represent models of cross-kingdom mutualism. The nitrogen fixing bacterium *Sinorhizobium meliloti* is the specialized rhizobial symbiont of the leguminous plant alfalfa (*Medicago sativa L.*), one of the most important crops commonly used as a forage or in rotation practices to contribute organic nitrogen to the soil. *S. meliloti* strains have multipartite genomes consisting of one chromosome and other large-sized replicons, such as chromids, megaplasmids and plasmids. In particular, most of the genes involved in symbiotic interaction are resident on a megaplasmid, called *pSymA*. Previous works have highlighted that *pSymA* is a hotspot for genome variability and that natural strains with genome differences on this megaplasmid, show also differences in the mutualistic interaction with the plant and, consequently, may have a role in biotechnologically relevant phentoypes, such as plant growth and nitrogen fixation.

In the last years we have investigated genome and phenotypic variability of *S. meliloti* strains, trying to reconstruct the pattern of variability of cellular networks devoted to symbiotic interaction and mutualism. We depicted a scenario where several genes present in the core and in the dispensable genome fraction of *S. meliloti*, but also regulatory elements may play a role in symbiotic efficiency and in mutualistic interaction. Evidences for genes related to cooperative and competitive interaction between strains were also collected, suggesting the presence of cheating and antagonism during formation of symbiotic structure in plant roots. Identified genetic determinants could be used for future “cis-genic” manipulation of *S. meliloti* strains for improving symbiotic efficiency.

Designing new flax plants by metabolic engineering

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Keywords: metabolic engeenering, flax

Flax (*Linum usitatissimum*) is a dual purpose crop plant with a great market potential. Oil and fibres are the main flax products and their quality improvement by metabolic engineering is the goal of our long-term studies. In the first instance selected genes involved in regulation of the main routes of secondary metabolite pathways have been identified. The key genes were then used for new plant generation. The new plant types were obtained by transforming flax with single and multi-genes constructs that results in genes overproduction or silencing and thus diversify compounds component of raw plant product. The manipulation designed for novel compounds biosynthesis was also exploited.

In several cases the goal was accomplished by overexpressing key gene (-s) and thus increasing the production of desirable component and also by re-directing metabolites by repressing one of the branches of metabolic pathways. For example manipulation of flavonoid genes expression affected lignin synthesis and cell wall arrangement. Squalene accumulation was increased by silencing carotene synthesis. The example of novel components is a production of a polyhydroxybutyrate (PHB). This was accomplished by simultaneous expression of three bacterial genes (βketothiolase, acetoacetyl-CoA reductase, PHB synthase). The PHB is co- synthesized with cellulose during fibre development resulting in a new quality of a flax fibre. Thus the gene identification, expression analysis in wide genes context and expression kinetics is all helpful for development of synthetic biology and might be exploited for new plant and thus new products generation.

Selected Talks

T.2.1. Global control of eukaryotic gene expression depends on chromatin remodeling by SWI/SNF complexes

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Keywords: SWI/SNF, Arabidopsis, human, metabolome, cancer

ATP-dependent chromatin remodeling complexes (CRCs) regulate the structure, activity and organization of chromatin. Different types of CRCs are defined by the type of central SNF2-type ATPase and unique composition of other subunits, including auxiliary proteins. The SWI/SNF class of CRCs, originally described in *Saccharomyces cerevisiae*, is conserved from fungi to mammals and plants. The SNF2 ATPase is associated with a small set of highly conserved „core" subunits including homologues of yeast SWI3 and SNF5-type proteins, which have full SWI/SNF remodeling activity *in vitro*. In multicellular eukaryotes, the lack or aberrant stoichiometry of individual core subunits causes embryolethality or severe defects in development, and in animals also leads to carcinogenesis. The studies of human CRCs represent an important area of cancer research. Depending on subunit composition, distinct mammalian SWI/SNF complexes can act as either activators or repressors of transcription and serve as the interface for integration of various processes. Genetic and molecular analyses confirm that different classes of plant SWI/SNF complexes are involved in regulation of specific processes.

Here we show by comparative functional analysis of plant and human SWI/SNF CRCs their conserved involvement in control of evolutionary most ancient signaling and regulatory processes, including those involved in regulation of metabolic status and energy homeostasis. Our findings suggest that impairment of SWI/SNF CRCs activity may be one of the main reasons of metabolic switch observed in some types of cancer. Our study justify using *Arabidopsis thaliana* as attractive model for investigation of molecular mechanisms controlling highly conserved processes in Eukaryotes.

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T.2.2. EcoFactory, a multipromoter explorer expression system for *Escherichia coli*

Zielińska J., Mokrzycka D., Lejman A., Osuch M., Szymańska M., Olejniczak O., Rabiasz A., Kopa M., Abramowski S., Bartoszewicz J., Nowicka M., Rżosińska K. and Nuc P.

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Keywords: expression, cloning, inducer, E.coli, GFP

Minimal, independently regulated promoters of different expression levels are important tools for synthetic biology to build complex but compact genetic systems. It is important for crystallographers to produce recombinant proteins of high purity and for pharmaceutical industry to make the whole process free of toxic compounds. Ideally the expression level should be tuneable, induction - non-toxic, mRNA translation – free of mistakes, with perfect folding, recombinant protein – soluble and homogenous, and finally its purification - fast and reproducible. We prepared a series of plasmids expressing his-tagged superfolder GFP (Pedelacq J-D. 2006) under the control of four nontoxic inducers: arabinose, melibiose, rhamnose and xylose. The four promoters are more controllable than the most popular lactose induced T7RNA polymerase dependent systems. The coding sequences are transcribed by the cellular RNA polymerase which is slower and more accurate than T7 RN A polymerase. SfGFP can be used to compare activities of standard, modified, and minimal promoters during growth on different carbon sources and as well as a probe of a fused protein solubility. We present a comprehensive comparison of the sfGFP expression driven by inducible promoters with the insulated, constitutive promoters proC and proD as standards (Davis 2009). Two pairs of primers should be sufficient to clone an ORF of interest as a protein fused to sfGFP or to replace it in all vectors of the multipromoter system.

T.2.3. Chloramphenicol acetyltransferase - new selectable marker for stable nuclear transformation of the red alga *Cyanidioschyzon merolae*

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Keywords: Cyanidioschyzon merolae; chloramphenicol acetyltransferase; transformation

Cyanidioschyzon merolae is an extremophile, unicellular red alga that thrives in acidic and moderately hot environments. It reveals the simplest cellular structure among eukaryotes, consisting of a minimum set of organelles, i.e. single nucleus, mitochondrion and plastid. This is why, the alga serves as a model organism for investigating a basic architecture of photosynthetic eukaryotes.

Several genetic engineering methods has been developed to transform *C. merolae* and obtain transient expression of heterologous genes, and only two examples of its stable transformation has been described so far. They based on a complementation of uridine 5'-monophosphate synthase deficiency in *C. merolae* spontaneous mutant by the wild type URA5.3 (*CMK046C*) gene. The aim of our investigation was to find a selectable marker that could be applied to perform stable transformation of wild type strain of *C. merolae*.

We have developed the system in which the product of the chloramphenicol acetyltransferase (cat) gene is used as a selectable marker in a stable nuclear transformation of *C. merolae*. As chloramphenicol arrests protein chain elongation by inhibiting the peptidyl transferase activity of bacterial ribosome 50S subunit, it is also toxic towards the alga due to a high enough similarity between bacterial and chloroplast ribosome 50S subunit. Thus, our system appears to be particularly useful for stable transformation of chloroplast.

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This investigation was financed by the grant Opus 5 (DEC-2013/09/B/NZ1/00187) from the Polish National Science Centre

Poster Presentations

P.2.1. *Agrobacterium*-mediated transient production of recombinant proteins in leaves and phloem exudates of luffa plants (*Luffa cylindrica L.*)

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Keywords: Cucurbitaceae, Luffa , agroinfiltration, phloem

Several physiological processes in plants, in particular relating to long-distance phloem transport, were previously studied with use of model plants belonging to *Cucurbitaceae* family. Despite this, there is lack of the well-optimized, highly efficient methods for genetic transformation of cucurbit species. The methods for stable transformation described in the scientific literature are time-consuming and complicated, wherein less effective. In this study we investigated the susceptibility of selected cucurbit species to agro-infiltration. The agro-infiltration is one of the simplest and most effective methods to achieve a transgene overexpression in such plant species as *Nicotiana benthamiana*, *Nicotiana tabacum*, *Arabidopsis thaliana*, tomato (*Lycopersicon esculentum* Mill.), lettuce (*Lactuca L.*) and potato (*Solanum tuberosum L.*). The technique involves the pressing of appropriately prepared suspension of genetically modified *Agrobacterium* into the intercellular space of plant leaves. The transgen activity in agro-infiltrated tissues can be detected within a few hours after infection.

In our work, we investigated the susceptibility of several cucurbit species to agro-infiltration. The results of our study indicate that leaves of luffa plants (*Luffa cylindrica*) can be agro-infiltrated and have the potential for the transient overproduction of recombinant proteins. Moreover, we observed that the bacterial β-glucuronidase (GUS) expressed locally in luffa leaves was transported in phloem tissue, and could be obtained dissolved in a so-called phloem exudates, directly from pre-cut petioles. We have shown that the expression of GUS protein is detectable in phloem exudates up to 10 days after agro-infiltration.

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P.2.2. Analysis of protein monolayers formed on the carbon surfaces by means of Langmuir- Blodgett and electro-deposition method

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Keywords: monolayers, electro-deposition, Langmuir – Blodgett

Monolayers are an extremely interesting subject of study. They are composed of a small number of molecules, often with precisely known properties. This fact allows for accurate monitoring their behavior, especially on the solid surfaces.

The most used technique of monolayers building is Langmuir – Blodgett method. In this approach, monolayers are formed on the water / air interphase by means of the Langmuir bath [Dynarowicz-Łątka et al., 2001]. Competitive method is a electro-deposition technique. It consists in applying a sufficient voltage to enable the migration of particles in accordance with the load, and thus adsorption on the surface of particles of opposite charge [Zoski, 2007].

The object of the presented research are monolayer formed from phycobilisomes and cytochrome c formed on the carbon surface. Monolayers are analyzed by Fourier Transform Infrared Spectroscopy (FTIR) and confocal microscopy [Stuart, 1996]. The distribution and surface coverage is determined using Atomic Force Microscopy (AFM). Redox properties of monolayers are then measured by cyclic voltamperometry. The possibility of further application of such monolayers in biosensors construction is discussed.

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P.2.3. GLP- Good Laboratory Practice- How it works

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Keywords: non-clinical, safety studies, health, environmental

Chemicals play a crucial role in many areas of human development, beginning with medicines and medical devices which prolong the life up to the application of pesticides or fertilizers, which contribute to improving the agricultural crop yields. The chemicals we use in order to make life easier for people. As time went it turned out that in addition to the expected positive action appeared a number of side effects.

Chemicals control legislation in OECD Member countries is founded in a proactive philosophy of preventing risk by testing and assessing chemicals to determine their potential hazards. The requirement that evaluations of chemicals be based on safety test data of sufficient quality, rigour and reproducibility is a basic principle in this legislation. The Principles of Good Laboratory Practice (GLP) have been developed to promote the quality and validity of test data used for determining the safety of chemicals and chemicals products. It is a managerial concept covering the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.

P.2.4. Imidazole-free purification of His3-tagged recombinant proteins using ssDNA aptamer-based affinity chromatography

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Keywords: aptamer, SELEX, protein purification

Availability of highly purified recombinant proteins is one of the factors inhibiting the development of an area of modern science. These proteins are used in a wide range of applications, including signal transduction and protein-protein interaction studies. They are also used as advanced tools in molecular biology. For this reason, research centers and biotechnological companies focus their efforts on the development of fast, efficient and simple methods of protein purification. Nowadays, immobilized metal affinity chromatography (IMAC) is often used for the preparation of recombinant protein samples. This method is based on the interaction between a protein containing a histidine tag (His-Tag) and immobilized metal ions e.g. Ni2+. For the purpose of chromatography, DNA aptamers, short single-stranded DNA molecules which can bind targets with high affinity and specificity, have recently been employed. The various immobilization methods, simple synthesis and the low production cost of DNA aptamers mean that they are promising ligands for affinity chromatography systems. In our studies we developed a method which can be used as an alternative to the IMAC technique. It is based on a DNA aptamer which can specifically bind a histidine tag composed of three histidines (His3-Tag). This system offers two major advantages. Firstly, it can provide higher His3-tagged protein purity when compared to standard Ni-NTA resin. Secondly it makes possible the sodium ion dependent elution of His3-tag from the ssDNA aptamer, thereby making the process imidazole-free.

P.2.5. On the way of synthetic biology of the furanocoumarin pathway

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Keywords: furanocoumarin, secondary metabolism

Furanocoumarins are compounds commonly highlighted in 4 higher plant families: Rutaceae, Fabaceae, Moraceae, and Apiaceae. These molecules are allelochemicals involved in plant defense mechanisms against insects and phytopathogens. As many secondary metabolites, furanocoumarins are also of particular interest for therapeutic applications. Due to their physicochemical properties some of them are used for the treatment of skin diseases (bergapten) or cancers. The production of such molecule in high amounts might therefore be of particular interest.

These compounds are synthesized through a complex cascade of enzymes involving cytochrome P450s, prenyltransferases, 2-oxoglutarate dependent dioxygenases and methyltransferases. Since 2007, most genes and enzymes involved in their biosynthesis have been described by our laboratory especially for the Apiaceae family. For this purpose we used several molecular biology strategies (published and non-published results).

To develop an efficient furanocoumarin production system, we will overexpress the genes involved in the synthesis of these molecules in heterologous plant systems or in microorganisms. To achieve this goal all the genes identified so far will be cloned into a single plasmid using the newly described Goldenbraid technology, based on type IIS restriction enzymes.

This challenging project will need to take into account the diversity of the enzymes involved in the biosynthetic pathway. Some enzymes were described to be cytosolic whereas others are known to be membrane bound. Some enzymes were highlighted in the cytoplasm whereas others are targeted to the endoplasmic reticulum or to chloroplasts. The expression level and the subcellular localization of each enzyme will also need to be optimized.

P.2.6. Purification of ELPylated protein from *N. tabacum* cv.BY-2 cell suspension culture

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Keywords: Elastin like polypeptides, ITC

At present, plants are an efficient and cost effective system for recombinant protein production. However, in many cases high costs of its extraction and purification is a major limiting factor for large scale production. One of the very promising solutions of this problem could be application of elastin-like polypeptide tag (ELP) for the nonchromatographic recombinant protein purification method. ELP are biopolymers containing repeats of Val-Pro-Gly-Xaa-Gly (VPGXG) pentapeptide, in which Xaa (the guest residue) can be any amino acid except proline. These polypeptides have a distinctive and very useful feature, which is the ability to transition from a soluble to insoluble form in response to changing environmental conditions (Inverse Transition Cycling). In this work we present the successful expression of ELPylated GUSPlus protein and procedure of its simple purification from transgenic plant cell suspension culture of *N. tabacum* cv. BY-2 obtained by an *A. tumefaciens* mediated transformation.

P.2.7. The abilities of potato pulp peroxidases to detoxify synthetic 2,4-dichlorophenol solutions

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Nicolaus Copernicus University

Keywrods: potato pulp, peroxidases, bioremediation, 2-4-dichlorophenol

Phenolic compounds are classified as priority pollutants of industrial effluents. They have adverse effects on human health, including but not limited to carcinogenesis and mutagenesis. It is therefore of great importance to find an effective method for their decontamination. Enzymatic bioremediation was proposed as an alternative to conventional methods. A new plant source of enzymes for this purpose is potato pulp - a waste product of starch industry. After starch extraction, an enzyme preparation immobilized on cell walls remains. In order to determine its usability in toxic pollutants decontamination, a series of experiments with 2,4-dichlorophenol was conducted. The optimal sample weight of potato pulp and hydrogen peroxide demand were established along with the pollutant concentration range from 1 to 6 mM. To determine the conditions for efficient 2,4-dichlorophenol detoxification , temperature and pH effects were tested. To verify, whether adding stabilizing a gents to the reaction medium would enhance the reaction efficiency, when high pollutant concentration were tested, the reaction mixtures were supplemented with polythelene glycol. To assess the toxicity of post-reaction solutions, two toxicity tests were performed: the phytotest on Lepidium sativum seeds and the bacterial test on *Escherichia coli* cultures.

P.2.8. Towards the simple and uncompromised assembly of genetic systems

Zielińska J., Mokrzycka D., Lejman A., Szymańska M., Osuch M., Olejniczak O., Rabiasz A., Kopa M., Abramowski S. and Nuc P.

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Keywords: method, CPEC, nebuilder, protein purification

An underlying necessity of synthetic biology is the ease, simplicity and possible automation of assembly of genetic systems. In 2009 J. Quan and J. Tian have developed the method for an efficient construction of combinatorial libraries (CPEC: circular polymerase extension cloning). At the same time D. Gibson and his co-workers from Craig Venter Institute have described another restriction site independent method of cloning and *de novo* assembly of large genomes from overlapping DNA fragments with a mixture of three enzymes: T5 exonuclease, Phusion DNA polymerase and Taq DNA ligase. The cloning system as well as the nebuilder software for design of the overlaps are available from New England Biolabs. Although Gibson Assembly is the method of choice in construction of larger genetic assemblies, we have found that CPEC can be successfully used for the assembly of smaller genetic systems composed of up to 5 DNA fragments with overlaps constructed with the nebuilder software. This cloning system is more flexible and faster than building more complex constructs from biobricks (2011 Shetty), more precise, more efficient, and cheaper than Gibson Assemby. Construct assembly can be completed in 2 -3 days by two rounds of PCR and transformation of electrocompetent cells. From a standard cloning we usually obtain several hundreds of colonies of which almost all contain a construct of the expected assembly and sequence. The expression system is under development for fast 15-min. purification of soluble recombinant his-tagged proteins in *Escherichia coli*.

SESSION 3: PLANT DEVELOPMENT AND GENE REGULATION

Plenary Lectures

MicroRNA functions in Arabidopsis embryos

Michael Nodine

Gregor Mendel Institute

MicroRNAs (miRNAs) are a class of small regulatory RNAs that repress key developmental regulators and are essential for plant embryo development. Previously we found that miRNA-deficient embryos exhibit widespread differentiation defects and precociously express maturation-phase genes. This indicates that miRNAs are required for both embryonic pattern formation and the timing of the morphogenesis-to-maturation phase transition. Because plant miRNAs typically repress transcription factors and other key developmental regulators they likely have a large influence on the gene regulatory networks that control plant embryogenesis. Our major goal is to understand how miRNAs shape the gene regulatory networks that govern plant embryogenesis, and I will present our progress towards this aim.

BABY BOOM induces somatic embryogenesis in a dose- and context-dependent manner via the LAFL pathway

Kim Boutilier

Wageningen University and Research Centre, Wageningen, Netherland

Somatic embryogenesis (SE) is an example of cellular totipotency, where embryos develop from vegetative cells rather than from gamete fusion. The *AINTEGUMENTA-LIKE* (*AIL*) transcription factor family comprises eight genes, which redundantly regulate meristem identity and growth. Ectopic expression of the *AIL* genes *BABY BOOM* (*BBM*) or *PLETHORA5/AIL5*, is sufficient to induce SE in *Arabidopsis thaliana* seedlings, but the roles of the other *AIL* genes in this process, as well as the signalling pathways underlying *AIL*-mediated SE, are not known. Here, we show that overexpression of all *AIL* genes, except for the phylogenetically-distinct *AIL1* and *AINTEGUMENTA*, induces SE, suggesting extensive overlap in *AIL* function. Using *BBM* and *PLT2* as representatives of AIL function, we show that *AIL*-mediated SE is dose-dependent, where a relatively high dose induces SE and a relatively low dose induces shoot (*BBM*) or root (*PLT2*) organogenesis. *AIL*-induced SE is also context-dependent, as early expression of *BBM* or *PLT2* induces SE directly from seedling tissues, whereas late expression induces SE indirectly from callus. Analysis of *BBM* regulatory pathways shows that *BBM* binds to and regulates genes with roles in maintaining embryo identity and/or somatic embryo induction including the *LAFL* genes, *LEC1*, *LEC2*, *FUS3* and *ABI3*, as well as *AGL15*. Mutant analysis identified these genes as positive regulators of *BBM*-mediated SE. Our results demonstrate that *AIL* proteins regulate overlapping pathways in a context- and dose-dependent manner to modulate plant development and place *BBM* upstream of other known inducers of SE.

A question my lab has become interested in is – what do genomes really encode?

Gordon G. Simpson

College of Life Sciences, University of Dundee, Scotland, UK

We have been studying RNA binding proteins that play crucial roles in regulating Arabidopsis flower development and discovered that they control where mRNA 3' ends are formed (Cell, Developmental Cell). We have used Direct RNA Sequencing to quantify shifts in 3' end formation mediated by these RNA binding proteins transcriptome-wide, revealing RNAs previously unannotated in the Arabidopsis genome as well as consequences of disrupting regulated RNA processing (NSMB, PLoS Genetics).

More recently, in an attempt to understand the mechanisms involved, we have developed in vivo interaction proteomics to identify the proteins that these RNA binding proteins associate with in living cells. Unexpectedly, this has led us into the emerging field of mRNA methylation and modification that has been called the epitranscriptome. Remarkably, this entire layer of gene regulation has been largely overlooked.

We have purified a conserved set of interacting proteins required for mRNA methylation in Arabidopsis and human cells, and associated them with specific developmental defects.

We think that in the same way that Arabidopsis has proven useful in the study of DNA methylation and RNA silencing, the study of this wonderful model plant will provide widespread insight into the neglected impacts of the epitranscriptome. Understanding how mRNAs can be modified and alternatively processed is crucial to understanding what genomes really encode.

Functional specialization amongst canonical plant poly(A) polymerases – An overlooked layer in plant gene expression control?

Michael Lenhard

Institute for Biochemistry and Biology, University of Potsdam

The poly(A) tail at 3' ends of eukaryotic mRNAs promotes their nuclear export, stability and translational efficiency, and changes in its length can strongly impact gene expression. The *Arabidopsis thaliana* genome encodes three canonical nuclear poly(A) polymerases, *PAPS1*, *PAPS2* and *PAPS4*. As shown by their different mutant phenotypes, these three isoforms are functionally specialized, with *PAPS1* modifying organ growth and suppressing a constitutive immune response. However, the molecular basis of this specialization is largely unknown. We have estimated poly(A)-tail lengths on a transcriptome-wide scale in wild-type and *paps1* mutants. The poly(A)-tail changes in *paps1* mutants predicted two novel functions of *PAPS1* in the response to cold stress and redox homoeostasis that were both confirmed experimentally. When overlaying the *PAPS1*-dependent effects on poly(A) tail length with coexpression analysis based on microarray data, the two clusters of transcripts that are most closely coexpressed with *PAPS1* show the strongest change in poly(A)-tail length and transcript abundance in *paps1* mutants. This suggests that their coexpression reflects at least partly the preferential polyadenylation of these transcripts by *PAPS1* versus the other two poly(A)-polymerase isoforms.

In addition to its role in the sporophyte, *PAPS1* is also essential for male gametophyte development and growth. We are using a fluorescent marking system to characterize the growth of mutant pollen tubes and study the role of *PAPS1* in the male gametophyte in detail.

Selected Talks

T.3.1. The miRNAs are involve in the plant cell dedifferentiation

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Keywords: dedifferentiation, miRNA, protoplasts

The mechanisms of plant cell dedifferentiation and the acquisition of totipotency are poorly understood. One of the methods to induce the dedifferentiation process in plant cells is removal of the cell wall. The protoplasts in specific conditions are able to proliferation, next differentiation and regeneration of whole plant. Previously we shown that reduced transcription activity and the eradication of RNA transcripts from the cytoplasm at the beginning culture of protoplasts is a crucial process in obtaining totipotency (Dełeńko et al. 2015). One of the post-transcriptional gene regulation mechanisms involved in the control of the amount of mRNA is RNA interference. It was also shown participated of miRNAs in dedifferentiation and rededifferentiation of animal cells. Our analysis of the amount of D-bodies (domain of biogenesis miRNAs) and the viability and the rate of divisions of mutant cells *dcl1-9* indicate participation of miRNA in differentiation plant cell s. Next we have analysed microRNA transcriptome. The transition from differentiated mesophyll cells, through protoplast to proliferating cells is accompanied by expression changes in 94 known microRNA molecules. Preliminary analysis indicate that the nature of stress associated with the removal of the wall may differ from other abiotic stresses. However, during division we observed decrease miRNA-related stress and increase those related in proliferation and development plant cells.

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Dełeńko K, Niedojadło J. et al. Dedifferentiation of *Arabidopsis thaliana* cells is accompanied by a strong decrease in RNA polymerase II transcription activity and poly(A+) RNA and 25S rRNA eradication from the cytoplasm. Protoplasma (2015) 252:537–546

T.3.2. Auxin related miRNAs are involved in somatic embryogenesis induction in *Arabidopsis*

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Keywords: miRNA, somatic embryogenesis, Arabidopsis, auxin

MicroRNAs (miRNAs) have been implicated to regulate a wide range of developmental processes in plants, involving zygotic embryogenesis (ZE). Beside in vivo development, miRNAs are also believed to control morphogenic processes induced in vitro including somatic embryogenesis (SE), i.e. formation of somatic embryos in culture of differentiated somatic tissue. Considering that auxin treatment isused to induce SE in many plant species, the study was focused on elucidation of a role of auxin-related miRNAs in SE induction. Accordingly, miR160, miR167, miR393, components of auxin signaling pathway were studied in *Arabidopsis* explants undergoing embryogenic transition. Target genes of the studied miRNAs were also analysed including those encoding TIR1/AFB2 receptors of a TAAR clade and AUXIN RESPONS FACTORS (ARFs).

Results indicated significantly modulated level of mature miR160, miR167 and miR393 molecules to be associated with early stages of embryogenic culture. In support, the expression of MIR167 and MIR393 in tissue engaged in SE was indicated with GUS reporter lines. In addition, RT-qPCR profiling of the gene expression suggested that a regulatory relation between the studied miRNAs and their targets is associated with SE induction. Involvement of auxin-related miRNAs in SE induction was further confirmed by the reduced embryogenic capacity of insertional mutant-derived cultures. The study provides several pieces of evidence that miR160, miR167 and miR393 operate in SE induction in *Arabidopsis* to regulate auxin signaling pathway.

T.3.3. The role of phytochromes in storage material mobilization during light-induced tomato seed germination

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Keywords: tomato, phytochromes, germination, starch, lipids

Phytochromes, red and far-red light photoreceptors, are known to control the germination of many plant species. However, very little is known about light regulation of storage material metabolism during germination and post-germinative growth. Tomato (*Solanum lycopersicum*) seeds store mainly lipids and additionally starch. Although many aspects of tomato plant development have been thoroughly investigated, information about storage material metabolism in germinating seeds is scarce.

In this study we analyzed the mobilization of storage reserves in germinating tomato seeds and investigated the role of phytochromes in this process. To elucidate the roles of different phytochrome species, single and multiple *phyA*, *phyB1* and *phyB2* mutants were used apart from wild type. Imbibed seeds were briefly irradiated with far-red or far-red followed by red light, and germinated in darkness. Starch and triacylglycerols were quantified in seeds and seedlings during the first 5 days after sowing. To investigate the fat-carbohydrate transformation, the activity of the glyoxylate cycle was assayed.

Our results confirm the role of phytochromes in controlling tomato seed germination. Phytochromes A and B2 were shown to play specific roles, acting antagonistically in far-red light. Triacylglycerol breakdown proceeded independently of light and no significant differences could be seen between wild type and phytochrome mutants. However, phytochrome control was visible in the next stages of the lipid-carbohydrate transformation. The key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, were regulated by phytochromes. This regulation was also reflected in differential starch accumulation in seeds irradiated with far-red or far-red followed by red light.

T.3.4. Competition between Spliceosome, Microprocessor and Polyadenylation Machinery in the biogenesis of miRNAs located within the first intron of host genes in *Arabidopsis thaliana*

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Keywords: intronic miRNAs, microprocessor, splicing, polyadenylation

In plants, miRNAs are encoded mostly by independent transcription units, but in Arabidopsis at least 29 miRNAs are embedded within introns of other genes.

To analyze the possible crosstalk between splicing and miRNA biogenesis we selected intronic miR402. We found upregulation of mature miR402 level in abiotic-stress conditions which correlated with the inhibition of splicing of miR402-carrying intron. Additionally the intronic proximal polyA site was activated. It suggests that miR402 is not processed from an intron, but rather from shorter transcript after selection of proximal polyA site.

To evaluate the role of splicing machinery during miR402 processing we generated constructs containing *MIR402* host gene with mutated splice sites. The strong accumulation of the mature miRNA was observed after 5'ss inactivation, which correlated with the splicing inhibition and activation of proximal polyadenylation site.

We also shifted pre-miR402 from the intron to the first exon of *MIR402* gene and we observed that the active 5'ss plays an opposite role in miRNA maturation depending on its position relative to this splice site. Moreover, shifting the pre-miR402 to the second intron abolished miRNA production. These experiments strongly suggest that at least in the case of the miR402 it cannot be produced after splicing.

Performed analyses point to the strong competition between spliceosome and plant microprocessor complexes during intronic miRNA production. We also revealed that the accessibility of polyA sites plays an important role in plant miRNA biogenesis.

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T.3.5. Spatial regulation of cytoplasmic snRNPs assembly during larch microsporogenesis

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Keywords: snRNA, Sm proteins, RNP granules

Small nuclear ribonucleoproteins (snRNPs) play crucial role in pre-mRNA splicing in all eukaryotic cells. Contrary to relatively broad knowledge on snRNPs assembly within the nucleus, spatial organization of this process in cytoplasm remains unrevealed. Here we present *in situ* investigation of cytoplasmic snRNPs distribution on cellular level, which provides new insights into spatial regulation of their assembly and maturation in plant cells. Larix decidua Mill. microsporocytes were used for this study, due to its natural periodic fluctuations in expression levels of splicing elements. For *in situ* investigation, multilabelling techniques of U4, U5 snRNA and Sm proteins were used, including *in situ* hybridization, immunolocalization, and tyramide signal amplification methods.

We have shown for the first time, that under physiological conditions the same cell model might establish 2 distinct spatial manners of cytoplasmic snRNPs assembly, depending on the rate of de novo formation of snRNPs in relation with steady state of these particles within the nucleus. During periods of moderate expression of splicing elements, cytoplasmic assembly of snRNPs occurs diffusely throughout the cytoplasm. High expression of Sm proteins and U snRNA triggers accumulation of these particles within distinct, non-membranous RNP-rich granules, referred to as snRNP-rich cytoplasmic bodies (CsBs). We propose, that these structures function as regulating platforms, which coordinate kinetics of de novo formation of snRNPs during intensive expression of splicing elements, as well as they regulate proper pool of accessible Sm proteins in relation with the level of U snRNA in the cytoplasm.

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T.3.6. SWP73A and SWP73B subunits of *Arabidopsis* SWI/SNF chromatin remodeling complexes play distinct roles during development

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Keywords: Arabidopsis, SWI/SNF, flower, leaf

Chromatin structure is dynamically affected by SWI/SNF- type ATP-dependent chromatin remodeling complexes (CRCs) in order to control regulation of gene expression. The functional core of SWI/SNF CRCs is composed of SWI2/SNF2- type ATPase, two SWI3 one SNF5 subunits. With this core complex frequently co-purifies the SWP73 subunit. *Arabidopsis* genome encodes two SWP73- type proteins: SWP73A and SWP73B.

So far, there is no data about the function of SWP73A in *Arabidopsis*. By contrast, plants with RNAi silenced SWP73B gene show dwarf phenotype. SWP73B is involved in control of flowering time by direct regulation of FLC and leaf growth through interaction with ANGUSTIFOLIA3 transcription factor.

During our study we found that SWP73A inactivation does not cause significant morphological defects, while *swp73b* mutants are characterized by retarded growth, severe alterations in leaf and flower development, delayed flowering and male sterility.

Interestingly, inactivation of either SWP73A or SWP73B doesn't modify global nucleosome occupancy, however the lack of SWP73B alters positioning of particular nucleosomes on loci of genes crucial for leaf (e.g. *AS1, AS2, KAN1*) and flower development (e.g. *AGL24, SOC1, LFY, AP1, AP3*). Subsequently, we identified, using chromatin immunoprecipitation approach, that SWP73B directly binds promoter regions of these genes. This findings are consistent with altered expression of these genes exhibited by *swp73b* plants.

Summarizing, our study showed that SWP73B appears to act as important modulator of major developmental pathways, while SWP73A functions in flowering time control indicating differential contribution of SWP73A and SWP73B containing SWI/SNF complexes to regulation of transcription networks controlling *Arabidopsis* development.

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Poster Presentations

P.3.1. 20S proteasome and protein decay play important role in regulation of light-dependent germination of Arabidopsis seeds

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Keywords: germination, proteasome, protein decay, seeds

Protein degradation is fundamental in many biochemical processes, from catabolism of proteins to the execution of more specialized tasks in living organisms. The 20S proteasome is a high-molecular weight complex of proteolytic enzymes present in the cytoplasm and nucleus within the cell. Seed germination is a complex, multi-stage process requiring the coordinated series of various processes resulting in the biosynthesis and/or catabolism of defined cellular components. The proteasome system is involved in rapid degradation of many regulatory, storage and abnormal proteins. There are strong presumptions suggesting that germination completion may be partly related also to the removal of inhibitory proteins during imbibition, particularly through the activation of the 20S proteasome. The germination tests using dormant Arabidopsis wild type seeds imbibed on water and in the presence of MG132 (an inhibitor of 20S proteasome) unravelled interesting germination phenotypes, indicating that for seed-related processes protein decay by 20S proteasome is important modulatory mechanism acting in light-dependent manner. These findings together with results of the qRT-PCR analysis of expression of genes coding particular subunits of 20S proteasome, examination of the 20S core proteins accumulation and the measurement of its activity provide significant discoveries in the field of biochemical and cellular processes. Taking all into account it is postulated that 20S proteasome and protein decay are key mechanisms through which seeds are able to quickly modulate their responses to environmental conditions, allowing the transition from a quiescent metabolic state in a dry mature seed, to an active metabolic state in a vigorously germinating seed.

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This project was financially supported by the POLONIUM 2014/15 Program of the Ministry of Science and Higher Education of Poland

P.3.2. A comparative view of architectural root modification of WT and *nrt1.1* and *nrt2.1* mutants of *Arabidopsis thaliana* underlying the contribution of plasma membrane H+ ATPase (AHA2) in response to N conditions

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Keywords: AHA2,NRT1,1,NRT2.1, plasma membrane proton pump, root, growth

The aim of this project is the molecular and physiological characterization of the one isoforms of the plasma membrane proton pumps (AHA2), and its role in nitrogen-dependent growth and development of roots of *Arabidopsis thaliana*. It is known that soil nutrient conditions strong affect root architecture and roots have developmental responses to optimize nutrient acquisition. However, the signal transduction mechanism, which enables roots to sense changes in different mineral component concentrations and match their growth and development patterns to actual conditions in soil, is still unknown. Molecular studies of *A. thaliana* have identified several genes involved in nitrate–dependent root architecture changes i.e. *NRT1.1* and *NRT2.1*. However, not all elements of the signal transduction pathway leading to anatomical changes of root phenotype are known. The results of this study suggest that the plasma membrane proton pump AHA2 play important role in the nitrate signal transduction pathway and participates in regulation of root growth in response to variable nitrogen source.

P.3.3. A molecular framework for auxin-dependent flower development in yellow lupine (Lupinus luteus L.)

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Keywords: lupine, flower, gene expression, auxin

Auxin is known for decades as a phytohormone that regulates various important processes, such as flowering. Its cellular perception is dependent on proteins belonging to the family of nuclear receptors TAAR (TIR1/AFB auxin receptor) and co-receptors belonging to the Aux/IAA family (Auxin/Indole-3-Acetic Acid). The interpretation of the signal involves ARF (Auxin Response Factors) transcription factors.There is a great body of evidence, that in *Arabidopsis thaliana* TIR1 and AFB1-31, as well as ARF6 and ARF82 are involved in flower development. However, we do not know if this is valid for crop plants.

The aim of this study was to determine the expression profile of genes encoding homologues of above mentioned proteins during flowering in yellow lupine (*Lupinus luteus* L.). Our results indicate that each of the analysed genes exhibits a specific expression pattern and its transcriptional activity change during he subsequent stages of flower development. We conclude, that the studied genes are involved in the regulation of flowering in yellow lupine.

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P.3.4. Analysis of interactions between CBC-SE and NEXT complex in *Arabidopsis thaliana*

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Keywords: SERRATE, NEXT, EXOSOME, CBC, miRNA

The *Arabidopsis thaliana* SERRATE protein (SE) is involved in two important pathways of RNA metabolism: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA biogenesis, where together with DCL1 (Dicer like 1) and HYL1 (HYPONASTIC LEAVES 1) form a core of the plant microprocessor. In this complex SE influences the accuracy of pri-miRNA cleavages catalyzed by DCL1. The Arabidopsis *se* null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the cap binding complex (CBC), have been also ascribed to splicing of pre-mRNA. In order to understand this dual role of SE in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the *se-1* mutant genetic background. The SE-bound prote ins were identified by mass spectrometry, and the putative interactions were confirmed by the yeast two hybrid system and pull-down experiments. Our results have clearly demonstrated that SE contacts directly both the NEXT complex and the polyadenylation machinery. We suggest that these interactions are important for the quality control of miRNA precursors and/or degradation of pri-miRNA fragments after miRNA excision.

P.3.5. AtPHR2, PHOTOLYASE/BLUE-LIGHT RECEPTOR 2, is localized in chloroplasts and is involved in chlorophyll biosynthesis in seedlings

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Keywords: Arabidopsis, chloroplast, photolyase, PHR2, seedlings

PHR2, encoded by the At2g47590 *Arabidopsis thaliana* locus, is described as a photolyase and/or blue light photoreceptor based on *in silico* analysis. However neither its physiological role nor its enzymatic activity have been tested yet. Complementation of the photolyase deficient *E. coli* strain CSR603 with AtPHR2 showed that this protein very strongly increases the survival of bacteria after UVB treatment. This result points to the photolyase activity of this protein. The steady-state mRNA level of AtPHR2 was about 4 times higher in leaves, siliques and stems as compared with roots and flowers. The expression of this gene was up-regulated by light in a photosynthesis-independent manner. PHR2 protein was localised in chloroplasts as showed using transient expression of GFP-tagged protein in *Nicotiana benthamiana* epidermal cells. When seeds of the SALK_116579C T-DNA insertional line were sown in soil only WT and heterozygous plants were found. The progeny of heterozygous plants gave 20% of albino seedlings when sown *in vitro* on B5 medium supplemented with sucrose.

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P.3.6. Blue-light induced changes in ABA metabolism and signalling in light-dependent germination of dormant Arabidopsis seeds

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Keywords: ABA, dormancy, germination, seeds, signalling

The successful propagation of plants allowing survival of species depends on seed germination, a complex process consisting of many changes at physical, biochemical and molecular levels. In contrary, dormancy is a disability of seed to germinate even under optimal environmental conditions. It has been proposed that a theory of hormonal regulation of processes occurring within a seed implies interactions between various internal (i.e. ABA) and external (i.e. light) factors, thus mediating dormancy release required for germination. Numerous data confirm that red light regulates germination of dicot seeds such as Arabidopsis, by modification of expression of genes regulating internal GA/ABA balance and signalling of these hormones. Interestingly, a blue light effect on germination of Arabidopsis seeds, as well as a putative role of cryptochrome signalling pathway in this process is not well understood. The germination tests performed in this particular study, using dormant Arabidopsis wild type (WT) and *abi* mutant seeds imbibed on water and in the presence of ABA unravelled interesting germination phenotypes, indicating that seed-related processes can be stimulated by blue and/or white light and the effect depends on the depth of dormancy. Moreover, results of the qRT-PCR analysis of expression profiles of genes responsible for perception and transduction of blue light signal (i.e. *CRY, HY5, CIB*) and involved in ABA metabolism, as well as signalling (i.e. *ABA1, NCED, CYP707A2, ABI, AIP1*) strongly suggest that light-dependent germination of dormant Arabidopsis seeds can be regulated by cryptochrome signalling and relies on changes in ABA metabolism as well as its signalling.

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P.3.7. Cajal bodies role in maturation of mRNA

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Keywords: Cajal bodies, mRNA, Sm proteins

Gene expression is a crucial and fundamental phenomenon in living cells of all organisms. Not much known process of post-transcriptional regulation of gene expression is the delay translation of the newly formed transcripts. This process may be associated with retention of both pre-mRNA and mature mRNA in the nucleus. Our studies have shown that in the larch microsporocytes in the diplotene stage takes place accumulation different types of mRNA in the Cajal bodies (CB). This process usually occurs after a period of high transcriptional in the nucleus precedes the appearance the mRNA in the cytoplasm We performed a detailed analysis of this process concerning the distribution and level of Sm proteins and mRNAs encoding Sm proteins. The initial period is characterized by a high level of transcription and the high level of the Sm mRNA in the nucleoplasm. Then, after decrease decline the transcriptional activity of the cells, the mRNAs encoding Sm proteins accumulate in the CB, and next are transported into the cytoplasm where they are translated. During this period in the cytoplasm there are many small structures that contain the Sm proteins, which often colocalized or are in close proximity to the accumulation of mRNAs. This distribution of both mRNAs and Sm proteins indicates the start of their biosynthesis. Then Sm proteins are transported to the nucleus before they are able to perform its splicing function. Our results showed functional link of the Cajal bodies in mRNAs maturation or modification.

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P.3.8. Calreticulin is a key regulatory element of the pollen tube growth

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Keywords: calreticulin, siRNA, pollen tube growth

Pollen tube growth requires coordination of several strongly interrelated processes: formation and maintenance of a zoned cytoplasm, functional regulation of actin cytoskeleton, cytoplasmic streaming, cell wall biogenesis, membrane trafficking, and signaling. Most, if not all, of these processes are thought to be controlled by spatial and temporal variations in the level of cytoplasmic calcium (Ca2+) in distinct pollen tube zones. However, the molecular mechanism of stabilizing tip-focused Ca2+ gradient in growing pollen tube has not been explained. Several pieces of evidence point to calreticulin (CRT) as a key Ca2+-binding/buffering protein involved in pollen germination and tube growth. We previously showed that in *Petunia hybrida* (Ph) germinating pollen and growing tubes, CRT is translated on the ribosomes associated with endoplasmic reticulum (ER) at pollen germinal aperture and subapical zone of elongating tube (Suwińska et al. 2015). We hypothesized that CRT c an serve as a mobile intracellular Ca2+ store and control its local concentration within the cytoplasm. Here, we demonstrate that post-transcriptional PhCRT gene silencing with the specific small interfering RNA (siRNA), selectively degrading PhCRT mRNA, inhibits pollen tube growth by disruption of actin-dependent cytoplasmic streaming. These siRNA tubes exhibit severe ultrastructural abnormalities and consequently die. Our results seem to support the idea that during pollen tube growth, CRT is translated on ER membrane-bound ribosomes in the regions where its activity is required for stabilization of tip-focused Ca2+ gradient.

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P.3.9. Combined influence of intermediate heat shock and cytochalasin D on microspore embryogenesis in oilseed rape

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Keywords: microspore embryogenesis, suspensor, cytochalasin D

Since the early 70s last century there has been obtained many androgenesis *in vitro* protocols for crop plants. Nowadays researchers are focused to gain androgenetic embryos with suspensor that highly mimic those giving rise in embryogenesis *in vivo* (Prem et al 2012). The key factor for suspensor's development in microspore embryogenesis is intermediate heat shock at the beginning of the microspore suspension (Supena et al 2008). Oilseed rape is one of important agriculture plants grown in Poland. We chose for our experiments Feliks cultivar which has a very high potential yields, strong vigor and dynamism of growth and development in early spring (seeds were kindly provided by The Plant Breeding and Acclimatization Institute). The main aim of our study was to check common influence of intermediate heat shock and cytochalasin D (inhibitor disrupting microfilaments) on frequency of microspore embryos' induction. For many cytoskeletal inhibitors has been demonstrated enhanced success of androgenesis *in vitro*, for example colchicine, oryzalin. It is known that F-actin is responsible for tethering the microspore nucleus in center (Zonia et al 1999), supporting symmetrical division essential for further embryo formation in culture conditions. Herein in this study we show that intermediate heat shock (32.5°C for 12h) combined with 0,5 ug/ml cytochalasin D significantly increases number of microspore embryos with suspensor than in control cultures. Prem et al. 2012. BMC Plant Biology 12:127.

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P.3.10. Comparison of pri- and mature- miRNA levels. A case study on SE induction in Arabidopsis

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Keywords: microRNA, somatic embryogenesis, Arabidopsis

microRNAs are post-transcriptional regulators of developmental processes in all eukaryotic organisms. In plants, microRNAs beside their well-documented role *in vivo*, are implicated to control *in vitro* induced morphogenesis, including somatic embryogenesis (SE). Accordingly, numerous primary transcripts of MIRNA genes (pri-miRNAs) were found differentially accumulated in embryogenic culture of Arabidopsis (Szyrajew et al. 2012). To reveal if the indicated transcriptional activity of MIRNA genes in SE results in production of regulatory molecules, accumulation of mature miRNAs in the embryogenic culture was examined. To this end, a specific stem-loop reverse transcription primer qPCR analysis was used. Mature miRNA molecules of thirteen MIRNA genes represented seven MIRNA families including, MIR156, MIR157, MIR169, MIR172, MIR319, MIR393 and MIR396, were evaluated. The substantial discrepancy in the levels of the pri-miRNAs and the relevant mature miRNAs was indicated for most of the genes evaluated at various time points during SE (0, 5 and 10 day of culture). The possible explanation of the observed inconsistency between accumulation of pri- and mature- miRNA levels are presented. In conclusion, the study showed that the profiling of MIRNA primary transcripts is of limited value for the identification of miRNAs involved in the studied process.

P.3.11. Comprehensive analysis of tRNA-derived small RNAs biogenesis in plants

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Keywords: tRFs, Arabidopsis thaliana

In the last few years different classes of endogenous small non-coding RNAs were identified and described in diverse species with the use of advanced high-throughput sequencing technology (NGS) and computational analyses. Among them, one originating from canonical tRNA molecules seems to be the most evolutionary conserved and thus represented in all three domains of life. tRFs (tRNA-derived fragments) of 15-26 nucleotides in length have long been regarded as random byproducts of tRNA biogenesis or degradation processes, but emerging evidence demonstrates that they are precisely generated through a specific cleavage pattern.

Using *Arabidopsis thaliana* as a model organism, we aim at identification and characterization of the components involved in tRNA and microRNA biogenesis pathways that may play a role in generation of tRFs. The RNAs sequences used for this analysis were obtained using sRNA libraries from over 30 *A. thaliana* mutant lines carrying mutations in genes associated with tRNA and microRNA biogenesis. Interestingly, bioinformatic analysis of our NGS data revealed the existence of small RNAs deriving not only from mature tRNAs but also from tRNA flanking regions embedded within longer noncoding transcripts synthesized by RNA polymerase II.

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P.3.12. Correlation between direction of cell differentiation and changes in cell wall composition during tomato (*Solanum lycopersicum* L.) stems autografting

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Keywords: Autografting, Cell differentiation, Cell wall

Cell wall is a rich source of signals which are involved in regulation of cell differentiation. During autografting of tomato (*Solanum lycopersicum* L.) stems, callus tissue arises due to former dedifferentiation of particular tissues. Next, callus cells redifferentiate, enabling reconnection of vascular system as well as ground and protective tissues between scion and stock. This experimental approach is abounding in different cell events leading to differentiation of callus cells into new directions. With the use of monoclonal antibodies recognizing various pectin, hemicellulose, arabinogalactan proteins and extensin epitopes and histochemical staining (callose, lipids) we show that: 1/ there is a correlation between the wall composition and the callus origin and its position within the stem, 2/ appearance of LM15 (XXXG motif of xyloglucan) and JIM12, JIM20, LM1 (extensins) epitopes as well as disappearance of JIM11 epitope (extensin) mark cells that rediffer entiate into tracheary elements; 3/ LM5 and LM6 epitopes (rhamnogalacturonan I, galactan and arabinan residues respectively) and JIM8 (AGP epitope) occur abundantly in cells undergoing PCD; 4/ cell separation is accompanied by the appearance of cell wall compounds, that were not present before (LM6 epitope – galactan, rhamnogalacturonan I; LM8 epitope – xylogalacturonan; LM1 epitope – extensin). Furthermore, we point out that graft union is an area composed of cells with specific phenotypes, different cell polarity including circular polarity and arrangement, what is valuable for studies of mechanism involved in cell differentiation.

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P.3.13. CsClCa and CsClCc are the tonoplast transporters with different substrate specificity

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Keywords: ClC family, tonoplast transporter, cucumber

The ChLoride Chanel superfamily (ClC) is a group of anion transporters expressed in nearly every cell of living organisms. In plant cell, ClC proteins are present in membranes of various organelles. Although most of them are involved in Cl- transport, recent studies on *A. thaliana* have revealed that at least tonoplast AtClCa can act as a NO3-/H+ exchanger, suggesting the role of ClC also in nitrate transport. Recently, the different behavior towards nitrate and chloride of ClC has been linked to the presence of serine or proline in conserved GXGIP motif in the selectivity filter. This work is taking the attempt to functional characterization of the CsClCa and CsClCc chloride transporters from cucumber. The transient expression of the CsClC fused with GFP revealed the tonoplast localization of both transporters. Expression of CsClCc but not CsClCa in *S.cerevisae gef1* mutant (the single ClC homologue in yeast) suppressed yeast sensitivity to chlorides and sulfates, suggesting a potential role of CsClCc in transport of both anions. However the kinetic studies of CsClCa and CsClCc in yeast tonoplast implied that both proteins can transport NO3- as well as Cl- ions. Furthermore, detailed analysis appeared the considerable differences in substrate affinity of these two proteins suggesting, that in plant cells CsClCa is responsible mainly for NO3- transport into the vacuole, whereas CsClCc is involved rather in transport Cl- and SO42-. Comparing the nitrate/chloride affinity of CsClCa and CsClCc with substitution at 165 serine/proline we concluded about importance of amino acid for anion selectivity of transporters.

P.3.14. Cuticle-like layer in phellem cells of *Cornus sanguinea* and *C. controversa*

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Keywords: phellem, periderm, phelloid, cuticle, Cornus

The functional adaptation of the protective tissues is the deposition of cutin and suberin in the cell walls. Cutin is a specific polymer, deposited exclusively in the outer cell walls of the epidermal cells, whereas suberin occurs in the cell walls of different cell types including phellem. Phellem is an external cork layer in the periderm, formed by the divisions of the meristematic phellogen. Its cells are characterised by the thick and suberized cell walls; additionally, in many woody species non-suberized phelloid cells can also occur.

The structure and formation of the periderm were analysed in stems of various Cornus species. Staining methods coupled with the autofluorescence in UV light were used to visualise the differences in the structure and composition of the periderm. An atypical layer of non-suberized phellem cells, formed at the end of growing season, was found in *C. sanguinea* and *C. controversa*, suggesting that this layer can contain the phelloid cells. However surprisingly, the structure and chemical composition of the external walls of phelloid cells resembled the cuticle. Since the cutin and suberin are both the (bio)polymers similar in the chemical composition, likely, the suberin biosynthesis pathway is switched into the cutin formation in the atypical phellem cells of the analysed species. The phellem cells covered by a cuticle-like layer have not been so far described in other woody plant species.

P.3.15. Development and ultrastructure of haustoria in the ovule of Sedum (Crassulaceae)

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Keywords: Embryogenesis, endosperm, haustoria, Sedum

The endosperm of numerous angiosperms produces special types of structures which function as haustoria. Usually, the endosperm haustoria are fast-developing, highly specialized and synthetically active structures which absorb food materials from the maternal tissues and transfer them to the endosperm. In the genus Sedum the endosperm conforms to the cellular type of development and several members of this family develop chalazal endosperm haustoria. The endosperm chalazal haustorium of Sedum is a huge cell containing one enlarged nucleus. At the chalazal end of the haustorium cell structure, ultrastructural study revealed the presence of a transfer wall forming wall ingrowths. Additionally, in the Sedum the suspensor basal cell forms haustorial protrusions, penetrating the ovular tissues, and is apparently the main source of nutritive substances for the embryo-proper. The walls of the haustorium and the micropylar part of the basal cell form the wall ingrowths typical for a transfer cells. The development of the suspensorial micropylar haustorium and endosperm chalazal haustorium in Sedum was investigated using light and electron microscopy. Our analyses of the development of both the chalazal endosperm chalazal haustorium and suspensor micropylar haustorium confirm that are an active transfer cells absorbing nutrients from the mother plant tissue, metabolizing and translocating them to the endosperm/embryo.

P.3.16. Distribution of fluorescent probes within the embryo and seed in Sedum

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Keywords: plasmodesmata, symplasmic transport, embryogenesis, Sedum

Plasmodesmata (PD) is part of the system of information exchange between plant cells. Molecules which pass through the PD are proteins, transcription factors and different classes of RNA. This suggests that plasmodesmata can participate in the coordination of plant growth and development and that cell-to-cell communication is a universal mechanism involved in regulation of cell differentiation. It is known that regulation of plant embryogenesis is based (among others) on changes in symplasmic transport between embryo cells, but involvement of this process in cell-to cell communication within seeds is less understood.

It is characteristic of the embryogenesis of many angiosperms that the early embryo differentiates into two parts: the embryo proper and the suspensor, which develops rapidly and is short-lived. This organ pushes the embryo proper into the endosperm cavity and connects the embryo proper to the surrounding maternal and endosperm tissues, thus serving as a conduit for the nutrients and growth regulators required for embryonic development.

Analysis of the symplasmic communication was performed on the Sedum seeds and embryos on different developmental stages with the use of low molecular weight fluorochromes (HPTSA and caged fluorescein) and fluorescence microscopy techniques including confocal microscopy. Performed analysis showed spatio-temporal changes in symplasmic communication between endosperm/suspensor and suspensor/embryo. This indicate that symplasmic communication changed during the seed development. Obtained results suggest the involvement of changes in cell-to-cell communication in the Sedum embryogenesis.

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P.3.17. Disturbances in root growth of tomato seedlings induced by non-protein amino acids are due to alterations in RNS emission

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Keywords: RNS, m-tyrosine, canavanine, toxicity

Meta-tyrosine (m-Tyr) and canavanine (Can) are non-protein amino acids (NPAAs). m-Tyr is an allelopathic compound synthesized by Festuca. Can is recognized due to its role in plants' reaction to herbivores, and is identified in Fabaceae. Can is an inhibitor of arginine-dependent NO formation in reaction catalyzed by nitric oxide synthase (NOS).

Our study was performed using roots of tomato (*Solanum lycopersicum* L.) plants. Seedlings developed from seeds germinated in water for 3 days were cultured for 72 hours in m-Tyr or Can at concentration inhibiting root growth in 50% or in 100%. Treatment of seedlings with NPAAs for 24 hours did not induce cell death. After 72 hours slightly lowered cell viability was detected in root tips of plants cultured in m-Tyr or Can at higher concentration. Emission and cytological localization of NO and ONOO- in tomato roots were determined by spectrofluorymetric and confocal microscopic methods. m-Tyr induced transient enhancement in RNS emission in roots after 24 hours. In prolonged experiment contribution of RNS in harmful effect of m-Tyr was less evident. Can inhibited NO emission in roots but slightly enhanced ONOO- level. Cytological observations of root tips indicated localization of NO and ONOO- in border cells and rhizodermis. For both NPAAs modifications in RNS emission correlated to NO2- level in roots.

RNS may act as signaling agents in toxicity of m-Tyr. Harmful effect of Can may be due to perturbation in RNS production, influencing finally pattern of root growth.

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P.3.18. Effect of ABA on the transcriptional activity of LlFT in *Lupinus luteus*

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Keywords: Lupinus luteus, gene expression, LlFT,ABA

Lupinus luteus seeds have a high content of storage proteins and of sulfur-containing amino acids (cysteine and methionine) but the most important feature is its ability to form symbiotic relationships with bacteria to bind atmospheric nitrogen and use it for growth and development. The research carried out in Poland in the 1960s and '70s revealed physiological and anatomical mechanisms of growth and development in *L. luteus*. Still, little is known about the molecular mechanisms of flower induction in this plant.

The induction of flowering is one of the most important stages of the development of higher plants. A lot of data indicates the necessity of cooperation of many factors both exogenous and endogenous to provide an optimum time of flowering. The FT gene plays a key role in the control of time initiation flower development. Transcriptional regulation of FT is crucial for integrating the information derived from external and internal factors, such as photoperiod, temperature, amount of hormones and developmental age.

In this study the influence of abscisic acid on transcriptional activity of LlFT has been defined. Determination of expression of this gene was performed in vegetative organs of yellow lupine by RT-qPCR using UPL probe. Our preliminary results suggest that exogenous ABA treatment cause marked decrease in the amount of mRNA studied gene. The LlFT inhibition were observed in all studied organ regardless of the age of plants.

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P.3.19. Effect of cold treatment on transcript level of raffinose synthase gene

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Keywords: raffinose synthase, raffinose, low temperature

Raffinose synthase (RS, EC 2.4.1.82) catalyzes the synthesis of raffinose from sucrose and galactinol. This enzyme is involved in second step of synthesis of raffinose family oligosaccharides (RFOs) which are known as reserve carbohydrates, membrane stabilizers and stress tolerance mediators. Recently RFOs were hypothesized to act as phloem-mobile signaling compounds as well as factors that contribute to cellular reactive oxygen species homeostasis. Genes encoding RS create gene family in Arabidopsis (model plant) as well as in rice and maize. However, only one RS gene (AJ426475) from pea has been cloned so far.

To evaluate the impact of raffinose in plant response induced by low temperature the five days old seedlings of *P. sativum L. cv. Hubal* (control sample that were grown in a day/night temperature of 20/10 °C), subjected to a day/night temperature of 10/5 °C for 24 hours, were exposed to 5/0 °C. The changes induced by cold treatment (5/0 °C for 1, 8 and 13 days) in the expression of *PsRS* gene, raffinose level and their precursor are presented. The level of mRNA *PsRS* transcript and carbohydrates were studied at the post stress recovery stage too when the cold treated (5/0 °C, 13 days) seedlings were transferred to the control condition (20/10 °C) for next 3 days. Additionally the 5' upstream region of *PsRS* gene was cloned by genome walking. The potential cis-regulatory elements present in nucleotide sequences of PsRS promoter are described.

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P.3.20. Effect of NPA on growth and proton fluxes in maize coleoptile cells

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Keywords: NPA, auxin, growth, maize, pH

1-N-naphthylphthalamic acid (NPA) is the most popular inhibitor of polar auxin transport (PAT) used to investigate the mechanisms of auxin action. At the cellular level NPA probably works in two different ways. The first is via NPA binding specific membrane proteins (Zazimalowa et al., 2003) affecting auxin movement. According to second hypothesis NPA is required for the proper positioning of the auxin efflux carriers at the plasma membrane (Lusching, 2000).

The effect of NPA on endogenous growth, growth in the presence of indoleacetic acid (IAA) and on proton extrusion in maize coleoptiles segments were studied. Growth kinetics and medium pH changes were recorded as previously described (Burdach et al., 2014).

It was found that endogenous growth and growth in the presence of IAA depends on the NPA concentration. This compound at 10 μM stimulated endogenous growth as compared to the control. All NPA concentrations tested inhibited growth as compared to control. Interestingly, NPA in the concentration of 10 μM increased growth rate solely in the first phase response to auxin.

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P.3.21. Effects of selected antibiotics on regeneration potential of Polish tomato cultivars

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Keywords: in vitro regeneration, tomato, antibiotics

Antibiotics are commonly used to eliminate or inhibit microbial growth in plant tissue cultures. Generally antimicrobial agents are frequently used in micro propagation techniques to obtain free elite clones or after genetic transformation to select a putative transformants. Despite their successful application to minimize bacterial contamination, they are phytotoxic and may differently affect the regeneration ability in plant tissue culture. Antibiotics also have been reported to exhibit plant growth regulator-like properties which may vary depending on the species. Here, we report on the effects of kanamycin (Km), cefotaxim (Cef), carbenicillin (Crb) and ampicillin (Amp) on morphogenesis of different Polish tomato cultivars cultured on Murashige and Skoog medium.

P.3.22. Ethylene-induced flower abscission in *Lupinus luteus*

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Keywords: ethylene, ACC, *Lupinus luteus*, flower abscission, phytohormones

The separation process occurs in a specialized abscission zone (AZ) usually formed at the base of the organ. The changing level of ethylene (ET) is the element responsible for coordinating the anatomical and physiological transformation that accompanied flower abscission. Exogenous ET has a significant stimulatory effect on flower abortion in *Lupinus luteus*, whereas 2-aminoethoxyvinylglycine (AVG) – ET biosynthesis inhibitor or norbornadiene (NBD) – inhibitor of ET action, applied directly onto the pedicels of *L. luteus* reversed the stimulatory effect of ET on flower abortion. We also showed that AZ activation is accompanied by a considerable increase both in 1-aminocyclopropane-1-carboxylic acid synthase (LLACS) and oxidase (LLACO) cDNAs and the ACC content. Obtained results suggest that ethylene-dependent pathway functioning in *L. luteus* plays an important role in flower abortion processes.

P.3.23. Expression of auxin signal transduction pathway genes during pod wall and seed development of yellow lupine (*Lupinus luteus* L.)

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Keywords: lupine, gene expression, auxin

Yellow lupine like other members of the Fabaceae family (Fabaceae L.), has important practical value. Development of lupine flowers and fruits are crucial for the productivity of these plants. Phytohormone auxin (IAA) plays an important role in the regulation of plant ontogeny, including apical dominance, the fruit maturation and aging. Auxin signal transduction pathway involves three families of genes: TAAR (TRANSPORT INHIBITOR RESPONSE 1 / AUXIN SIGNALING F-BOX PROTEIN 1-3), AUX / IAA and ARF (AUXIN RESPONSIVE FACTOR) encoding receptors, repressors and transcription factors responding to IAA, respectively. Studies in other plant species have shown that, for example, ARF6 and ARF8 transcription factors regulate the morphogenesis and development of flowers and fruits. Another gene, namely ARF2, can affect the size of the formed seeds. The aim of this study was to determine the expression profile of selected genes of auxin signal transduction pathway during pod wall an d seed development of yellow lupine (*Lupinus luteus* L.). The results indicate that each of the analysed genes exhibits a specific expression pattern and its transcriptional activity change during the subsequent stages of growth the pods. The results indicate the involvement of the studied genes in the regulation early stages of development of yellow lupine pods.

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P.3.24. Female biased sex ratios in two Rumex species under *in vitro* conditions

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Keywords: Rumex, sex ratio, in vitro

Rumex thyrsiflorus Fingerh. (thyrse sorrel) and *R. acetosa* L. (common sorrel) are dioecious plants with polymorphic sex chromosomes (XX in females, XY1Y2 in males). Female-biased sex ratios have been observed in natural populations of these species. It is expected that in plants with chromosomal sex determination sex ratio should be equal or similar to 1:1 thus the deviation from that balance in sex ratio of these species is a topic worthy of attention.

In this study we examined the sex ratio and viability of *R. thyrsiflorus* and *R. acetosa* seedlings, cultured explants and explants with morphogenetic potential under *in vitro* conditions.

The hypocotyls of both species were isolated from 11-day-old seedlings and were cultured on MS basal medium supplemented with either 2.4-D, BAP or TDZ applied in different concentrations. Sex was analyzed using PCR-based methods with sex specific DNA markers located on Y chromosomes.

Indirect organogenesis (adventitious shoots) and somatic embryogenesis were revealed using histological analysis of tissue stained with periodic acid Schiff/naphtol blue black (PAS/NBB) for both studied species and Scanning Electron Microscope (SEM) analysis for *R. thyrsiflorus*.

The results of our experiments showed female-biased sex ratio in analysed seedlings, cultured explants and explants with morphogenetic potential. The comparative studies on sex ratio among *R. thyrsiflorus* and *R. acetosa* explants under *in vitro* conditions are in progress.

P.3.25. Fluorescent auxin conjugates and their transport in *Arabidopsis thaliana* tissues

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Keywords: auxin, transport, conjugates, IAA-FITC, IAA-RITC

The tissue-specific distribution of auxin, which is related to the various pathways of auxin transport, plays an important role in plant developmental processes. However, the relationship between the pathway of auxin transport and its specific impact on plant cells is still poorly understood, mostly because of the lack of efficient research tools for auxin transport detection. To overcome this problem, we conjugated auxin – IAA, with the two fluorescent dyes which differ in their transporting pathways: symplasmic fluorescein isothiocyanate (FITC) and apoplasmic rhodamine isothiocyanate (RITC). The obtained auxin conjugates enable the visualization of auxin transport directly in the plant tissues and therefore are a promising novel tool for plant experimental biology. Both compounds IAA-FITC and IAA-RITC have a biological activity in different developmental processes similar to free IAA and induce the auxin response genes. The conjugates are also transported simil arly to the free IAA forming uneven tissue-specific distribution patterns with e.g. characteristic, auxin-like, maxima of concentration in the shoot and root apical meristems, lateral meristems and leaf hydathodes. Therefore, in our opinion, auxin conjugates may be the substrates for auxin carrier proteins and consequently can be used, in studies concerning processes regulated by auxin in different plant species.

P.3.26. Functional analysis of AtYY1 and AtWDR55, homologs of epigenetic regulators in mammals, during somatic embryogenesis in Arabidopsis

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Keywords: developmental plasticity; *in vitro* culture; somatic embryogenesis

The process of embryo formation from plant somatic cells, termed as somatic embryogenesis (SE), manifests developmental plasticity of differentiated cells. Thus, the identification of the genetic factors involved in SE greatly contributes to the knowledge on genes that control cell pluripotency. However, an open question remains on the similarities between genetic mechanisms that control reprogramming in animal and plant somatic cells.

The study was focused on the analysis of *AtYY1* (*YIN YANG 1*) and *AtWDR55* (*HUMAN WDR55 (WD40 REPEAT) HOMOLOG*), that encode proteins with functional domains homologous to epigenetic regulators involved in dedifferentiation of somatic cells in mammals. Expression level of *AtYY1* and *AtWDR55* was evaluated in embryogenic culture of Col-0 with the use of qRT-PCR and both genes were found to be differentially expressed in explants undergoing embryogenic induction. Moreover, the *yy1* and *wdr55* insertional mutants were indicated to be significantly impaired in the embryogenic response induced *in vitro*. A strong reduction in SE efficiency and SE productivity was observed in culture of these mutants. The results imply the involvement of AtYY1 and AtWDR55 in embryogenic transition of somatic cells in Arabidopsis. In conclusion, some similarities in genetic regulators of the reprogramming induced in somatic cells of animals and plants can be expected.

P.3.27. Functional analysis of genes involved in root hair development in barley (*Hordeum vulgare* L.)

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Keywords: root hairs, TILLING, barley

Root hairs are specialized epidermal cells which are important for nutrient and water uptake from the rhizosphere. There is a limited knowledge on molecular control of root hair morphogenesis in monocots, including major cereals. Therefore, we conducted the analysis leading to identification of new genes related to root hair development in *Hordeum vulgare* and determination of their function using TILLING technique (Targeting Induced Local Lesions IN Genomes).

In the microarray experiment, conducted in Department of Genetics, University of Silesia, 48 genes differentially expressed in the roots of a root-hairless mutant *rhl1.a* and the wild type parent cultivar 'Karat' were identified. These genes provided a pool of candidates from which a three genes were selected for functional analysis in the presented study. We applied a new approach for identification of genomic structures of selected genes. We used combinatorial bioinformatic analyses which allowed to identify the genomic structures of selected genes without gene cloning. Bioinformatic analysis included the theoretical description of genomic sequences/mRNA/proteins of 48 genes, analysis of conservation of genomic and protein sequences and analysis of patterns of gene expression in barley and other species using the available microarray data. These analyses provided supportive information about putative functions of selected genes. TILLING strategy was used to identify mutations in three genes: *HvRIC7* (ontology: pollen tube growth), *HvEXPB4* (ontology: plant-type cell wall loosening), *HvFMN* (ontology: oxidreductase activity). Functions of these genes have not been described yet in any model or cultivated species.

P.3.28. Functional analysis of miR164 in control of somatic embryogenesis induced *in vitro* in Arabidopsis

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Keywords: miR164, CUC1, CUC2, somatic embryogenesis

In planta miR164 regulates CUP-SHAPED COTYLEDON1 (CUC1) and CUC2 transcription factors, the members of the NAC family involved in shoot apical meristem (SAM) formation and cotyledon separation. miR164 was hypothesized to be also involved in somatic embryogenesis (SE) induced *in vitro* in culture of Arabidopsis explants considering differential expression of *MIR164* genes indicated in embryogenic culture (Szyrajew et al. 2012). To verify this assumption the present analysis was conducted and a drastic down-regulation of mature miR164 during SE induction was indicated. The hypothesis about the involvement of the miR164 in SE was further verified with the use of insertional mutants and the *mir164a*, *mir164b*, *mir164c* and *mir164abc* mutants were found to negatively affect explant capacity for SE. In addition, *in vivo* targets of miR164 were analysed and a regulatory relation between miR164 and *CUC* genes during SE is assumed considering a significant up-regulation of *CUC1* and *CUC2* in embryogenic culture. Moreover, GFP reporter line analysis indicated an enhanced expression of *CUC1* and *CUC2* in explant areas involved in embryogenic transition (cotyledons and SAM). In conclusion, the results indicated that miR164 regulates SE induction in Arabidopsis possibly through the control of *CUC* genes.

P.3.29. Genetic modification of hairy roots of *Crambe abyssinica* for wax esters synthesis

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Keywords: FAR, WS, hairy roots, transformation

Hairy roots, induced by *Agrobacterium rhizogenes*-mediated transformation, are used for the production of secondary metabolites and recombinant proteins. The analysis of lipids of *Crambe abyssinica* hairy root cultures revealed that palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids as well as 18:1 trans acid were found in the highest amount. Wax esters are esters of long chain fatty alcohols and fatty acids used as high pressure lubricants, components of pharmaceuticals, cosmetics, ink and emulsions. The final step of wax biosynthesis includes fatty alcohols formation from long chain fatty acids carried out by fatty acyl-CoA reductases (FAR) and esterification reaction of a fatty acyl-CoA and a long chain fatty alcohol catalysed by wax synthases (WS).

The aim of the project was to investigate the possibility of the introduction of genes encoding FAR and WS to hairy roots of *C. abyssinica* in order to obtain wax ester synthesis. The series of binary plasmids carrying genes encoding FAR and WS from different organisms were constructed and transformed into wild type *A. rhizogenes* strains (ATCC 31798 and ATCC 15834). Hairy roots were observed 1-4 months after the inoculation of *C. abyssinica* leaves. The presence of T-DNA as well as genes encoding FAR and WS in hairy roots was verified by PCR amplifications. The analysis of the lipid content and composition of transformed roots was performed using gas chromatography.

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P.3.30. Hydropriming supplemented with melatonin modifies corn (*Zea mays* L.) embryo proteome during seed germination under optimal temperature conditions

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Keywords: melatonin, priming, germination, proteome analysis

Melatonin (N-acetyl-5-methoxytryptamine) has a great potential for plant biostimulation. Its role in plant physiology is intensively explored and its important function in plant stress defence frequently underlined. Melatonin is particularly effective when applied as an additional factor of seed priming. In the presented research hydroconditioning was chosen experimentally as optimal for corn (*Zea mays* L.) seeds. The following seed variants were compared: non-treated (nt) and hydroprimed with water (H) or with melatonin water solutions 50 and 500 µM (HMel50 and HMel500). In order to identify changes in corn seed proteome after applying the above hydropriming techniques, purified protein extracts of germinated seed embryos (24 h, 25 °C) were separated by two-dimensional electrophoresis. Then proteome maps were graphically and statistically compared using PDQuest software and selected protein spots were qualitatively analysed using mass spectrometry techniques and iden tified using the Mascot protein databases. Qualitative research helped to identify hydropriming-associated proteins, and for the first time those which were expressed only in the presence of melatonin. Quantitative studies also indicated the directions of changes in protein metabolism affected by priming and melatonin in the embryos of germinated maize seed. The possible roles of these proteins for maize seed vigour and its quality improvement were discussed.

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P.3.31. Identification of candidate AUXIN RESPONSE FACTORs involved in shoot organogenesis induced *in vitro* in Arabidopsis

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Keywords: AUXIN RESPONSE FACTORs; Arabidopsis thaliana; shoot regeneration

AUXIN RESPONSE FACTORS (ARFs) are the major regulators of auxin response genes of a key role in developmental processes in plants. In contrast to in vivo development, our knowledge on auxin signaling components that control *in vitro* induced plant development is very limited. The study was performed to identify *ARF* genes expressed during shoot organogenesis (ORG) induced *in vitro* in culture of Arabidopsis explants, immature zygotic embryos. With the use of Real-Time qRT-PCR the expression level of 22 functional *ARF* genes encoded in Arabidopsis genome was analysed at different time points of ORG process. The results indicated that majority (14) of *ARFs* is expressed during ORG. Most of the *ARFs* expressed in ORG (12) displayed the modulated transcript level and the genes up-regulated (*ARF5, ARF10, ARF16, ARF17, ARF19*) and down-regulated (*ARF1, ARF2, ARF3, ARF6, ARF9, ARF11, ARF18*) in ORG were identified. ORG-modulated *ARFs* were found to differ in expression profiles. A high transcript accumulation in callus induction phase of ORG followed by a reduced expression during shoot regeneration was observed for *ARF1, ARF10* and *ARF16*. Expression of *ARFs* in explants induced towards ORG was also monitored with the use of GFP reporter lines. Moreover, capacity for ORG was evaluated in culture of arf mutants and a significantly reduced embryogenic potential was observed in *arf1*, *arf6* and *arf19* mutants suggested the respective genes (*ARF1, ARF6 and ARF19*) to be involved in shoot organogenesis.

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P.3.32. Identification of Nuclear Localization Signal of *Arabidopsis thaliana* Cyclin-Dependent Kinase C;1

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Keywords: CDKs, transcription, nuclear localization signal

Cyclin-dependent kinases (CDKs) are a family of evolutionarily conserved serine/threonine protein kinases discovered in the 1960s by Leland Hartwell. By the definition all CDKs require the binding of a regulatory cyclin subunit for their enzymatic activation. CDKs can be generally classified into two major groups based on whether they control cell cycle progression or regulate gene transcription by phosphorylation of the C-terminal domain of RNA polymerase II.

Plant cyclin-dependent kinase C;1 (CDKC;1) is a homolog of mammalian CDK9. The CDKC;1/CycT complex is localized in the nucleus. It is suggested to have a role similar to the mammalian CDK9/CycT complex, which is a component of transcription elongation factor b (P-TEFb) involved in the positive regulation of RNA synthesis.

In our research using NLS mapper we first analyzed the Arabidopsis thaliana CDKC;1 protein sequence to identify the region with the putative nuclear localization signal. Next, the sequence identified was mutated. Finally, the analysis of CDKC;1_WTNLS-GFP and CDKC;1_mutNLS-GFP revealed that CDKC;1_WTNLS-GFP was observed only in the nucleus in contrast to the CDKC;1_mutNLS-GFP which was in the nucleus and cytoplasm.

P.3.33. Interactions between FLS2 and BRI1 receptor proteins and gamma-secretase complex in plant cells

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Keywords: gamma-secretase, presenilin, endocytosis, FLS2, BRI1

Gamma-secretase is a complex of four proteins with intramembrane protease activity. In most cells, the complex includes: presenilin (PS1 or PS2), nicastrin (NCS), APH-1 (anterior pharynx-defective 1) and PEN-2 (presenilin enhancer 2). Presenilins and γ-secretase have been very extensively investigated in animals, this complex may be involved in the regulation of endocytosis, recycling and degradation of selected membrane receptors . Best known function of the complex is participation in the process of regulated intramembrane proteolysis of the Notch receptor and the amyloid precursor protein. Mutations in the γ-secretase complex are directly correlated with an etiology of Alzheimer's disease. In animal cells mutations of any presenilin result also in reduced endocytosis of membrane receptors, such as transferrin, Notch or EGFR.

The roles of gamma-secretase complex in plants are almost completely unknown. In *Arabidopsis thaliana* protoplasts, the existing data confirm localization of γ-secretase subunit homologs within endomembrane system. Interestingly, however, none of known major substrates of the animal γ-secretase complex, has been identified in plants. Presenilins and the whole γ-secretase complex could therefore be considered as proteins involved most probably in the regulation of vesicular transport.

Here we show localization of presenilins in living plant cells and unravel some interactions between γ-secretase complex and FLS2 (flagellin-sensitive 2) and BRI1 (brassinosteroid insensitive 1) receptor proteins.

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P.3.34. Involvement of S1/P1-like nucleases in plant programmed cell death

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Keywords: nuclease, PCD, RNA, DNA, senescens

Programmed cell death (PCD) is one of the most important cell process, plays crucial role in morphogenesis, defense responses to pathogens and other stress condtidion. Although in animals and plants, degradation of cellular DNA and RNA is a inherent step of PCD, role and mechanism of this process it's different. Previous studies have demonstrated that S1/P1 nucleases are mainly responsible of nucleic acids degradation in plants. As a result of their high homology, to well described fungus nucleases S1 and P1 with catalytic activity in low pH and in presence of zinc-ions, it was assumed that the same conditions occurs during hydrolysis of the plant's DNA and RNA. In spite of this, our research exhibits that plant S1/P1 nucleases have a high diversity of the catalytic requirements. Some of them demonstrate activity in neutral pH and in presence of calcium or manganese ions, while low pH and zinc ions inhibit their activity. These information could lead to described a exact step of PCD and when degradation of nucleic acids occurs. For this reason we established studies to determine evolutionary and molecular causes leading to such high diversity of catalytic requirements in plant nucleases. Our data, shows that S1/P1 nucleases evolved from enzymes activated in neutral pH and in presence of manganese ions to recent diverseness of catalytic capabilities.

P.3.35. Looking inside the SAM of *Arabidopsis thaliana* plants

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Keywords: shoot apical meristem, fluorescence, *A.thaliana*

The maintenance of the properly organized and functioning shoot apical meristem (SAM), throughout the life, is of crucial importance for plant growth and development. Our aim was to visualize the changes within the SAM with the use of fluorescent techniques. In general, *in vivo* analyzes within the shoot apical meristem are difficult to accomplish due to its inaccessibility or e.g. small size. We have used an approach coupling a hand dissection of the SAM and the Confocal Scanning Laser Microscopy (CSLM) in order to localize the distribution of various signals, like fluorescent probes and markers. The method can be used to analyze the ontogenetic changes or differences between various genotypes during vegetative growth of the *A. thaliana* plants. The results of representative cases will be presented.

P.3.36. *Medicago truncatula* somatic embryogenesis induction is regulated through LEAFY transcription factors and probably gibberellin mediated signal pathway

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Keywords: Somatic embryogenesis, LEC2, Gibberellin biosynthesis

Somatic embryogenesis (SE), a process of embryo formation from somatic cells described the first time in 1958 up to date remains unresolved. Among other factors regulating SE, transcription factors (TFs) become the most desirable research target. The best analyzed *LEAFY COTYLEDON* group genes are known to be an important part of the regulation mechanism resulting competence acquisition during SE. Hormones like auxin and gibberellin are regarded as being regulators or/and being regulated by those TFs what especially for *Arabidopsis thaliana* gibberellin biosynthesis and deactivation genes *GA3ox3* and *GA2ox6* was confirmed. Currently accepted mechanism of action suggests that with the increase in the LEC2, FUS3 and AGL15 activity, endogenous gibberellin content decreases.

Somatic embryogenesis were conducted with the two step method on *Medicago truncatula* cv Jemalong M9 - not embryogenic (NE) and M9-10 - embryogenic (E) lines where all analysis was performed during the first step, namely induction. Gene expression profiling confirmed presence of *MtrLEC2* and *MtrFUS3* transcripts after one week only in EL and raising to the end of induction. Interestingly transcripts of *MtrAGL15* gene were identified in both lines just after two days of induction but only in EL was visible the maximum of expression at day 7. Results of LC-MS indicated that amount of active gibberellin GA3 in E line dramatically increase starting from day 14 up to 10 times higher amount compared to NE line. Within all tested *Medicago truncatula* gibberellin biosynthesis genes only *MtrGA3ox1* and *MtrGA2ox3* expression were correlated to *LECs/AGL15* gene regulation model.

P.3.37. Micropropagation *in vitro* of *Inula germanica* L.

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Keywords: Inula germanica, micropropagation, BAP

Inula germanica is extremely endangered species and listed as being under critical thread (CR) on Polish red lists (Zarzycki et al., 2006). *I. germanica* inhabits the central and south-eastern Europe and locally Asia Minor. In Poland there is only one single siteBielinek on the Oder River. The survival of this species will be improbable if the risk factors are not eliminated or if efficient biotechnological methods fail to prepare succesful reproduction and further development of the plant in order to increase their numbers.

The aim of this study was to analyze the applicability of a protocol of efficient *in vitro* micropropagation system developed for the Asteraceae species for regeneration of *Inula germanica*. The experimental material consisted of few-day old sterile seedlings, from which shoot tips and fragments of cotyledons, hypocotyls and roots were isolated. Explants were exposed on MS medium containing 1 mg•l-1 BA and NAA 0,1 mg•l-1. The highest number of shoots was recorded for shoot tips (12,0/explant). Fragments of root were not able to produce shoots by organogenesis. After 4 weeks of culture, shoots were isolated and transferred to fresh proliferation media. In three subsequent subcultures multiplication rate decreased (4-6 shoots/explant whatever type of explant). The shoots obtained were rooted on solid and liquid MS medium. The highest rooting efficiency was recorded for liquid medium. Microcuttings were hardened for 8 weeks to field condition. In the first year plants were capable for flowering.

P.3.38. Overexpression and purification of *Arabidopsis thaliana* CDKF;1

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Keywords: CDKF, cell cycle, phosphorylation

Cyclin-dependent serine/threonine kinases (CDKs) regulate cell cycle progression at the embrionic stage of many different organisms. In Arabidopsis seven types of CDKs were identified including, CDKA, CDKB, CDKC, CDKD, CDKE, CDKF and CDKG. The activation of cyclin-dependent kinases is not only regulated by the presence of proper cyclin, but also by the phosphorylation of the CDK T-loop domain. The aim of this work was the overexpression and purification of Arabidopsis CDKF;1. The open reading frame coding for the CDKF;1 protein (479 amino acid residues) was cloned into the pET59DEST vector and transformed into the *Escherichia coli* BL21pRIL strain. Next, the optimal conditions for protein overexpression were determined. Recombinant CDKF;1 protein was purified using Ni-NTA resin. The purity of the protein was verified by SDS-PAGE and Coomassie staining. The gel filtration analysis confirmed the formation of a homodimer by CDKF. The purified protein will be biochemically characterized and potential new protein substrates will be verified.

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P.3.39. Pattern of DNA metylation and histone acetylation in mature pollen grain and growing pollen tube of *Hyacinthus orientalis*

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Keywords: pollen tube, DNA methylation,histone acetylation

Epigenetic mechanisms are one of crucial factors in regulation of transcriptional activity and chromatin structure. It'is known that mature pollen cells are transcriptionally inactive. However, in time of pollen tube growth reactivation of transcriptional activity took place in those cells (Zienkiewicz et al 2008).

The aim of the study was to investigate the pattern of DNA methylation and histone acetylation in the cells of male gametophyte *Hyacinthus orientalis*. Using immunofluorescence methods we examined the localization of heterochromatin (5metC and HDT1) and euchromatin (acH4) markers in the mature pollen grain and in vitro growing pollen tube.

Our research has shown that after anthesis during the male gametophyte growth there are changes in the distribution of epigenetic markers which were tested. In the pollen tube and generative cell nucleus we observed a reduction in the level of 5metC and increased levels of acH4. This indicates that in the nuclei of these cells DNA demethylation and euchromatynization takes place.These processes are accompanied by changes in the distribution pattern of the enzyme HDT1. After the division of the generative cells in both the vegetative and sperm nuclei we observed increase in DNA methylation and progressive decrease in the level of acH4 as well HDT1. Those processes are probably associated with the strong chromatin condensation and transcriptional inhibition of those nuclei.

Our observations in the confocal microscope showed that exceptional place of changes in the distribution pattern of analyzed epigenetic markers are areas of vegetative and generative nucleus which are located close to each other.

P.3.40. *Phaseolus vulgaris* – A plant regeneration system for common bean

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Keywords: common bean, regeneration, direct organogenesis

The common bean is the most widely cultivated of Phaseolus species worldwide since it is a very important source of protein and calories. However, a number of biotic and abiotic stresses severely affect the yield of this crop. At present, several genetic engineering approaches are used for common bean improvement, but still plant regeneration employing published protocols remains uneasy to reproduce. Therefore, here we present the results of optimization of regeneration procedure for Polish varieties of *Phaseolus vulgaris*. Six commercial cultivars of common bean: Czerwona, Laponia, Ibiza, Złota Saxa, Gold Pantera and Plus were tested for regeneration efficiency.

P.3.41. Polyamines-dependent regulation of NO metabolism during dormancy alleviation and germination of apple embryos

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Keywords: dormancy, germination, GSNOR, nitration, polyamines

Apple (*Malus domestica* Borkh.) seeds are characterized by deep embryonic dormancy, leading to inhibition of germination. Dormancy of apple embryos may be overcome by treatment with phytohormones (e.g. gibberellins) or other plant growth regulators, including NO and polyamines (PAs: Putrescine-Put, Spermidine-Spd). Moreover, the application of these PAs leads to alteration in RNS emission, namely NO and ONOO- (the product of the reaction of NO and O2•-). In contrast, Spermine (Spm), similarly to NO scavengers, is responsible for dormancy maintenance and lowers tissue NO content.

Level of RNS depends on their biosynthesis, scavenging or reactivity with other cellular compounds. Therefore, the goal of our work was to investigate: 1) the activity of enzymes engaged in NO metabolism, 2) NO-dependent posttranslational modification of protein (nitration), influencing RNS level during PAs regulated dormancy elimination and germination of apple embryos. Stimulation of embryos germination by PAs and NO was associated with increased NOS-like and nitrosogutathione reductase activity. Activation of germination correlated also with fluctuation in protein nitration pattern.

We assume that RNS level alteration is due to PAs induced regulation of NO metabolism and is necessary for dormancy alleviation and germination of apple embryos.

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P.3.42. Regulation of LEC transcription factors through PICKLE and gibberellins during induction of somatic embryogenesis in *Medicago truncatula* cv. Jemalong

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Keywords: PICKLE, somatic embryogenesis, gibberellins

Plant somatic embryogenesis is process of forming embryos from somatic cells of the plant explants without the fusion of gametes. During the induction phase development program is switched from somatic to embryogenic, what is associated with the temporary activation or deactivation of different sets of genes, including *LEAFY COTYLEDON (LEC)* genes. Process of gene inhibition can be carried out by chromatin remodeling factors (CHR), i.e., PICKLE (PKL), PICKLE RELATED 1,2 (PKR1,2) or CHR5. PKL interacting with gibberellins is involved in the suppression of embryogenic characteristics during seed germination. Inhibition of gibberellins biosynthesis in *pkl* seedlings resulting in an enhanced expression of embryonic traits. *Arabidopsis thaliana* protein sequences of selected genes were used to protein BLAST searching against *Medicago truncatula* 4.0.1 database and four candidate genes after bioinformatics analysis were selected for further study. Genes expression profiles during SE were analyzed at five time points of the induction in two *M. truncatula* lines: non-embryogenic (NEL), embryogenic (EL) and embryogenic after GA3 treatment (EL+GA). The results indicate activity of *PKL*, *PKR1* and *CHR5* during SE induction phase. Transcription level of these genes in EL was lower for 2 days and increased in the following days of the induction compared to NEL. Despite a similar transcription level of *CHR* genes in both lines, *LEC* genes (*LEC1, L1L, LEC2, FUS3, ABI3*) were activated only in EL. Expression all of *LEC* genes were reduced after GA3 treatment. Results might suggest that regulation of *M. truncatula* SE probably is not PKL but might be gibberellin dependent.

P.3.43. RNA sequencing as a method of choice for the identification of genes differentially expressed between male and female gametophytes producing sex organs in simple thalloid liverwort *Pellia endiviifolia* sp B

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Keywords: liverworts, Pellia endiviifolia, sexual reproduction

Among liverworts, the most basal lineage of bryophytes, there is almost no data about the genes controlling the transition from vegetative to generative phase of life cycle. This puts liverworts in critical evolutionary position to investigate the genetic basis of key innovations which allowed them to survive in demanding terrestrial environment and to give fertile offspring.

We have chosen *Pellia endiviifolia* species B, a dioecious liverwort from class Jungermaniopsida to profile the differences in transcripts level between different stages of the male and female thalli development. We applied RNA-sequencing to identify genes engaged in the antheridia and archegonia production using four different developmental stages: the male thalli i) producing or ii) without antheridia, the female thalli iii) producing or iv) without archegonia. To select genes with the highest differences in expression between the male/female thalli producing/not producing sex organs bioinformatics analyses were performed with criterion log2_fold_change ≥ 10: 72 DEGs were selected. Out of 10 genes up-regulated in sperm-producing male thalli, 8 are also expressed in the vegetative male thalli; out of 62 up-regulated genes in archegonia-producing female thalli, 46 are also expressed in the vegetative female thalli. Real-time PCR analysis was performed which validated 9 male and 47 female specifically expressed genes.

Our studies provide possibility to learn about the gene expression regulation within the representative of genus Pellia, which is recognized as the one of the most basal lineage of simple thalloid liverworts.

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P.3.44. Searching for the roles for *A.thaliana* AlkB dioxygenases in alkylating lesions repair and beyond

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Keywords: AlkB, ALKBHs, Arabidopsis, alkylation

AlkB protein, primary discovered and studied in *Escherichia coli*, is a non-heme iron (II) and α-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in nucleic acids recovering natural bases A and C, respectively. Our and others bioinformatical analysis has shown the presence of *E.coli* AlkB homologs in almost all organisms. In *Arabidopsis thaliana* we identified 13 homologs of bacterial AlkB protein (AtALKBHs).

We have found four proteins: AtALKBH1C, AtALKBH6, AtALKBH9A and AtALKBH9C changing their cellular localization after MMS treatment from cytoplasmatic to nuclear, indicating possible involvement of these proteins in alkylating lesions repair in DNA. We have also characterized the expression level of all 13 AtAlkB homologs before and after treatment with alkylating agents: MMS, CAA, and MNNG, and potent DNA crosslinker – Mitomycin C. We found that the expression level of *ALKBH9A, ALKBH10A* and *ALKBH10B* is increased after CAA treatment likely indicating the role of mentioned proteins in ε-adducts repair in DNA. Using fluorescence-monitored thermal unfolding, we have confirmed that the ALKBH10B protein binds its cosubstrate - α-ketoglutarate and cofactor - Fe(II), thus, it may act as dioxygenase. However, it does not show oxidoreductase activity towards 3meC and εA under the conditions tested. Additionally, we found 362 potential partner proteins of AtALKBH10B protein using Pull-Down and Mass Spectrometry approaches, and we selected 10 potentially interesting interactors for further analysis in YTH system.

Our results indicate that the multiplicity of ALKBHs in Arabidopsis may correspond to their functional diversification and besides alkyl lesions repair, involvement in other cellular processes.

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P.3.45. SERRATE - a key protein involved in the crosstalk between microprocesor and spliceosome

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Keywords: microRNA, splicing, SERRATE, U1snRNP

MicroRNAs (miRNAs) are small noncoding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. Most of plant *MIR* genes are independent transcription units that encode long primary miRNA precursors which usually contain introns. For two *miRNA* genes, *MIR163* and *MIR161*, we showed that introns were crucial for the accumulation of proper levels of mature miRNA163 and miRNA161. Removal of the intron in both cases led to a drop-off in the level of the mature miRNA. We also demonstrated that the stimulating effect of the intron mostly reside in the 5'ss rather than on a genuine splicing event. Our results have suggested that in the communication between the spliceosome and the miRNA biogenesis machinery U1 snRNP is involved. To characterize this molecular interplay we decided to test interactions of key factors of the plant miRNA machinery with all ten *A. thaliana* U1snRNP proteins. We have found that the SERRATE protein (SE) interacts with PRP39b, PRP40a, PRP40b and LUC7rl of U1 snRNP. Our results suggest that the interplay between CBC, SE and U1 snRNP is crucial for the crosstalk between the plant spliceosome and the miRNA biogenesis protein complex. Our findings on the functional connection between splicing and miRNA biogenesis in plants are biologically significant as the presence of functional splice sites in the *MIR163* gene appears mandatory for pathogen triggered accumulation of miR163 and proper regulation of at least one of its targets.

P.3.46. Shoot apical meristem arrest due to the oxidative damage in Arabidopsis lacking FtsH4 protease

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Keywords: AtFtsH4 gene, SAM, oxidative stress

Shoot apical meristem (SAM) is necessary for the continuous growth and development of a plant, ranging from the vegetative phase, through the inflorescence formation, until setting-up seeds. We study the cellular and molecular mechanisms underlying the SAM arrest during growth of Arabidopsis, lacking mitochondrial protease AtFtsH4, under unfavorable conditions. This protease belongs to the family of the AAA-proteases, and it is strongly up-regulated during the transition to flowering, what correlates with its predominant impact on the SAM indeterminate growth. As investigated macroscopically, 10% of *ftsh4* mutant plants were not able to form inflorescences and the inflorescence stems of remaining plants were significantly reduced. Our results indicate that the *ftsh4* mutants, due to internally accumulating oxidative damage, abruptly terminate the SAM, either without affecting meristem organization, or by using up its cells for primordia formation. Ultimately this leads to the female sterility. The results of our research will be discussed in the context of the role of *AtFtsH4* gene for the indeterminate SAM growth by preventing the oxidative damage.

P.3.47. Study of the interaction between Arabidopsis CPK3 and 14-3-3 omega using a two-hybrid approach

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Keywords: Calcium-dependent protein kinase, 14-3-3 proteins

In our study, the interaction between CPK3 and 14-3-3 omega was evaluated. In this interaction, the roles played by a putative 14-3-3-binding site identified in CPK3 and by a 14-3-3 site phosphorylated by CPK3 were assessed. For this purpose, we performed a yeast two-hybrid assay using the AH109 *Saccharomyces cerevisiae* strain. All vector constructs were prepared using Gateway technology and point mutations in CPK3 and 14-3-3 omega were obtained by site-directed mutagenesis. Six different forms of CPK3 were tested: the wild type protein, a catalytically inactive version (K107M), a constitutively active truncated form lacking the regulatory calcium-binding domain and its inactive version (K107M), mutated or not, in one or two serine residues of a putative 14-3-3-binding site. Concerning 14-3-3 omega, three forms were tested: the wild type protein and two versions where the serine residue at the position 62 is mutated to a non phosphorylatable alanine (S62A) or to a pho sphomimetic aspartate. We also examined the homo/heterodimerization of these different forms of 14-3-3omega.

Due to a possible deleterious effect in yeast observed with the constitutively active form, only interaction results involving variants of the truncated catalytically inactive CPK3 form are presented.

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P.3.48. The abscisic acid content and expression of its metabolic genes during maturation of triticale grains

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keywords: abscisic acid, metabolism, maturation, grains

Abscisic acid (ABA) is a plant hormone that plays crucial role in seed development, acquisition and maintanace of dormancy. The content of ABA in seeds is the result of balance between two simultaneously occurring processes, its biosynthesis and catabolism. 9-cis-epoxycarotenoid dioxygenase (NCED) is the key enzyme in the biosynthesis pathway of ABA, however in seeds, zeaxanthin epoxidase (ZEP) may also play an important role. Inactivation of ABA occurs either by hydroxylation, mainly catalyzed by 8'-hydroxylase (OH), or conjugation with glucose which is catalyzed by glucosyltransferases (GTs).

An increase of ABA content in embryos of triticale grains occurred during mid-maturation phase and reached the highest value on 35 day after flowering (DAF). The ABA content decreased on 40 DAF and remained at relatively low level until grains reached full maturity. The ABA concentration was higher in embryos of cultivar more resistant to pre-harvest sprouting (Fredro) than in cultivar more susceptible to this adverse phenomenon (Leontino). The changes in ABA content were correlated with the expression of genes encoding TsNCED1, TsNCED2 and putative TsGT1, whereas the expression of *TsZEP, TsOH1, TsOH2* did not change significantly during maturation phase. The highest level of *TsNCEDs* mRNA, was observed in the period preceding and during the peak of ABA accumulation and was higher in embryos of Fredro cultivar, while the highest mRNA level of putative TsGT1 was observed at late-maturation phase of both cultivars. The obtained results suggest that TsNCEDs and putative TsGT1 are involved in the regulation of the ABA content in triticale grains.

P.3.49. The analysis of antioxidants, catalase (CAT) activity and glutathione (GSH) concentration in 2,4-D-induced embryogenic culture of Arabidopsis

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Keywords: oxidative stress, somatic embryogenesis, 2,4-D

Stress conditions are believed to significantly impact plant development and in support stress factors were indicated to trigger embryogenic development in tissue cultured *in vitro*. Accordingly, 2,4-D used commonly to induce SE is assumed to trigger a stress response in cultured tissue resulting in induction of embryonic development. Thus, to elucidate a relation between 2,4-D treatment, SE induction and oxidative stress level, activity of antioxidants were analyzed in cultured explants. To this end, activity of catalase (CAT) and concentration of glutathione (GSH) was evaluated in Arabidopsis explants cultured on 2,4-D (E5) and hormone-free (E0) medium and the cultures were sampled at 0; 1; 3; 5; 10 and 15 day.

A drastic surge of the CAT activity and GSH concentration was observed in explants cultured in vitro, regardless of hormonal composition of the medium used. However, explant cultures induced on E5 and E0 media were found to substantially differ in activity of these antioxidants. Accordingly, we observed a lower CAT activity and higher GSH concentration in 2,4-D treated explants undergoing SE induction (E5) in comparison to the explants developing seedlings on hormone free E0 medium. These results imply the increased level of oxidative stress, related to production of reactive oxygen species (ROS), to be associated with 2,4-D-induced embryogenic culture.

P.3.50. The contribution of lipoxygenase to activation of flower abscission processes in *Lupinus luteus*

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Keywords: lipoxygenase, LlLOX gene, jasmonates, lupin

Lupin (*Lupinus luteus* L.) is one of the most widely distributed crops in Europe due to its beneficial health and nutritional properties. The manipulation of abscission in this species and genetic modification of particular genes involved in the regulation of the flower detachment are therefore crucial from agricultural and economic point of view and could contribute to increases in both the quantity and quality of yield.

Recent studies indicate that generation of membrane breakdown products affects their functionality and integrity and is the first step in the lipid signaling pathways that lead to abscission. Genes encoding this pathway components, are up-regulated in the abscission zone (AZ) including: the genes encode a lipid binding protein, GDSL lipase/hydrolase, fatty acid desaturase, lipid transfer protein and oleosin. Several researchers have reported that application of jasmonates, generated via the lipoxygenase pathway, hastened organ abscission (or loosening of fruit) in crops such as citrus and tomato, which confirms their contribution in separation processes.

The main purpose of our research was to analyze the role of lipoxygenase, enzyme of jasmonate biosynthesis, in *L. luteus* AZ activation. We showed that induction of abscission is correlated with strong *LlLOX* cDNA accumulation and also protein content and activity. Additionally, LOX protein was specifically localized within AZ cells and that changes are tightly linked with elevated endogenous JAs (JA and MeJA, methyl jasmonate) content.

On the basis of our results we suggest, that lipoxygenase may be a potential key player in lipid-signaling pathway directly controlling *L. luteus* floral AZ activation.

P.3.51. The effect of BAP and kinetin on butterworts (*Pinguicula* sp. L.) micropropagation

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Keywords: micropropagation, in vitro, pinguicula, cytokinins

In this study the effect of two cytokinins on *in vitro* micropropagation of 6 different species of cold temperate Pinguicula was tested.

Seeds were germinated on Orchimax medium supplemented with 15 mg/L GA3. After germination seedlings from each species were transferred onto Orchimax medium supplemented with 1 mg/L kinetin or 2 mg/L BAP. After every 3 weeks plants were passaged on the fresh medium. Finally the effectiveness and productivity for both methods were calculated based on the number of regenerated plants.

The highest germination rate (70%) was observed for *P. lusitanica* and *P. macroceras*. The micropropagation study showed that the highest productivity rate was estimated for *P. lusitanica* with nearly 100 plants produced per 1 seed independently from cytokinin used to supply the medium. *P. longifolia* subsp. *longifolia* and *Pinguicula vulgaris* f. *bicolor* showed very low germination rate, nevertheless significantly higher productivity was observed for medium supplied with BAP compared to supplementation with kinetin. Our study showed that presence of investigated cytokinins efficiently induced regeneration of Pinguicula species, although more distinctive effect of micropropagation was observed in plants treated with kinetin, compared to BAP.

Various species of cold temperate Pinguicula could be successfully micropropagated using our *in vitro* protocols. However effectiveness for seed germination and productivity of micropropagation is highly species-specific. Micropropagation could be considered as a fast and cost effective method for Pinguicula cultivation which can be used in restoration of declining natural populations.

P.3.52. The effect of brassinosteroid on the growth, chlorophyll, monosaccharide and protein content in cucumber (*Cucumis sativus* L.) seedlings

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Keywords: cucumber seedlings, phytohormone, 24-epibrassinolide

Brassinosteroids are plant hormones that play an important role in the plant growth and development. They demonstrate high biological activity and are significant for many physiological processes, because they participate in cellular development and multiplication, vascular differentiation, male fertility, and even in leaf development and aging. The effect of 24-epibrassinolide (24-epiBL) in the range of concentrations 10-12-10-5 M on the growth and biochemical response of cucumber (*Cucumis sativus* L.) seedlings was studied. The study involved changes in the length of shoots and roots, and the contents of photosynthetic pigments (chlorophylls and carotenoids), proteins, and sugars. The study confirmed that at concentrations 10-10-10-7 M 24-epiBL stimulated the seedlings growth, and increased the proteins, sugars, and photosynthetic pigments content. Simultaneously, the highest affect of 24-epiBL was observed at the concentration of 10-8 M. When 24-epiBL was used at 10-10-10-11 M, the length of shoot and root and the biochemical parameters remained at the control levels (untreated plants). The length of shoot and root and the contents of pigments, proteins and sugars were slightly inhibited in plants treated with 10-12 M 24-epiBL. In the contrast, the application of 10-5 M 24-epiBL led to lower shoot and root length, protein, chlorophyll, carotenoid and monosaccharide contents in comparison with control. These results suggest that 24-epiBL at low concentrations (10-10-10-7 M) has a stimulating effect on the growth of cucumber plants. The compound can be used as a bio-regulator in agriculture and horticulture.

P.3.53. The impact of exogenous gibberellins on SVP gene expression in *Lupinus luteus*

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Keywords: Lupinus luteus, gene expression, LlSVP,GA

In plants, the initiation of flowering is an important developmental transition as it represent the beginning of the reproductive phase. The transition from vegetative growth to flowering is induced by molecular pathways that respond to environmental and internal cues.

The MADS box transcription factor SHORT VEGETATIVE PHASE (SVP) is one of critical inhibitor of flowering that directly represses transcription of integrator genes. Reduced integrator genes expression results in the maintenance phase of the vegetative growth of the plant.

Research on the identification and the SVP activity has been conducted on yellow lupine, varieties Taper. This species has a high potential yield, limited by excessive abscission generative organs. The reason for this negative phenomenon are both plant hormones and the prevailing environmental conditions.

The aim of this study was determine expression of SVP in vegetative organs of yellow lupine. Determination of transcriptional activity was performed by RT-qPCR using UPL probe. To determine SVP expression the plants were grown in natural conditions in several variants with different sowing date. Preliminary results show the changes in gene expression SVP of yellow lupine vegetative organs under the influence of applied exogenous gibberellins.

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P.3.54. The impact of plant hormones on expression of FLD gene from the flowering autonomous pathway in *Lupinus luteus*

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Keywords: FLD, Lupinus luteus

The flowering time is one of the factors providing reproductive success. Seven genetically defined pathways have been identified that control flowering: the vernalization and ambient temperature pathway, the photoperiod and quality of light pathway, the gibberellin or hormonal pathway, the physiological maturity pathway and the autonomous pathway. All pathways control of flowering cooperate regulation of key flowering genes which name 'integrator genes' or 'central floral pathway integrators'.

The autonomous pathway of flowering induction genes include 7 genes. All these genes are negative regulators of FLOWERING LOCUS C (FLC) - main inhibitor of generative development.

FLOWERING LOCUS D regulate *FLC* expression by chromatin remodeling. FLD is demethylase of histone H3K4. FLD is a part of component of a histone deacetylase complex (HDAC) initialize demethylation and deacetylation of locus *FLC* and repress the expression of this gene.

In this study, expression of *FLD* gene was quantitative used RT-qPCR technique. Plants were cultivated in natural conditions. The research materials were vegetative organs. Before collected plants were sprayed aqueous solutions of abscisic acid or gibberellins. Plants were collected after 1, 4, 24 hour and seven days after sprayed.

Our results obtained here will enable us to determine *FLD* expression pattern in vegetative organs of *L. luteus* cultivars. It will also facilitate to characterize the role of these genes in the regulation of development of *L. luteus* crops in different growth conditions.

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P.3.55. The involvement of miR169 in somatic embryogenesis induced in vitro in culture of Arabidopsis explants

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Keywords: embryogenic transition; miR169; NF-YA; Arabidopsis

In plants, it has been shown that miRNAs play a key role in regulation of different aspects of development and stress responses. Accordingly, it is assumed that miRNAs control somatic embryogenesis (SE), a developmental process resulted in formation of somatic embryos in *in vitro* cultured explants. In support, numerous genes of different *MIRNA* families were found differentially expressed during SE in Arabidopsis. Among them *MIR169* genes were indicated to be highly modulated (Szyrajew et al., 2012). The study aimed at the verification of the involvement of miR169 molecules in SE.

The analysis showed that during SE the increased level of mature miR169 was related to down-regulation of four target genes, *NF-YA1, NF-YA3, NF-YA5* and *NF-YA8*. The target genes encode NF-YA subunit of nuclear factor Y (NF-Y) that potentially binds to a vast number of eukaryotic genes and control variety of developmental processes including postgermination growth and stress responses. The assumption on the miR169 involvement in SE was further strengthened by observation that explants carrying mutations in *MIR169i* and *NF-YA3* genes are strongly impaired in the capacity for SE. The results infer the relation between miR169 and SE induction however, further analysis is needed to reveal other genetic elements involved in this regulatory pathway.

P.3.56. The localization of auxin gradient and expression of *AtPIN1*, *4 7* genes in the transgenic callus of *Arabidopsis thaliana*

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Keywords: auxin, DR5::GUS, AtPIN genes, callus

The mechanism of auxin transport and the formation of its gradient in the callus tissue is very important for the growth and differentiation of the callus cells *in vitro* conditions.

The ability of leaf explants transgenic plants and *pin* mutans to form callus were tested. It has been shown that the leaf explants of all studied lines showed similar ability to form callus (ranging from 93,68% to 99,07%). The overexpression of *AtPIN1* gene in tested transgenic line 35S::PIN1 or lack of functional proteins AtPIN1, AtPIN4 in mutant lines also did not have significant importance in the process of callus formation. In contrast, it has been found an approximate 31,81 % less fresh weight of callus obtained from leaf explants of *pin7* mutants, compared to the control line Wt (Col). It indicates that the *AtPIN7* gene plays an important role in the formation of callus *in vitro* conditions. The localization of the auxin gradient and selected *AtPINs* genes expression during the callus formation *in vitro* conditions was determined using the GUS reporter protein. Blue product of the GUS activity, providing a higher level of auxin, was observed only in the four outer layers, intensively proliferating of DR5rev::GUS callus cells. The localization of *AtPIN1* gene expression included more external layers of callus cells than DR5rev::GUS. In contrast, the localization of the *AtPIN7* gene expression was identified only in cells that most likely will come into being conductive vascular tissue cells in the future.

P.3.57. The new approach for manipulation of gene expression

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Keywords: gene regualtion, epigenetics, β-1,3-glucanase

In order to manipulate a gene expression, genetic engineering is the most commonly used tool to produce genetically modified organisms. Owing to targeted action this is the most effective method but also causes the interruption of the continuity of GMO's genome. Recently the new non-vector technology has been exploited. The epigenetic modification is based on the introducing into the plant cell a short (18-nucleotides), single stranded DNA fragment (OLIGO) homologous to the targeted gene to silence or activate its expression. The aim of this research was to broaden the knowledge of this non-invasive technology by the structural analysis of OLIGO and their influence on the target gene expression, its methylation profile and the expression of epi-genes such as coding for methylases and demethylases and those involved in the histone modification. We conducted a comprehensive analysis of the β-1,3-glucanase gene treated with OLIGOs that are homologous to gene region such as promoter, coding, non-coding, 3'UTR and 5'UTR regions to ascertain the most affected gene region. The analysis of flax seedlings treated with OLIGOs (EMO plant) revealed changes in the expression pattern of β-1,3-glucanase gene and epi-genes. The level of changes depend upon the gene region for which OLIGOs were designed. Thus the OLIGO method is promising for gene function analysis and new EM plant generation.

P.3.58. Transcriptional activity of *Arabidopsis thaliana* female gametophyte cells

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Keywords: embryo sac,trancription, RNA Pol II

The female gametophyte of most flowering plants is composed of seven cells with different biological function. Two synergids attract the pollen tube and one of them becomes the site of delivery of sperm cells. The egg cell and central cell participate in the process of double fertilization. Three antipodal cells have somatic roles and have already begun to degenerate before fertilization. All previous studies (usually performed *in vitro*) show that the nuclei of all these cells are probably characterized by distinct pattern of gene expression and different regulation strategies may be present.

The aim of the present investigations was to determine in vivo the total transcriptional activity of the *A. thaliana* embryo sac cells by analyzing the level and distribution of 1) newly formed transcripts using immunolocalization of incorporated 5'-bromouracil and 2) hypo- (initiation) and hyperphosphorylated (elongation) forms of RNA Pol II. The obtained results have shown that during anthesis in the female gametophyte cells was not observed a characteristic pattern of transcriptional activity. In the mature embryo sac significant differences in the labelling of the nucleus and of the cytoplasm of individual cells were present. In cells targeted by sperm cells a high level of newly formed transcripts and both forms of RNA Pol II as well as their transcriptional silencing were detected. It is possible that observed in vivo different transcriptional activity of these cells is probably related to the maturation of the ovule in the ovary.

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P.3.59. Ultrastructural analysis of plastid biogenesis during *in vivo* development of barley pollen and zygotic embryos

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Keywords: barley, plastid biogenesis, microspore culture

One of the major problems of *in vitro* microspore culture in barley (*Hordeum vulgare* L.) , is the occurrence of albino plantlets among androgenic regenerants. Albinisms may be caused by the degradation and/or rearrangement of plastid genome, but also by the modification of expression of genes located in the nuclear genome, involved in the regulation of plastid biogenesis. Albino plantlets contain non-functional plastids, what prevents their development in the normal growth conditions, beyond the *in vitro* culture. However, the proportion of chlorophyllous and albino plantlets produced in the culture is highly variable among cultivars and in some cases up to 90% regenerated plantlets are deprived of functional chloroplasts. Plastids of haploid androgenic plant cells derived from proplastids of the microspores which were used in the culture. Therefore we decided to analyze the in vivo plastid biogenesis in pollen of two barley varieties: 'Jersey' and 'Mercada' that significantly differ in their ability to regenerate green plants from microspore culture. 'Jersey' produced up to 90% of chlorophyllous plantlets, whereas 'Mercada' provided less than 3% of non-albino plantlets. To compare the *in vivo* plastid biogenesis between those two cultivars, the ultrastucture of plastids was analyzed under different stages of pollen development: tetrad, early stage, middle-late stage, and mature pollen, as well as in zygotic embryo of both cultivars was investigated. Using transmission electron microscope, the total number of plastids and number of dividing plastids was counted, additionally, the process of *in vivo* plastid biogenesis in barley pollen and zygotic embryo was described.

SESSION 4: PLANT-MICROBE INTERACTIONS

Plenary Lectures

Signaling in plant immunity

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Plants detect potential pathogens in their environment via pathogen-associated molecular patterns (PAMPs) that are recognized by plant plasma membrane receptors. Typical PAMPs include the bacterial flagellin-derived flg22 peptide, the elf18 peptide of the bacterial elongation factor EF-Tu, bacterial peptidoglycans and lipopolysaccharides, as well as fungal chitin oligomers and glucan fragments from oomycetes. PAMP-binding to their receptors initiates complex signaling networks that activate a multi-component defense response and thereby establish PAMP-triggered immunity.

One of the earliest detectable responses after PAMP perception is the activation of ion channels at the plasma membrane. Using a transgenic Arabidopsis line with the calcium reporter, aequorin, increases in cytosolic calcium levels are detected after PAMP application. To identify signaling network components, seeds of aequorin-expressing lines were mutagenized and the population screened for mutants with changed calcium elevation (*cce*) in response to different PAMPs. Among several receptor complex components, a lectin S-domain receptor kinase was identified, which mediates lipopolysaccharide sensing in *Arabidopsis thaliana*.

MAPK cascades are essential for controlling defense responses. The elements that prevent erroneous signaling crosstalk may include expression patterns of the MAPK components, the presence of pathway-specific protein complexes or the MAPK substrate diversity. Different strategies have been employed to isolate MAPK interacting proteins. Several VQ-motif containing proteins are MAPK substrates and regulate immune responses.

Pathogenomics of *Verticillium* wilt diseases

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Verticillium dahliae is a soil-borne pathogen that aggressively colonizes hundreds of host plants, including high-value crops such as tomato and potato, leading to the formation of vascular wilt disease. Resistance factors in the host population exert selective pressure on the pathogen forcing the rapid evolution of adaptive traits to participate in the arms race with the host. By comparative genomics on a *V. dahliae* population, we recently revealed extensive genomic rearrangements that facilitate the gain and loss of genetic material and the establishment of highly dynamic lineage-specific (LS) regions. LS regions are enriched for transposable elements (TEs) and in planta-induced effector genes encoding secreted proteins that significantly contribute to aggressiveness towards the host, and thus have been hypothesized to contribute to the genome plasticity required for adaptive genome evolution. However, factors that drive genome plasticity in *V. dahliae* remain enigmatic. Using single molecule real time sequencing, we re-sequenced two *V. dahliae* strains and analyzed the previously identified genomic rearrangements in unprecedented detail, revealing multiple genomic breakpoints at the nucleotide level. We established that genomic breakpoints are flanked by multiple TEs, suggesting that these elements play essential roles in their formation. Currently, we are using Nanopore sequencing to generate long reads that can be used to improve genome assemblies of *V. dahliae* strains. The aim is to generate genome assemblies in which highly repetitive LS regions are fully assembled. Using the improved genome assemblies, we will investigate the origin of the LS regions and their role in determining host specificity of *V. dahliae*.

Foundations of Quantitative Disease Resistance in Cereals and Brassicas

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There have been tremendous advances in our understanding of the plant immune system in recent years. Our challenge now is to use this knowledge to improve disease control in crops. Breeders are increasingly using quantitative disease resistance (QDR) which is considered broad-spectrum and durable. Our research is focussed on the first layer of active defence in plants, the perception of pathogen (or microbe) associated molecular patterns (PAMPs/MAMPs) leading to PAMP-triggered immunity (PTI). PAMPs are essential molecules, conserved in entire kingdoms of microbes, and are recognised by pattern recognition receptors (PRRs) in plants. Since these PAMPs are essential, and cannot be lost or mutated, resistance based on PTI is potentially durable and broad spectrum. We have developed methods to study PTI in brassicas and cereals in order to study how this contributes to QDR.

One of the earliest responses to PAMPs is the production of reactive oxygen species (ROS) which can readily be measured using a luminol-based assay widely used in Arabidopsis research. We developed the ROS assay and other methods to study PTI in *Brassica napus* (Lloyd et al. 2014 MPMI 27: 286). Using a diversity panel of 94 *B. napus* cultivars, we demonstrated wide variation in ROS production and also resistance to *Pseudomonas syringae* and *Botrytis cinerea*. Transcriptome sequences of all cultivars in the diversity panel are available enabling associations between PTI, disease resistance and expressed genes to be made. In collaboration with European partners, we are now developing this method of 'Associative Transcriptomics' to establish how PTI contributes to QDR against the major pathogens of *B. napus*, including *Sclerotinia sclerotiorum*, *Leptosphaeria maculans, Alternaria brassicicola* and *Verticillium longisporum*. This work should lead to the identification of QDR gene loci that can be used for breeding disease resistance against these important pathogens of *B. napus*.

We found that the ROS assay was not reliable for measuring PTI in cereals, and so developed a method based on early gene induction to investigate PTI in wheat and barley. One of the well-studied PRRs in Arabidopsis is the Elongation Factor-Tu (EF-Tu) Receptor (EFR). The EF-Tu epitope elf18 is detected by EFR in Arabidopsis to elicit PTI. We generated transgenic wheat plants expressing EFR and showed that they gained the ability to detect elf18 (Schoonbeek et al 2015, New Phytol. 206: 606). The transgenic wheat plants also had enhanced resistance to *Pseudomonas syringae*, a pathogen of wheat. The work demonstrates that PTI signalling components are conserved between monocots and dicots. Arabidopsis genes known to be involved in PTI could therefore have similar functions in dicots and monocots, and so this research could provide new insight into the basis of QDR in cereals.

A hidden pathogen with uncommon properties – reviewing the state-of-the-art of *Verticillium longisporum* on oilseed rape

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Keywords: vascular diseases, premature senescence, cultivar resistance, pathotypes, drought resistance, resistance mechanisms

Verticillium longisporum (VL) is a soilborne vascular pathogen of oilseed rape (OSR; *Brassica napus*) which causes premature ripening associated with potential significant yield losses. First reports on the disease date back to the 1960s in Sweden and the 1980s in North-East Germany, but more recently outbreaks of the disease were observed in the UK, France and Poland. In 2014, occurrence of VL has been confirmed for the first time in Canada (Manitoba). The pathogen is an amphihaploid hybrid form of Verticillium and host-specific on Brassicaceae. Three different hybrids representing three genetically distinct lineages have been identified so far and were shown to differ in host specificity (Inderbitzin et al. 2015).

VL survives with microsclerotia in the soil which germinate in close vicinity to roots upon stimulation by root exudates. Hyphae of VL attach to the roots in the root hair zone and penetrate the rhizodermis to colonize the root parenchyma intra- and intercellularly (Eynck et al. 2007). The lack of ROS generation or cell death responses by the plant during these early stages of interaction implies a biotrophic or even endophytic relationship. Plants are colonized systemically in a conspicuous tri-phasic manner. Following root invasion, the fungus remains strictly xylem-limited and colonizes the hypocotyl, where intense plant responses are triggered as expression of cultivar resistance (Eynck et al. 2009). Resistance derives from the C-genome of *B. napus* (AACC). Expression of cultivar resistance in the hypocotyl consists of massive parenchymatic accumulation of cell wall-bound phenols, lignin and vessel occlusions, obviously halting the pathogen from further spread into the shoot.

None of the drought stress parameters analyzed (stomatal conductance, transpiration rate, gas exchange, photosynthesis rate, proline content, leaf water content) responded to infection, thus substantiating that VL does not induce any wilt and indicating that responses in resistant genotypes to infection do not compromise drought resistance.

Upon VL infection, salicylic acid (SA) dependent genes PR-1 and PR-3 are up-regulated while the jasmonic acid/ethylene dependent PDF1.2 is not. VL induces conspicuous elevations in levels of SA/SAG in xylem sap and stem parenchyma, which surprisingly correlate with susceptibility and disease severity. Increased levels of SA/SAG in diseased plants may indicate a fungus-induced redirection of precursors from the phenylalanine/cinnamate pool towards SA synthesis depriving the synthesis pathway towards CW-bound phenolics crucial for resistance. In contrast, SA deficient nahG transformed OSR plants exhibit a strongly elevated susceptibility to VL. These findings imply a dual role of SA in basal and cultivar-specific resistance of *B. napus* to VL.

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Selected Talks

T.4.1. Elucidation of mechanisms underlying virulence function of *Pseudomonas syringae* HopQ1 effector in plant cells

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Keywords: Pseudomonas syringae, MAPKs, TTSS effector

HopQ (for Hrp outer protein Q1) is a type three effector secreted by many strains of *Pseudomonas syringae*, a gram-negative bacterium that infects a wide range of plant species. After delivery into plant cells, HopQ1 is phosphorylated and binds to host 14-3-3 proteins. This interaction affects stability and subcellular localization of the effector. However, a mutation that prevents binding between 14-3-3 and HopQ1 only slightly compromises bacterial growth in plants. Thus, 14-3-3 proteins seem to play an accessory role in HopQ1 virulence function, possibly in bridging the effector to a bona fide virulence target. Since HopQ1 has been previously shown to inhibit MAPKs activity, whose function depends on 14-3-3 proteins, we hypothesize that the effector co-opts 14-3-3 proteins to interfere with MAPKs immune signaling in plant cells. Interestingly, two strain-specific variants of HopQ1, that differ only at a few amino acid positions, show considerable differences in their ability to suppress MAPKs activities. Furthermore, the HopQ1 variants are diversely susceptible to proteolytic cleavage by an unknown plant protease, which might regulate function of the effector in plant cells. These data prompted us to test over 100 P. syringae strains to find amino acids involved in strain-specific functions of HopQ1. It is proposed that the differences between HopQ1 homologs are a consequence of an adaptation of the effector to a given host of bacterial strain. We are currently testing this hypothesis.

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T.4.2. Screening of potato plants (*Solanum tuberosum L.*) and water samples for the presence of pectinolytic bacteria originating from potato fields in Poland

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Keywords: Dickeya, Pectobacterium, blackleg, soft rot

Poland is 7th top producer of potatoes worldwide (FAO 2012). Economic losses connected with soft rot and blackleg diseases caused by bacteria classified to genera *Pectobacterium* and *Dickeya* can reach even 30% of total crop yield (Czajkowski et al., 2011). Importantly, there are no chemical or biological methods to treat these diseases, so prevention approach became crucial. Therefore, our group monitors potato fields and surface waters annually, in search of pectinolytic bacteria. We perform cultures of plant homogenates and water samples obtained from The State Plant Health and Seed Inspection Service in Poland on selective CVP medium in 28°C. Identification of isolated *Pectobacterium atrosepticum* (*Pba*), *Pectobacterium carotovorum* (*Pcc*)/*Pectobacterium wasabiae* (*Pwa*) and *Dickeya* sp. (*Dsp*) is based on multiplex PCR (Potrykus et al., 2014) and PCR reactions with specific primers for Pwa (De Boer et al., 2012) and *D. solani* (*Dsol*) (Prichard et al., 2012). Percentage of different pectinolytic bacterial species was determined for plant and water samples in 2013 and 2014 in comparison to the results from previous years. Interestingly, most virulent species, *Dsol* and *Pwa*, were identified only in plant material, not in water samples. Besides, *Pectobacterium* spp. are widespread in Poland, while *Dickeya* spp. are limited to certain provinces. The level of virulence determinants pivotal for plant tissue invasion (motility), iron acquisition (siderophores) and maceration of potato tissue (pectinases, proteases, cellulases) was determined for *Pwa* strains that were the most abundant species in Poland in 2013.

T.4.3. Effect of ectomycorrhizal symbiosis with two different strains of *Paxillus involutus* on the features of Populus x canescens seedlings

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Keywords: Ectomycorrhiza, poplar, symbiosis, in vitro

Ectomycorrhizal (ECM) fungi, essential partners of plants, are most often found in symbiosis with vascular plants. Moreover, such symbiosis is obligatory for almost all tree species common in Poland. In consequence, the ECM symbiosis plays a very important role in responses of trees to environmental conditions and should be taken into account, particularly in analyses of poplar response to various stresses.

It is well known that the ECM symbiosis generally enhances growth of the poplar host as well as its resistance to stress conditions, e.g. due to improvement of the plant nutrient and water uptake, in exchange for carbohydrates. However, interactions between the two living organisms are varied and depend on numerous conditions, especially on the characteristics of particular symbiotic partners, which additionally may vary during long-term cultivation.

In this work, we present the results of an experiment performed on poplar, *Populus x canescens*, growing *in vitro* in control conditions and in the presence of lead ions and inoculated with two strains of *Paxillus involutus*. The field-collected strains were selected according to theirs values of indices of tolerance for lead ions in the pure culture. Those strains *Paxillus involutus* showed also significantly different levels of root mycorrhization of poplar roots. The differences not only affect the biometric features of inoculated Populus x canescens, but also in the mineral composition and leaf proteomes of poplars.

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T.4.4. Are the *Phytophthora infestans* population, R gene or effector expression changes responsible for the potato resistance decline within single vegetation season?

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Keywords: Phytophthora infestans, potato, plant resistance

Phytophthora infestans (Mont.) de Bary, which belongs to Oomycetes, is the most destructive potato (*Solanum tuberosum L.*) pathogen worldwide. This organism causes late blight. *P. infestans* secretes hundreds of proteins, which promote infection. Effectors are pathogen's molecules that manipulate host cell structure and function facilitating infection and, when recognized by the host resistance proteins, triggering defense responses. To find out why plants with *Rpi-phu1* gene show disease symptoms at the end of the vegetation season we monitored *P. infestans* population structure in the experimental field. Moreover, R gene and effector expression were evaluated in infected potato leaflets.

Samples with single lesions were collected in 2014 year from field in Młochów. Half of a leaflet was frozen in liquid nitrogen and used to analyze the R gene and effectors expression. The remaining part was used to obtain pure cultures of *P. infestans*. Total number of tested isolates was 89. Mating type, mitochondrial haplotype and diversity of microsatellite markers were determined by PCR. Resistance to metalaxyl was tested on rye A agar media. For virulence scoring, 11 Black`s differentials and new resistance sources were applied in detached leaflets. Expression of effectors and R gene was analyze by a RT-qPCR.

In *P. infestans* population mating type A2 (73%) and mitochondrial haplotype IIa (75%) were dominating. Most of the isolates were resistant to metalaxyl (55%). Analysis of effectors, R gene expression, virulence and SSR are in progress. Getting to know pathogen population structure within single vegetation season is helpful in integrated potato production management.

Poster Presentations

P.4.1. A kinetics of induced antioxidative defence in pea leaf cells upon infestation of pea aphid at varying population size

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Keywords: signaling molecules, flavonoids, pea, aphid

A complex signaling network contributes to a coordinated regulation of gene expression leading to the specific stimulation of plant defence responses to aphid attack. We revealed a differential induction of defence signaling molecules in response of *Pisum sativum* L.cv. Cysterski seedling leaves to the infestation of *Acyrthosiphon pisum* (Harris) at a varied population size. In time from 0 to 96 h after *A. pisum* infestation these signal molecules accumulated transiently. The convergence of signaling pathways was observed. The present study revealed a kinetics of induced antioxidative defence (the accumulation of flavonoids, including pisatin) in the context of allocation of carbon in pea leaf cells upon infestation of *A. pisum* at varying population size. A strong accumulation of flavonoids was mainly detected in whole cells, while in the early stage of infestation in leaf cells the high content of these metabolites was mainly recorded in the cell wall. Additionally, has been demonstrated to an induction of the expression of the flavonoid biosynthetic genes in leaf cells of pea in response to infestation of *A. pisum*. The innovative also was to show the effect of exogenous NO on the feeding process of pea aphids on leaves of pea seedlings using electronic feeding graphs (EPG), and determining whether NO may modulate defence responses in pea leaves.

These results contribute to a better understanding of the regulatory mechanisms during plant-aphid interactions bringing new knowledge to for contemporary plant biology.

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P.4.2. Are green islands photoperiod-dependent? *Brassica juncea-Alternaria brassicicola* compatible interaction

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Keywords: biotic interactions, green islands, photosynthesis

Formation of green islands is rather a rare phenomenon during biotic stress, especially during plant-fungus interaction. Although we investigated black spot disease of different Brassicas species, green islands were observed only on *Brassica juncea* leaves infected with *Alternaria brassicicola*.

In this work green islands formation have been investigated under different photoperiod treatment: 8 h day/16 h night, 12 h day/12 h night, 16 h day/8 h night and under continuous light. Plants were infected with *A. brassicicola* spore suspension at 4-5 leaf stage. We have discovered two patterns of green islands formation at 48-72 hours post inoculation. Expended green islands were formed mainly on first and second leaf under continuous light and 16 h day/ 8 h light, only small necroses on younger leaves (third and fourth) were observed. Green islands restricted to the ring round the necrosis were observed on first and second leaves of plants grown under all light conditions and occasionally also on the third leaves. It has to be emphasized that not all infected plants shown this phenomenon. Analysis of chlorophyll content and fluorescence and also changes in basic photosynthesis parameters were performed for first and third leaves of plants grown under all light conditions and microarray analysis was performed for first and third leaves of plants grown under continuous light. In addition starch analysis was also conducted.

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P.4.3. Between phytohormone response and symbiotic interactions– exploringthe role of ABC transporters

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Keywords: ABC transporters, cytokinins, symbiosis, Medicago

Legume plants have a unique ability to form intimate, intracellular associations with nitrogen fixing rhizobial species. Establishment of symbiosis and formation of highly organized root organs, called nodules, arestrictly controlled by phytohormone factors. Cytokinins play crucial role in symbiosis, acting as a positive and/or negative regulators on different nodulation stages. Activation of the cytokinin signaling pathway in the root cortex leads to the cell division and formation of the nodule primordium and it has been proposed to be a consequence of an active transport process of this phytohormone. Whether this activation is due to increased cytokinin concentrationor sensitivity or both has not been directly determined yet [1]. A major function fulfilled by ABC (ATP-binding cassette) proteins is transport of diverse compounds via cellular membranes. Intriguingly, recent studies in Arabidopsis suggest that ABC transporters might translocate cytokinins[2].

We have identified and classified at least30 full-size ABC transporters from the G subfamily in *Medicago truncatula*.Currently we address a question about a putative role of two selected, root expressed full-size ABCG transporters in the modulation of nitrogen-fixing symbiosis. The conducted ddPCR (Droplet Digital PCR) analyses revealed that expression of these transporters is significantly up regulated upon cytokinin and *S.meliloti* treatment. Understanding the role of ABC transporters in nodulation process and linking it to phytohormone translocation, might contribute to improving existing symbioses and extending them to nonlegumes

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2) Zhang et al (2014) Nat Commun

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P.4.4. Determination of genomic profiles of low and highly virulent *Pectobacterium wasabiae* strains on potato

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Keywords: plant pathogenic bacteria, fingerprinting, plant

Pectobacterium wasabiae (Pwa) is a plant pathogenic, pectinolytic bacterium responsible for soft rot and blackleg disease on economically important plants, including potato (Solanum tuberosum L), horseradish (*Armoracia rusticana*) and ornamental plants. Untill 2009 Pwa strains infecting potato plants were wrongly classified to *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc). After the reclassification of Pcc 3193 strain isolated in Finland to Pwa 3193, virulent Pcc were classified to Pwa species on the basis of their high ability to macerate potato tissue, sequences of recA genes and MLST analysis.

The aim was to define genomic variability of Pwa strains isolated in Poland, Belgium, Finland, The Netherlands and South Africa and to search for a molecular marker(s) of strains with elevated ability to macerate potato tissue. Genomic profiles were obtained in repPCR with BOX, REP and ERIC primers and due to PFGE analysis. Performed analysis revealed the presence of strains exhibiting 6 different REP, variable ERIC, 2 BOX profiles and several PFGE profiles. Because of low variability within BOX and too high in ERIC profiles, REP profiles were chosen for further analysis. Results showed that 10 Polish and 5 Belgian strains indicating the highest ability to macerate potato tubers tissue belong to the REP profile III. The lowest maceration ability was shown for the strains of REP profile I that was isolated from horseradish only. REP profile II characteristic for Pwa 3193 isolated and 18 Polish strains indicates intermediate virulence on potato. Analysis of PFGE fingerprinting is ongoing.

P.4.5. Differential expression of plasma membrane aquaporins of mycorrhized and non-mycorrhized maize in response to drought stress

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Keywords: arbuscular fungi, water stress, aquaporins

The benefit of arbuscular mycorrhizal fungi to plants is mainly attributed to increased uptake of crucial nutrients, especially phosphorus but also, to some extent, nitrogen. Higher tolerance of plants to drought stress may result from symbiotic improvement of aquaporin activity. Their high molecular diversity in plants is related not only to symplastic water movement but also to transport of small organic and mineral solutes, including transfer of CO2 into chloroplasts. Therefore these proteins have a significant influence on a number of physiological processes as photosynthesis, seeds germination and growth, lateral roots formation and maintenance of homeostasis of cells and tissues under stress conditions.

The analysis made previously have shown differential protein levels of plasma membrane aquaporins of two types PIP1 and PIP2 in mycorrhized and non-mycorrhized plants in response to drought stress. In this study we present root and leaves transcriptional expression profiles of six aquaporin isoforms, pip1;1, pip1;2, pip1;5 and pip2;1, pip2;4, pip2;5 which differ according to more complex patterns than at protein level.

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P.4.6. Effect of application of salicylic acid on symptom development caused by *D. solani* on *in vitro* grown potato (*Solanum tuberosum* L.) plants

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Keywords: Blackleg disease, biological control, SAR

Salicylic acid (SA) is widely known as a signaling molecule that plays a key role in establishing a defense response against various pathogenic infections and induces systemic acquired resistance (SAR) in plants. The purpose of this study was to investigate whether application of SA on *in vitro* grown potato plants would reduce infection symptoms caused by *D. solani*. Potato plants were grown for 14 days on MS medium with or without 25 or 50 µM SA prior to inoculation with the pathogen. Plants were analyzed after next 14 days post or without inoculation with GFP-tagged *D. solani* for blackleg development and colonization of potato plants by the bacteria. Our results showed that at 14 days post inoculation 100% of control plants grown on medium without SA showed severe disease symptoms, whereas plants grown on medium supplemented with 50 µM SA did not express any symptoms. The GFP-tagged bacteria were detected by confocal laser scanning microscopy on the surface of the roots of control plants but not on the surface of the plants treated with 50 µM SA 14 days after inoculation.

P.4.7. Involvement of CDPKs and ROS in response of Solanum species to stresses

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Keywords: Solanum, *Phytophthora infestans*, wounding, CDPKs

Plants are constantly exposed to different stresses. Among components of plant signal transduction pathways, Calcium-Dependent Protein Kinases (CDPKs) and Reactive Oxygen Species (ROS) play a significant role in plant defence strategy.

The CDPKs activities, their expression profiles, and ROS production were determined in Solanum species exhibiting different level of resistance in response to the *Phytophthora infestans* that causes late blight. Moreover, expression profiles of CDPKs were estimated in Solanum leaves after wounding. We have examined resistant Solanum species: *S. scabrum* and *S. tuberosum* cv Bzura; and susceptible - *S. tuberosum* clone H-8105. Leaves of Solanum species were treated with culture filtrate of *P. infestans* (elicitor, CF) or wounded with scalpel. Activities of CDPKs were determined using "in gel kinase assay". The expression levels of CDPKs were measured by method of RT-PCR and QPCR. ROS production was estimated using nitroblue tetrazolium (NBT).

CDPKs activities increased after elicitor treatment, were positively correlated with plant resistance, however varied with respect to intensity and timing. The induction of expression of CDPKs were differentiated, dependent upon the level of resistance. Also mechanical wounding of potato leaves stimulated of expression of CDPKs reaching the highest level at 6 h after stress. After CF treatment, susceptible genotype showed the highest increase in ROS production in comparison with resistant ones.

The obtained results indicate involvement of CDPKs and ROS in defence strategy of Solanum species against stresses.

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P.4.8. Involvement of virus capsid protein and tubulin in *Prune dwarf virus* particles trafficking

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Keywords: PDV, viral transport, tubules,CP

Prune dwarf virus (PDV) is member of family Bromoviridae and Ilarvirus genus. PDV is dangerous pathogen widely spread all over the world, which infects over hundred species in Prunus genus including: plum sweet cherry and peach. The virus is transmitted via seeds, pollen and through vegetative reproduction. Mechanism of local and systemic transport of viral particles in natural and diagnostic plant hosts are still unknown. All viruses in Bromoviridae family uses tubules in cell to cell transport in form of: virion or ribonucleoprotein complex (viral proteins-viral RNA, vRNP). The aim of our study was firstly presentation of phylogenetic comparison of amino acid sequences of several isolats of PDV CP and sequences of CP of Bromoviridae with described transport mechanism like*:* AMV (*Alfalfa mosaic virus*), BMV (*Brome mosaic virus*), CMV (*Cucumber mosaic virus*) and CCMV (*Cowpea chlorotic mottle virus*) and secondly ultrastructural examination of tubules and PDV coat protein (CP) involvement in virus transport in the mechanical infected tobacco plant leaf tissues. To consider the participation of viral and host proteins in PDV local transport, the immunolocalisation of PDV CP and plant tubulin were investigated.

P.4.9. New unexpected functions for ACC deaminase genes in the *Sinorhizobium meliloti*

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Keywords: Sinorhizobium meliloti, Symbiosis, Ethylene, ACC

The gene encoding the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (acdS) is present in the dispensable genome of the plant symbiotic rhizobium *Sinorhizobium meliloti*. AcdS is supposed to be involved in the sequestering and cleaving of plant-produced ACC, the precursor of the plant stress hormone ethylene. However, the function of acdS in symbiotic bacteria has not been fully clarified and no definitive conclusion on the role of such gene on the efficiency of the symbiotic interaction have been drawn.

To clarify this issue, comparative genomic analyses of acdS orthologs were performed in *S. meliloti* and functional studies were carried out by expressing *acdS* from natural strains in the model strain *S. meliloti* Rm1021, which lacks *acdS* gene. Symbiotic and endophytic phenotypes of recombinant vs parental strain were evaluated with respect to competition for root nodule occupancy, plant colonization and modulation of ethylene production by the host plant. Additionally, phenotype microarray experiments were performed to investigate the metabolic function carried out by AcdS. Data showed that the acdS spread in *S. meliloti* through horizontal gene transfer. No increase in fitness for nodule occupancy was found overexpressing *acdS*, as well as faint effects on the modulation of plant ethylene levels were observed. Surprisingly, AcdS was shown to confer the ability to utilize formamide as sole nitrogen source. We conclude that acdS in *S. meliloti* could be more related to the exploitation of unusual nitrogen sources, in connection with rhizospheric colonization or endophytic life-style than to the symbiotic interaction.

P.4.10. Nitric oxide modulates redox-mediated defense in potato challenged with *Phytophthora infestans*

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Keywords: NO, ROS, redox, late blight

We investigated how nitric oxide-dependent signaling activated in potato leaves after inoculation was related to defense expression against avirulent (avr) and virulent (vr) isolates of *Phytophthora infestans*. Obtained data revealed that exclusively in an incompatible response, early NO and superoxide (O2•-) generation guided peroxynitrite (ONOO-) formation and together with hydrogen peroxide (H2O2) production synchronized with SOD activity induced effective defense against avr pathogen. Early oxidative and nitrosative bursts triggered an imbalance in redox homeostasis in inoculated tissue. To counteract that effect, a total antioxidative capacity, ascorbate and sulfhydryl (-SH) group compounds increased both synergistically and markedly, confirming the precise mechanism of redox re-adjustment in avr oomycete-potato interaction. Moreover, the NO-coded message was stored and converted into an enhanced total SNO pool and particular S-nitrosylation of targeted proteins . Overall, we identified 104 candidate-proteins typed for S-nitrosylation in potato leaves. The S-nitrosoproteome structure comprised a wide repertoire of proteins, i.e. defense- and redox-related. Finally, only in the incompatible interaction, NO-based signal was re-written on the rapid PR-1 gene and PR-2 protein activation and was tuned with a limitation of late blight disease symptoms.

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P.4.11. *Phytophthora infestans* generates nitric oxide to colonize potato leave

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Keywords: nitric oxide, Phytophthora infestans, potato

Generation and the potential functions of nitric oxide (NO) during plant-pathogen interactions have so far been analyzed solely from the point of view of the host plant. However, there is evidence that pathogenic microorganisms are capable of synthesizing NO as well. In this study we showed that the oomycete phytopathogen *Phytophthora infestans* (Mont.) de Bary is able to generate NO growing on the medium and *in planta*. Using a NO-specific fluorochromes, we observed the presence of NO in hyphae and zoospores of *P. infestans* growing on a synthetic medium, indicating that the signaling molecule might govern the pathogen development. When analyzing NO during plant-pathogen interaction, we found that the virulent isolates of the microorganism in contact with the potato leave tissues acquired the ability to generate much greater amounts of NO and other reactive nitrogen species. In strongly infected leaves hyphae of the blight pathogen showed an elevated NO production. The obtained results clearly indicate that *P. infestans* attempting to colonize plant tissues set in a program of boosted NO formation which cause a massive nitrosative and oxidative stress, leading to late blight development on potato leaves.

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P.4.12. Solution structure of the HSP90-SGT1 complex with ADP

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Keywords: HSP90, SGT1, solution structure, SAXS

Heat Shock Protein 90 kDa (HSP90) is a molecular chaperone that is involved in protein folding and maturation. HSP90 plays important role during heat shock and other stress conditions but is also engaged in maintaining protein homeostasis during normal growth of the cell. In plants HSP90-SGT1-RAR1 chaperone complex plays essential role in disease resistance against many pathogens. It is thought that HSP90 together with SGT1 and RAR1, plays role in the maturation or stabilization of the R proteins in the absence of recognized effector molecules. Although structure of the HSP90 protein and CS domain of SGT1 protein are known there is lack of detailed information about the structure and conformation of the complex of the full length proteins.

In this study we investigated complex formation between C-terminally truncated SGT1 protein from barley and HSP90 protein from wheat in the presence of ADP using small angle X-Ray scattering technique. Using MCR-ALS method and series of SAXS data for the: SGT1ΔSGS and HSP90 proteins and the complex at various stoichiometry we obtained pure scattering curve for the complex. Comparison of the molecular weight obtained from SAXS revealed presence of 2:1 HSP90:SGT1 complex. Our results are unexpected because each of the HSP90 monomer possesses one binding site for CS domain of SGT1. Moreover HSP90 in the complex with SGT1 exists in open conformation so there is no interaction between HSP90 monomers that could disrupt SGT1 binding.

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P.4.13. Subcellular localization of 14-3-3 protein in *Potato virus Y* (PVY) – Solanaceous plants compatible and incompatible interactions

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Keywords: hypersensitive response, plant-virus interaction, PVY

14-3-3 proteins (termed as general regulatory factors) play roles in multiple signalling pathways, including those controlling metabolism, hormone signalling, cell division, and responses to abiotic and biotic stimuli. Increasing evidence supports a prominent role of 14-3-3 proteins in regulating plant immunity against pathogens at various levels. Some 14-3-3s change their transcriptional expression, their protein abundance or their properties in response to pathogen detection or infection. Significantly, some 14-3-3s are targeted by pathogen effector proteins, which strongly suggest a function of these 14-3-3s in plant resistance. Conducted ultrastructural investigations focused on localization 14-3-3 in hypersensitive response to PVY infection and in susceptible host tissues. Experimental data providing a functional link between the detected abundance changes and a subsequent impact on plant defence responses. This protein accumulation was most marked in the epiderm is and phloem tissues of the infected leaflets and petioles, especially when PVY particles and inclusions were present. 14-3-3 was mainly thoughts as cytoplasmic protein in insoluble fraction. In PVY-tobacco and potato interaction 14-3-3 epitope was identified not only in protoplast, but also in apoplast regions of mesophyll and phloem or xylem cells. Nevertheless, in necrotic strains of PVY - potato interactions it was identified also nuclear fraction. However, in tobacco- as well as potato- PVY interactions 14-3-3 has been located inside chloroplasts (in stroma and on thylakoids). In hypersensitive reaction regulator factor was mainly documented inside mitochondria in mesophyll or phloem cells. Subcellular localization indicated the deposition of 14-3-3 epitope along endoplasmatic reticulum connected with PVY cytoplasmic inclusions.

P.4.14. The diversified metabolic activity of different cell types in *Medicago truncatula* root nodules

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Keywords: symbiosis, metabolic activity, nitrogen fixation

Legume plants form symbiotic interactions with nitrogen-fixing bacteria, commonly called rhizobia, through which they gain access to a source of nitrogen. The symbiosis leads to the formation of root nodules containing symbiotic cells infected with endosymbiotic rhizobia. The nitrogen fixation zone consists of infected and uninfected cells. Although the tissue appears to have a similar structure, the metabolic activity of the exterior is higher in comparison to the interior. Inside the infected cells a symbiosome is formed of the rhizobium cells surrounded by the membrane produced by the host cell. Bacteria inside the symbiosomes undergo a transformation into much bigger cells called bacteroids which, under the conditions created by the host plant, are able to fix atmospheric nitrogen.

We visualised the dehydrogenases activity using TPTC (TriPhenylTetrazolium Chloride) and succinate dehydrogenase using NBT (Nitro Blue Tetrazolium) labelling. Additionally, we analysed the esterase activity using FDA (Fluorescein Diacetate). By microscopy, we have detected a significant increase in dehydrogenases and esterases activity in infected cells neighbouring the parenchyma cells and in meristem. The outer part of the nitrogen fixation zone has a higher metabolic activity than its centre. Moreover, enormously high mitochondrial dehydrogenase activity was observed in pericycle cells which surrounds the vascular bundle. These results demonstrate that meristem, pericycle and outer part of the nitrogen fixation zone are highly metabolic parts of the root nodule. The diversified metabolic activity stems from their specific structure and, above all, function.

P.4.15. The involvement of sugars in the regulation of the level of endogenous signaling molecules engaged in defence responses of *Lupinus luteus* L.cv. Juno on *Fusarium oxysporum*

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Keywords: signaling molecules, lupine, Fusarium oxysporum

Sugars such as sucrose, glucose and fructose in plant-host cells may induce the metabolic signal influencing expression of many genes, including defence genes. These metabolites function within a complex network with many phytohormones as bioactive molecules and reactive oxygen species. After infection with a pathogenic fungus activation of receptors may induce defence responses, which are highly coordinated with the sequence of changes at the molecular level, including the synthesis of signaling molecules such as phytohormones, which independently or in a dialogue induce successive defence mechanisms. The aim of study was to determine the involvement of sugars as signaling molecules in the regulation of the levels of signaling molecules participating in defence responses of *Lupinus luteus* L. cv. Juno to *Fusarium oxysporum* f. sp. *lupini.* Additionally, the obtained results indicate involvement of sugars in triggering of effective defence mechanisms in embryo axes of ye llow lupine in relation to a hemibiotrophic fungus *F. oxysporum*. The initiation of defence reactions was synchronized with an enhancement of generation of the investigated signaling molecules and the activity of selected enzymes involved in their biosynthesis. The protective role of sucrose and monosaccharides in embryo axes of yellow lupine cv. Juno was also manifested in the limited development of infection and fusariosis.

These results provide evidence for the enhanced generation of signaling molecules both by carbohydrates alone and in the cross-talk of sugars and infection caused by *F. oxysporum*. The duration of post-infection generation of these molecules in yellow lupine was varied, which influenced other defence responses.

P.4.16. The role of thioredoxin peroxidase in potato response to *Phytophthora infestans*

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Keywords: Thioredoxin peroxidase, peroxynitrite, plant defence

Thioredoxin peroxidases (TPxs) belong to the peroxiredoxin family of antioxidant enzymes, which use a thioredoxin (THx) as an immediate electron donor for reactive oxygen species reduction. Recent research has shown that TPxs also react with peroxynitrite ($ONOO^-$), a reactive nitrogen species generated by the reaction of nitric oxide (NO) and superoxide in one of the most rapid reactions known in biology. Peroxynitrite is an important biological oxidant and nitrating compound contributing to oxidative and nitrosative stress in living cells; however, lower $ONOO^-$ concentration can activate signaling pathways and initiate defence responses. In this study we proposed TPx as a good candidate for controlling the balance between harmful or beneficial effect of $ONOO^-$ in plant cells challenged by the pathogen. An experimental approach involved two cultivars of potato (*Solanum tuberosum* L.) leaves i.e. highly resistant and susceptible to the oomycete pathogen *Phytophthora infestans*. As we found, the potato-avr *P. infestans* model system exhibited a transient program of boosted $ONOO^-$ formation coincident with a gradually increasing TPx gene expression. In turn, the compatible interaction revealed a 24-h delay of $ONOO^-$ formation; however, TPx up-regulation was recorded within the earlier stages of pathogen infection. Mentioned changes were temporary tuned with mRNA accumulation coding for THx gene. Taken together, TPx is engaged in the regulation of $ONOO^-$ concentration during potato - *P. infestans* interaction.

P.4.17. The structure of feeding side induced by juvenile J2 of *Nacobbus aberrans* in tomato and potato roots

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Keywords: Nacobbus aberrans, feeding site

The structure of the swelling induced in tomato and potato roots by two races of *N.aberrans* has been examinated by light and electron microscopy. Juveniles of both races (tomato and potato)of *N. aberrans* were able to induce feeding sites in tomato or potato roots. In infected roots the juveniles were located in a cavity formed among cortical cells. Ten days after inoculation the cavity was very large and composed of enlarged cortical, endodermal and pericyclic cells, in tomato roots infected with juveniles of both races and the juveniles themselves were strongly swollen. At the same time point after inoculation in the potato roots the cavities were very small and composed of enlarged cortical and endodermal cells and the juveniles were still thin. Cytoplasm of all feeding site cells proliferated and became electron dense and central vacuoles were substituted by numerous small ones. All feeding site cells were uninuclear. Within cytoplasm, proliferation of free rybo somes, mitochondria, structures of smooth and rough endoplasmic reticulum, Golgi apparatuses and plastid occurred. The number of plastids increased in feeding site cells. Plastids possessed weakly developed system of thylakoids and the most of their volume was occupied by large starch grains. Number and size of the starch grains is related to combination of host plant and race of *N. aberrans*. In all combinations cells incorporated into feeding sites revealed distinct local cell wall thickenings. No cell wall opening was found between feeding site cells. Neighbouring feeding site cells were interconnected by plasmodesmata.

P.4.18. The survival of rhizobacteria in lyophilisats

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Keywords: plant growth promoting rhizobacteria, lyophilisation

Lyophilisation proceeding at a low temperature maintains the chemical properties of the products. This process is widely used to preserve biological materials, such as: bacteria and fungi, as well as in the production of probiotics. Little information about freeze-dried rhizosphere bacteria are available in the literature. The study examined the effect of freeze-drying process, temperature and storage time of bacterial lyophilisats on the survival of six bacterial strains. It is known that these strains are characterized by high metabolic activity and the ability to stimulate the growth of rape in natural condition and in the presence of trace elements ions. The conducted experiments showed that the lyophilisation process resulted in significant reduction in the survival of analyzed rhizobacteria. It was found that both the temperature and time of storage of lyophilisats affected on viability of the microorganisms, which decreased with time and temperature increasing. All analyzed strains showed the best survival at 4°C. Survival of plant growth promoting rhizobacteria in lyophilisats depends on the species. The ability to make growth on solid medium by the freeze-dried bacteria are listed in the following order: Bacteroidetes bacterium = *Serratia* sp. > *Bacillus* sp. > *Massilia* sp. = *Pseudomonas fluorescens* = *Variovorax* sp. The highest survival after freeze-drying process, was characterized by a strain of *Serratia* sp. regardless of the temperature and time of preparations storage.

P.4.19. Transcription profile of PIN genes in root nodules and root tip of *Medicago truncatula*

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Keywords: Medicago, root nodule, auxin, PIN

Polar auxin transport is dependent on PIN proteins family, which are membrane transporters of anionic indole-3-acetic acid (IAA-). We assume that polar auxin transport is essential in the development and meristematic activity maintenance of *Medicago truncatula* root nodules. To test this hypothesis, we analyzed the expression pattern of all *M. truncatula* PIN genes. Using qPCR, we assessed and compared the transcription profile of PIN genes in root nodules and the root tip. On this basis we selected those PINs, which expression in root nodules was higher with respect to the root tip. This analysis indicated an essential contribution of polar auxin transporters in the development of root nodules. However, to confirm the role of PINs in this process, we are now preparing genetic constructs with green fluorescent protein (GFP):β-glucuronidase (GUS)-fusion under selected PINs promoters to investigate their tissular and subcellular localization in transformed *M. truncatula* plants.

P.4.20. Unraveling the role of MtABCG10 in medicarpin biosynthesis pathway

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Keywords: ABC transporters, phytoalexin, biotic stress

Members of the so called G subfamily of ABC transporters have been shown to be involved in numerous physiological processes associated with the plant response to biotic and abiotic stresses. There are at least 30 full size ABCGs identified in a model legume *Medicago truncatula*. Among them MtABCG10 has been reported as: (i) a plasma membrane protein accumulating in leaves and roots of Medicago upon pathogen infection and elicitor treatment, (ii) a protein engaged in the modulation of medicarpin biosynthesis. Medicarpin is a phytoalexin of Medicago and a product of a legume specific isoflavonoid biosynthesis pathway (IBP). Interestingly, the expression pattern of genes encoding phenylalanine ammonia - lyase (PAL) and isoflavone synthase (IFS), representing key enzymes from IBP, goes along with that of MtABCG10 upon biotic stress. Silencing of the *MtABCG10* expression results in lower accumulation of the IBP -derived medicarpin and its precursors. This effect can be averted by exogenous application of an early precursor isoliquiritigenin onto MtABCG10 - silenced hairy roots. Heterologous expression of *MtABCG10* in tobacco suspension cell lines and conducted transport experiments of various Medicago phenolic compounds support hypothesis that MtABCG10 is responsible for membrane translocation of medicarpin precursors. In view of the presented data, a new potential role for plant ABCGs as modulators of isoflavonoids level in legumes during biotic stress can be postulated.

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SESSION 5: PLANT RESPONSES TO ABIOTIC STRESS

Plenary Lectures

Multifunctional roles of sugars during plant stress responses

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Small soluble sugars take a central position in plant growth, development, and stress responses. They not only function as transport compounds (mainly sucrose) and as carbon skeletons to deliver energy, they also act as signals that cross-talk with hormonal signals and may regulate hormone biosynthesis. Sugars may be central in “sweet immunity” responses involved in stress adaptation mechanisms. Moreover, evidence is accumulating that, besides their role in membrane and protein stabilization, sugars can directly scavenge hydroxyl radicals as exemplified both from *in vitro* and *in vivo* studies. For this purpose, the fate of sucralose, an artificial sucrose analogue, was followed in the model plant Arabidopsis.

Role of the antenna complex LHCII in remodeling of the membranes of the photosynthetic apparatus of plants

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The thylakoid membranes of the photosynthetic apparatus of plants are characterized by particularly high fraction of proteins with respect to lipids, accounting for ca. 70 % of the membrane surface. This percentage is particularly high in the grana structure of a chloroplast and intermolecular interactions between the protein components are proposed to be responsible for stacking of adjacent thylakoid membranes. Light-harvesting pigment-protein complex of Photosystem II (LHCII) is a major protein constituent of the thylakoid membranes of plants and plays a prominent role in grana formation. According to the results of numerous studies, trimeric organization of LHCII is a crucial factor that might be involved in the grana-stabilizing activity of LHCII. Another important factor in such activity is a protein phosphorylation level. Interestingly, light-intensity-controlled phosphorylation level of LHCII is highest at relatively low light intensities, below the saturation level of photosynthesis. We applied fluorescence lifetime imaging microscopy (FLIM) to study molecular organization of LHCII, both phosphorylated and non-phosphorylated, in model protein-lipid membranes and in intact chloroplasts. The results of our studies confirmed importance of trimeric organization of LHCII in inter-membrane interaction between adjacent protein-lipid membranes. Moreover, we found that the light-induced remodeling of membrane stacks can be mediated via the LHCII trimer-monomer transition. During the talk a model will be discussed, which integrates involvement and interplay of the processes of LHCII phosphorylation and trimer-monomer transition in light-controlled remodeling of the thylakoid membrane architecture in plants.

Reactive oxygen species as a central hub in sensing and response of seeds to environmental cues

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Reactive Oxygen Species (ROS) are key players in seed biology. They are produced from embryogenesis to germination, but also during seed after-ripening and storage, and their homeostasis regulates the potential of seeds to germinate. The concept of the "oxidative window for germination" alllows explaining the role of ROS in seed germination. It restricts the occurrence of the cellular events associated with germination to a critical range of ROS level, enclosed by lower and higher limits. Above or below the "oxidative window for germination", weak or high amounts of ROS, do not permit progress toward germination. Here we will present data obtained with seeds of crops and Arabidopsis showing that ROS act as sensors of environmental cues such as temperature, osmotic pressure or heavy metals, and that they can translate environmental signals to endogenous signals regulating germination. The perception of environmental factors by ROS also regulates the expression of seed dormancy. We will show that their effect on germination can result from an interplay between with hormone signaling pathways, thus leading to changes in gene expression, but that also modify cell functioning through the oxidation of targeted cellular components.

Ribosome assembly and protein translation in light-dependent germination of dormant Arabidopsis seeds

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Translation is a biochemical process conserved in all eukaryotes, tightly linked to the regulation of growth and development. In seeds, germination requires de novo translation and its regulation by dormancy is likely to be related to the association of specific transcripts to polysomes. The translation machinery consists of complexes of proteins coded by several genes i.e.: RACK1 (Receptor for Activated C Kinase 1) which protein product control ribosome assembly and protein synthesis via interaction with Eukaryotic Initiation Factor6 (eIF6). Despite detailed knowledge about signal transduction pathways mediated by light, there is a limited number of information about genes involved in regulation of translation in response to light availability during germination. In this particular study, germination tests using dormant Arabidopsis wild type (WT) and *rack* mutant seeds imbibed on water and in the presence of anisomycin (an inhibitor of translation) unravelled interesting germination phenotypes. The qRT-PCR analysis indicated that expression of *RACK1* in germinating seeds was positively regulated by the presence of light while the transcripts level of *eIF6* was not light-dependent, suggesting that RACK1 are among key players controlling translation process in dormant seeds exposed to various light conditions. Moreover, the fractionated and characterized with microarrays polysome-associated mRNAs revealed that the translatome differs in germinating seeds in light-dependent manner. These findings provide new insights into the role of light availability in mediation of dynamic regulation of ribosome assembly and selective recruitment of mRNAs to polysomes during germination of dormant seeds.

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Selected Talks

T.5.1. Methylome-transcriptome relationships during drought stress in model and crop monocots

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Keywords: epigenetics, methylation, transcriptome, drought, crop

As sessile organisms, plants need to continuously adjust their responses to external stimuli to cope with stress conditions. It was found that epigenetic modifications, including DNA methylation, histones modifications and small RNA-based mechanisms constitute an essential layer of machinery that regulates gene expression networks. Recently, numerous studies have provided valuable insights into the epigenetic control of stress adaptation in plants. However, most of these studies decipher epigenetic mechanisms of stress response in model species with specific, small genomes, or describe particular layer of epigenetic regulation, e.g. changes in DNA methylation or differential expression of smallRNAs without broader, system-level or evolutionary context. To address this issue, genome-wide integrated methylome-transcriptome analyses have been carried out during drought stress in two closely related monocot species: *Brachypodium distachyon*, a model for functional genomics research in temperate grasses and cereals, and in *Hordeum vulgare*, an important cereal crop with a large genome. Whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) methods were used to study DNA methylation profiles and their changes upon drought stress in *B. distachyon* and in *H. vulgare*, respectively. These analyses were coupled with complementary, comprehensive transcriptome profiling carried out using mRNA-Seq and smallRNA-Seq approaches. Here we report a potential role for DNA methylation in regulation of the stress-response transcriptome. We describe the drought stress-induced variation in DNA methylation pattern and provide examples of stress-induced differentially methylated regions associated with differentially expressed genes. We also discuss general conclusions regarding correlations between methylation of genomic features and the gene expression potential.

T.5.2. New *Arabidopsis thaliana* microRNAs and their genes expression regulation in response to selected abiotic stresses

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Keywords: miRNA, pri-miRNA, abiotic stress

Arabidopsis microRNA expression regulation was studied in a wide array of abiotic stresses such as drought, heat, salinity, copper excess/deficiency, cadmium excess, and sulfur deficiency (Barciszewska-Pacak et al., 2015). A home-built RT-qPCR mirEX platform for the amplification of 289 *Arabidopsis* microRNA transcripts was used to study their response to abiotic stresses. Small RNA sequencing, Northern hybridization, and TaqMan® microRNA assays were performed to study the abundance of mature microRNAs. A broad response on the level of primary miRNAs (pri-miRNAs) was observed. However, stress response at the level of mature microRNAs was rather confined. The data presented show that in most instances, the level of a particular mature miRNA could not be predicted based on the level of its pri-miRNA. This points to an essential role of posttranscriptional regulation of microRNA expression. New *Arabidopsis* microRNAs responsive to abiotic stresses were discovered. Four microRNAs: miR319a/b, miR319b.2, and miR400 have been found to be responsive to several abiotic stresses and thus can be regarded as general stress-responsive microRNA species.

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T.5.3. Hydrogen peroxide and nitric oxide production during the cell cycle of *Chlamydomonas reinhardtii*

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Keywords: Chlamydomonas, NO, H2O2, cell cycle

Intracellular production of H2O2 and NO, known as the signaling molecules, was evaluated during the cell cycle of green alga *Chlamydomonas reinhardtii*. We focused on the mutual relationships of H2O2–NO and established them in the developmental processes of cell, with respect to the expression of enzymes involved in generation and scavenging of these molecules, as well as changes in the photosynthetic activity. Fe-SOD, Mn-SOD3 and APX were found as the enzymes involved in chloroplastic H2O2 metabolism and Mn-SOD5 and CAT as ones assigned to its mitochondrial generation. In *C. reinhardtii* cells NR activity occurred to be the main source of NO and the increase of NO production was associated with decrease of the photosynthetic activity or NiR activity decline. The increase of NO concentration vs H2O2 level in the 7th – 8th h of cell cycle was following by termination of cell cycle: G1/S and S/M check point passage (expression of CYC A1, B1 and CDK A1, B1). Tw o peaks of H2O2 incensement – 0-1st h and 10-11th h of cell cycle, were found to exhibit different source and implication. First one was a cell response to the intense illumination of young autospores, where H2O2 originated mainly from chloroplastic Fe-SOD activity. Second, was linked with mitochondrial Mn-SOD 5 activity and overlapped the induction of autopores release. Thus, we suppose ratio of H2O2 and NO play significant role in the progress of cell cycle of *C. reinhardtii*.

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T.5.4. Lichen response to CO2 abiotic stress- ionic liquid bioindicator of air pollution

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Keywords: Evernia Prunastri, ionic liquid, CO2

Lichens are organisms comprising both algae and fungi. Lichens are found on rocks and trees, and they respond to different environmental changes like climate and air quality. They are growing well in not polluted environment.

This work is showing impact of different doses of carbon dioxide on extract of the dried lichen *Evernia Prunastri* in so called green chemistry solvent - ionic liquid. The 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate ionic liquid was selected for this purpose, after synthesis and careful purification. The model of lichen extract in ionic liquid (*in vitro* study) is a good CO2 bioindicator well mimicking properties of lichen *in vivo*. This study presents the model of lichen based on extracted and synthetized functional biomaterials: (+) usnic acid, chlorophyll a and β-carotene in ionic liquids.

The technology of the CO2 quantitative measurements of carbon dioxide introduced into the samples during the process of saturation was developed. Also optimum of saturation process was found and the procedure of determination of mass of CO2 gas dissolved in the solution of functional biomaterials in ionic liquid was proposed. The spectroscopic methods: fluorescence emission and electronic absorption were used. Concluding - experimental results indicate that our novel system is very sensitive to the CO2 doses and it can be use as molecular bioindicator for CO2 pollution of air.

T.5.5. Early signaling events in soybean seedlings subjected to short term cadmium stress

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Keywords: cadmium, signaling, ethylene, ROS, NO

Contamination of the environment with heavy metals, including cadmium, is a serious problem of modern world. In the case of plants this pollutant causes disturbances in photosynthesis and respiration, growth inhibition, protein and nucleic acid damage and eventually cell death. The earliest reaction of plants to cadmium is activation of signal transduction pathways leading to the mobilization of plant defense system.

Present study concerns signaling events activated in soybean seedlings within first 24 hours of cadmium stress. The research shows that cadmium causes changes in the expression of several signaling-associated genes, encoding proteins involved in ethylene and nitric oxide synthesis, mitogen-activated protein cascades and gene regulation. The results also indicate that ethylene, reactive oxygen species and nitric oxide are engaged in early response to cadmium. The cross talk between mentioned signaling elements is discussed.

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T.5.6. The relevance of multiple steps of plant mitochondrial biogenesis in temperature stress and recovery

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Keywords: functional analysis, mitochondria, proteomics, stress

It was estimated that at least 22% of stress-responsive organellar proteins comprises the ones targeted to mitochondria. However, complex approaches to elucidate the biological relevance of plant mitochondrial responses to temperature stress and stress recovery are very limited.

We investigated the biogenesis of the cauliflower curd mitochondria under cold, heat and after the subsequent stress recovery.

Using various approaches, in heat and after heat recovery we noticed variations in abundance of subunits of respiratory complexes (CI, CIII) as well as components of import machinery. The presence of unassembled subunits of ATP synthase was accompanied by impairment in mitochondrial translation and partial disintegration of matrix complexes. Interestingly, the transcription profiles of mitochondrial genes were uncorrelated in cold and heat. The ultrastructure of mitochondria was significantly altered only in stress recovery. Contrary to general stability of respiratory chain complexes in heat, functional studies showed that their activity and the ATP synthesis yield were affected. Heat stress resulted in lowered OXPHOS efficiency. It also increased AOX activity, protein, while heat recovery reversed AOX level and activity. Cold stress, however, led to the opposite effects, which were reversed after cold recovery. Overall, cauliflower AOX was only induced by heat stress. Contrary to AOX activity, the activity of rotenone-insensitive internal NADH dehydrogenase was lowered in heat.

We conclude that cauliflower mitochondria are actively engaged in the response to various temperature treatments. However, their biogenesis at multiple steps is not equally affected by investigated conditions.

Poster Presentations

P.5.1. ABA transport as an essential regulatory factor in nodulation process

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Keywords: ABC transporters, ABA, drought, nodulation

It seems that ABA movement within plant can determine proper ABA perception and subsequently trigger adequate responses to environmental stressors. There is a strong evidence that ABC transporters belonging to the G subfamily participate in ABA translocation upon abiotic stress conditions. It was shown that in Arabidopsis the half-size AtWBC26 and full-size AtPDR12 function as an ABA exporter and importer respectively. Almost nothing is known about the ABA transport and ABA transporters in legumes. What is interesting, recently it was reported that in *Medicago truncatula* which is a model legume plant, ABA affects as a negative regulator, nodulation process, to reduce costly establishment of nodules under stressful conditions. We want to answer the question whether proper distribution of the ABA by the ABC transporters can have an impact on nodulation efficiency. We have just started to recognise this issue. Based on phylogenetic tree of the half-size WBC proteins we have selected potential ABA exporter from Medicago. We have shown that its expression is strongly induced upon drought stress, mimicked by PEG and ABA. A spatial expression pattern analysis with reporter gene revealed that *MtWBC20* promoter is active mostly within vascular bundles where ABA is predominantly biosynthesized. The corresponding protein is located in the plasma membrane. Finally, we have observed that this ABA-inducible gene acts independently of the ABA core signalling pathway. In addition what supports our assumption is that the MtWBC20 functions as an abscisic acid efflux carrier.

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P.5.2. Anatomical adjustments induced by water deficit in leaves of barley (*Hordeum vulgare* L.)

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Keywords: Hordeum, leaf anatomy, drought acclimation

Plants acclimate to drought by modifying their structure and metabolism. The ability for adjustment in leaf anatomy, however, has not been sufficiently studied. We examined plastic modifications of leaf anatomy induced by a period of controlled drought in a range of barley genotypes (cultivars and single-seed descended lines) grown in pots. For analyses all genotypes were treated as one population.

Leaves produced under the influence of drought were shorter and narrower in comparison with well watered controls. The reduction in leaf size was correlated with smaller sizes of epidermal cells and resulted in increased stomatal and vein densities. Leaves influenced by drought also had thinner laminas with smaller thickness of mesophyll and both epidermal layers. Drought also inhibited the development of sclerenchyma and caused a reduction in cross sectional areas of the midrib and xylem as well as lower diameters of metaxylem vessels. Majority of these trait modifications were explained by reduced leaf size, however, reduction in vessel diameter was greater than expected from leaf size reduction alone suggesting an acclimative hydraulic response. Interestingly, one of the cultivars exposed to drought produced vessels with smaller pits than in controls. Trait modifications induced by drought in barley were overall consistent with an enhanced water conservation.

P.5.3. Cadmium affects peroxynitrite generation and tyrosine nitration in soybean root seedlings

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Keywords: cadmium, peroxynitrite, tyrosine nitration, soybean

The effect of moderate (85µM CdCl2) and sublethal (170µM CdCl2) short-term cadmium (Cd) stress on endogenous peroxynitrite ($ONOO^-$) generation in the roots of 3-day old soybean (*Glycine max* L.) seedlings was investigated. The control roots showed a relatively high level of $ONOO^-$, whereas Cd-mediated $ONOO^-$ formation was detected only in more intensive Cd stress conditions that was accompanied by enhanced levels of nitric oxide (NO) and superoxide ($O2^{\cdot-}$). The contrasting response appeared during moderate stress which provoked a huge NADPH-oxidase dependent $O2^{\cdot-}$ accumulation with slightly elevated reactive nitrogen species formation. The accumulation of proteins undergoing tyrosine nitration in soybean roots was strictly dependent on the stress intensity. The most pronounced changes within the pool of 3-nitrotyrosine targets revealed proteins involved mainly in primary metabolism that are not involved in plant defense strategy. Moreover, sequential treatment with exogenous $ONOO^-$ and Cd was tuned with diminished Cd toxicity and up-regulation of gene coding for peroxiredoxin (Prx). Taken together, soybean seedlings mainly exposed to lower intensity of Cd stress, showed enhanced Pxr gene expression promoting direct scavenging of $ONOO^-$ what finally affects post-translational protein modification via tyrosine nitration.

P.5.4. Carotenoids shield algal cells from excess radiation

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Keywords: carotenoids, photoprotection, raman&fluorescence spectroscopy

Carotenoids are pigments that in plants and algae play very important role. One of the functions is photoprotection. It is well known that in the intense light conditions the pigment composition changes significantly, and in many cases, increased production of carotenoid pigments are observed.

Studies were performed on the different strains of algae illuminated during growth with the high and low light intensities. Research carried out with fluorescence lifetime imaging microscopy (FLIM) and the Raman imaging microscopy shows the different distribution of carotenoid pigments within the cells of algae incubated in high and low light. Detailed analysis suggests that carotenoids creates structures forming shell around the cells. Shield forming pigments have different molecular organization compared with carotenoids located in photosynthetic structures. Such structures can play important role in protecting internal algae organelles against excessive radiation.

P.5.5. Changes in polar metabolites in seedlings of pea (*Pisum sativum* L.) under osmotic stress

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Keywords: pea, osmotic stress, metabolite profiling

In the present study the effect of osmotic stress (at different osmotic potential: from -0.5 up to -2.0 MPa) on polar metabolites in 7-day-old pea seedlings was studied. Pea seedlings were exposed to osmotic stress by immersion of roots in PEG8000 solutions for 48h and then transferred to water for 24h. The metabolites were analyzed separately in cotyledons, epicotyls and roots.

The extraction of polar metabolites from lyophilized plant material was done in two steps by using solution of methanol:water (1:1; v:v) and follow pre-chilled chloroform. Polar fraction was derivatized. Separation and identification of polar metabolites were carried out by high resolution gas chromatography.

There were 93 detected compounds and 47 of them were identified. Carbohydrates (including polyols and phosphates of monosaccharides), amino acids, organic acids, amines and phosphoric acid were groups of identified metabolites. Glucose and sucrose dominated in the fraction of carbohydrates and citric acid in the fraction of organic acids. Homoserine was the main amino acid.

The accumulation of sucrose, proline and raffinose was the common response to osmotic stress in both roots and epicotyls. The amounts of accumulated metabolites correlated with the increasing osmotic potential. No such changes occurred in cotyledons. After recovery, the level of sucrose, proline and raffinose dramatically decreased.

P.5.6. Characterisation of barley (*Hordeum vulgare* L.) leaves microtranscriptome under water deficiency conditions and subsequent recovery

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Keywords: miRNAs, drought, microtranscriptome, gene expression

Plants exposed to adverse environmental conditions employ complex regulatory networks in order to induce a variety of defense mechanisms. MicroRNAs play an important role in stress responses by regulating target genes expression at the post-transcriptional level. In this study the results of detailed characterisation of barley leaves microtranscriptome and its modulation during drought stress and subsequent recovery are presented. Moreover, putative targets of detected miRNAs, predicted relying on mutual analysis of small RNA and mRNA sequencing and resulted putative regulatory networks are discussed.

By using small RNA deep sequencing and stringent bioinformatics analyses we detected 22 conserved miRNA families, comprising 41 *MIRNA* genes, expressed in leaves of barley seedlings. Among these families, we found a wide range of mature miRNAs that were differentially expressed in drought-stressed plants. We identified 19 up-regulated miRNAs and 15 down-regulated. A subset of these genes showed opposite expression pattern during plant recovery after re-watering, and thus the recovery phase allowed us to narrow down the set of miRNAs and to identify those specifically involved in drought response. Interestingly, their target genes were mainly involved in regulation of transcription. Importantly, 38 novel MIRNA candidates and their putative targets were predicted. The detailed analysis of microtranscriptome changes induced by water-deficiency stress in barley leaves, coupled with mRNA transcriptome analysis allowed for identification of the subset of drought-responsive genes, which expression is presumably regulated with the involvement of miRNAs.

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P.5.7. Chemically induced stress affects the level of CAH3 and PsbO proteins in *Chlamydomonas reinhardtii* during the cell cycle

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Keywords: Chlamydomonas, CAH3, PsbO, chemical stress

The lumenal carbonic anhydrase (CA) - CAH3 and PsbO protein located at the lumenal surface of thylakoid membranes, play an important role in optimal function of the Oxygen Evolving Complex and therefore are essential for photosynthetic process. The aim of this work was to evaluate the role of CAH3 and PsbO proteins in photosynthetic activity of the synchronous cultures of green alga *Chlamydomonas reinhardtii* treated with anthropogenic contaminations: cadmium (Cd) and anthracene (ANT).

The cultures of the wild type (WT) *C. reinhardtii* strain (CC-1690) were synchronized by alternating light and dark periods (10/14 h). The cells were exposed to Cd (95 µM) and ANT (5 µM) at the beginning of the light period of cell cycle. In the following hours, measurements of: a) photosynthetic O2 evolution, b) total CA activity, c) chlorophyll a fluorescence (OJIP test), d) proteins (CAH3, PsbO) amount (WB analysis) were performed.

The inhibitory effect of Cd on photosynthetic parameters in *C. reinhardtii* cells was visible during the whole light period of cell cycle, whereas ANT was toxic only within first 3-4 h of the cycle. Both toxicants reduced also CAH3 and PsbO proteins levels as well as total CA activity. Thus, it could be assumed that the low level of CAH3 and PsbO proteins in Cd- and ANT-treated cells is one of the reasons of photosynthesis inhibition caused by these substances.

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P.5.8. Cold and high concentration of sugar as enhancers of plant female organs development

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Keywords: Cold stress, sugar, plant reproduction

Stress of low temperature is one of the most important reasons causing the decrease in quantity and quality of crops (Yadav 2009). Apart from various phenotypic symptoms in response to cold stress, it also severely hampers the reproductive development of plants. Leaf redness through anthocyanin accumulation is also commonly considered as a stress response (Chalker-Scott and Scott 2004).

Sugar stress usually means too low or high concentration of soluble sugar, which induce either starvation or physiological drought caused by very high osmotic pressure. However, plant sugars are considered the crucial players in oxidative challenge during abiotic stress (Keunen et al 2013).

High concentrations of sugar and cold are factors useful for reprogramming generative plant cells and producing fertilization-less embryo and endosperm (Chen et al 2011).

We show that unpollinated pistils, in response to high exogenous sugar concentration (6 -12%), had a high content of anthocyanin, resulting in a red color. Coloration was more pronounced when donor plants were additionally cooled (at 10 °C). Furthermore, cold together with sugar stress clearly increased the survival rate and quality of female gametophytes and the development of autonomous endosperm was induced. The effect of cold and sugar stress was closely correlated with genotype.

We presume that the antioxidative cascades, in which sugars are also involved, and stress-induced epigenetic changes are activated and can increase the resistance of generative organs against cold and then can promote gametophytic or apomictic-like development. Our assumptions are currently being verified.

P.5.9. Cucumber Metal Tolerance Protein 7 (CsMTP7) is a mitochondrial transporter involved in detoxification of cells from Fe excess

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Keywords: heavy metals, iron, Metal ToleranceProteins,cucumber,mitochondria

Cucumber MTP7 protein belongs to the cation diffusion facilitator (CDF) family of divalent metal ions transporters, which is ubiquitous in all kingdoms of life. Plant MTP7-like transporters form a separate subgroup within the CDFs, which has not been characterized yet. Cucumber MTP7 gene is composed of 14 exons and 13 introns and encodes a putative protein of 520 amino acids with 5 transmembrane domains. CsMTP7 expression analysis revealed the presence of two alternative gene transcripts in cucumber: one variant (*lCsMTP7*) with retained intron between exon 1 and exon 2, and one variant (*sCsMTP7*) without introns. Interestingly, the occurrence of intron in *lCsMTP7* results in non-sense-mediated decay. The protein coding transcript sCsMTP7 was detected in hypocotyls, cotyledons and leaves under control conditions, and in roots of plants grown upon Fe excess. These results indicate organ-specific and Fe-mediated posttranscriptional regulation of CsMTP7 activity. When expressed in yeast, CsMTP7 localized in mitochondria and restored the growth of Fe-sensitive yeast mutants on high Fe concentration, suggesting the function of cucumber protein in Fe transport and detoxification. Studies on protoplasts isolated from *A. thaliana* cells confirmed that CsMTP7 localizes to mitochondria, consistent with the presence of a mitochondrial targeting peptide (32 aa) in the N-terminus of cucumber protein. Overall, the results identify CsMTP7 as the mitochondrial iron transporter involved in Fe detoxification that is post-transcriptionally regulated by different Fe availability.

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P.5.10. Dark-induced leaf senescence and point of no return

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Keywords: senesence, PCD, leaf, barley

The results obtained in the work provide new data concerning the organization of knowledge of the senescence process of leaf. Dark –induced barley leaf senescence is a highly regulated process of series transformations: molecular, biochemical and cytological and involving genetic re-programming, cessation of photosynthesis, degradation of leaf protein, loss of chlorophyll, remobilization of nitrogen and carbon and decomposition of chloroplast. At the end, disruption of other organelles such as nucleus and mitochondria also takes place. The very last stages of senescence include typical PCD symptoms - shrinking of the protoplast, chromatin condensation, DNA fragmentation and disintegration of the cell membrane.

However the cell death programme itself starts before visible yellowing and this decision could be reversed. Results show the two phases of the process. One is the stage of senescence reversibility and the other is irreversible stage. Dark-induced senescence mechanisms allow a starving plant to recycle materials first from proteins and chlorophyll without destroying whole organelles, then by destroying single organelles but even the organelle portion is removed from the cell some basal function of the organ could be maintained under starvation condition. If degradation will not go too far and environmental conditions subsequently improve the leaf could be rejuvenated and resume normal function.

P.5.11. Determination of membrane protein composition in lupine roots exposed to lead

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Keywords: lupine, lead, membrane proteins

Heavy metal phytotoxicity may result from alterations of membrane structure and permeability. Therefore determination of membrane protein composition in lead-treated plants may contribute to a better insight into the mode of action of metal ions. The gel electrophoresis of membrane proteins is considered to be difficult. Protein separation can be improved by using a cationic detergent, benzyldimethyl-n-hexadecylammonium chloride 16-BAC.

The purpose of our study was to investigate the influence of lead on the composition of membrane proteins isolated from lupine roots (*Lupinus luteus* L. cv. Juno) exposed to 150 and 350 mg l-1 Pb(NO3)2 for 48 h. The proteins were analyzed by one-dimensional 16-BAC gel electrophoresis followed by staining with CBB (Hartinger et al. 1996). The selected bands were excised from the gel and subjected to the LC-MS analysis. Qualitative and semi-quantitative comparison between some proteins from control and lead-exposed lupine roots was undertaken.

Numerous proteins were identified by this approach, including membrane proteins or proteins associated with membranes e. g.: 1) GTP-binding proteins assigned to families: Rab, Ran and Sar that are involved in signalling pathways and in vesicular trafficking 2) actin and tubulin, which are tethered to the membrane proteins and contribute to vesicle movement along cytoskeletal networks 3) aquaporins whose functions include water transport in and out of the cell 4) cytochrome C oxidase and mitochondrial ATP-ases engaged in formation of proton gradient across the inner mitochondrial membrane and ATP synthesis 5) lipoxygenase involved in the oxidation of fatty acids and the synthesis of signaling molecules.

P.5.12. Differences in the physical properties of thylakoid membranes from diatoms, green algae and higher plants as revealed by EPR-spin probe technique

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Keywords: Fluidity, Thylakoids, Diatoms, Chlamydomonas, Spinach

Fluidity of thylakoid membranes isolated from diatom *Phaeodactylum tricornutum*, green alga *Chlamydomonas reinhardtii* and higher plant *Spinacia oleracea* were measured by EPR spectroscopy using two spin labels: 5 doxylstearic acid (5-SASL) and 16 doxylstearic acid (16-SASL). The EPR spectra were recorded in the temperature range of 0 - 40°C and selected parameters: central field line width (ΔH0), order parameter (S) and rotational correlation times (T2B, T2C for 16-SASL) were analyzed.

5-SASL spectra showed statistically significant differences between tested organisms in low (2,5 to 22,5°C) and higher (35 to 40°C) range of temperatures as far as the ΔH0 and S parameters are concerned. Thylakoids of *Ch. reinhardtii* and *S. oleracea* tested with 5-SASL were slightly more fluid than thylakoids of *Ph. tricornutum*. These results indicate that fragments of lipids molecules localized close to membrane surface are more ordered in *Ph. tricornutum* than in *Ch. reinhardtii* and *S. oleracea* thylakoids.

Statistically significant differences were also recorded for 16-SASL when *Ph. tricornutum* membranes were compared to *Ch. reinhardtii* and *S. oleracea* . Values of T2B, T2C and ΔH0 parmeters for the latter species were higher than those for *Ph. Tricornutum*, whereas the S parameter calculated for *Ch. reinhardtii* and spinach thylakoid membranes was similar but different from that of *Ph. tricornutum*. Obtained results reveal, that lipids forming hydrophobic core of *Ch. reinhardtii* and *S. oleracea* thylakoids are more ordered than lipids of the same region of *Ph. tricornutum* which is related to different fatty acid composition.

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P.5.13. Early light induced proteins (ELIPs) and photosynthetic activity of *Chlamydomonas reinhardtii* cells under chemically induced stress

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Keywords: ELIP, Chlamydomonas, abiotic stress

Plant cells can acclimatize to low toxicants' concentrations, thus being able to survive in the contaminated environment. Inducible stress tolerance results from complex mechanism, of which the early-light-induced-proteins (ELIPs) induction is consider to be important element. ELIPs are supposed to play significant role in chloroplast protection against photoinhibition caused by reactive oxygen species, generated in stress conditions. This work aim to evaluate the role of ELIPs in cells of green alga Chlamydomonas treated with anthropogenic contaminants: anthraquinone (ANTQ, polycyclic quinone), chloridazon (CHD, herbicide) and cadmium (Cd, heavy metal), in concentrations that disturb cell homeostasis but do not significantly inhibit population growth (toxicological EC10 values).

C. reinhardtii wild type (cc1690) was grown at low light (LL, 60-70 umol photons×m-2×h-1) or high light (HL, 125-135 umol photons×m-2×h-1). The cells were exposed to CdCl2 (3.19 mg×dm-3), ANTQ (0.02 mg×dm-3) or CHD (0.09 mg×dm-3). Physiological state of the cells was characterized by their photosynthetic activity examination (O2 evolution, chlorophyll a fluorescence in vivo). To estimate ELIPs role in cells response to stress, level of ELIP proteins (western blot) was examined.

The control cells of both HL- and LL-cells showed similar photosynthetic activity. At the same time, all toxicants caused stronger photosynthesis inhibition in LL- than in HL-cells. Because the western blot analysis allowed us to confirm the high level of ELIP proteins in HL-cells, we assume that ELIPs are involved in cells defense against stress induced by anthropogenic contaminants of the environment.

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P.5.14. Effect of light/dark transition on photosystem I-associated antenna from *Cyanidioschyzon merolae*

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Keywords: phycobilisomes, light, PSI, darkness

Cyanidioschyzon merolae is unicellular, ultrasmall, autotrophic red alga living in very acidic sulfate-rich hot springs, contains associated with Photosystem I (PSI) phycobilisomes and chlorophyll a binding polypeptides (Lhcr) where they serve as functional antennae. The light-harvesting antennae of photosystem II (PSII) contains only phycobilisomes. Light quality and quantity are key factors affecting growth, pigment, composition and structure of antenna. Information about function of Lhcr proteins and phycobilisomes under different light conditions are limited. Analyzed 77K fluorescence spectra of *C. merolae* thylakoids indicate that there is much more of unbound phycobilisomes during dark period then in the light. Phycobilisomes are bound to PSI, only after light period. These results were confirmed by sucrose gradients separation of PSI fraction. Thylakoids isolated following the light period showed higher PSII activity (by about 50%) and lower PSI activity (by abo ut 20%), as compared to thylakoids isolated after the dark period.

These results indicates high mobility of phycobilisomes what may play a role in the regulation of light harvesting efficiency and in adaptation to light conditions.

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P.5.15. Enhancement of iridoid and phenylethanoid glycoside production in hairy roots of *Rehmannia glutinosa* Libosch. following elicitation by abiotic elicitors

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Keywords: hairy roots,iridoid glycosides, methyljasmonate,phenylethanoid glycosides

Rehmannia glutinosa Libosch. (Orobanchaceae) naturally occurs in China, Japan and Korea. Roots of the plant (*Rehmanniae radix*) have been listed in the Chinese Pharmacopoeia as well as widely used in Traditional Chinese Medicine mainly as anti-senescence, hypoglycemic and anti-inflammatory agents (Zhang et al. 2008). The pharmacological properties of the plant roots have been attributed mainly to the presence of iridoid glycosides such as catalpol, aucubin and phenylethanoid glycosides (verbascoside and isoverbascoside). The effect of methyl jasmonate (MeJa) and salicylic acid (SA) on hairy root biomass (FW and DW) accumulation (g/flask) as well as the biosynthesis of the iridoid and phenylethanoid glycosides were measured. It was found that elicitor kind and its concentration as well as the time of exposure to the elicitor affected the metabolite production in the tested hairy roots. Among iridoids, improved accumulations of harpagid (7.2-7.5-fold higher) and catalp ol (2-fold higher) were achieved when the 23-day-old hairy roots were exposed to 150 or 200 µM of MeJa for 3 and 5 days, respectively. Catalposide content (0.095 mg•g-1 DW) was also improved after elicitation with 150 µM of MeJa for 5 days. Among phenylethanoids, verbascoside production was enhanced ~10-fold when hairy roots were treated with combined 50 µM of MeJa and 50 µM of SA or 150 µM of MeJa for 3 days. Isoverbascoside accumulation increased 6.4-times compared to the control followed by elicitation with 200 µM of MeJa for 3 days. Elicitation did not improve aucubin and loganin accumulation.

P.5.16. Excitation energy transfer control in the LHCII antenna systems by xanthophylls

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Keywords: Photosynthesis, LHCII, Photoprotection, Excitation energy transfer, Langmuir-Blodgett

Photosynthesis is a sole process able to convert the energy of electromagnetic radiation to the forms which can be directly utilized by living organisms to drive biochemical reactions.

Efficient and fluent operation of photosynthesis is assured by the presence in the photosynthetic apparatus of pigment-protein complexes called antenna, which absorb light quanta and transfer electronic excitations towards the reaction centers. The major photosynthetic antenna complex, referred to as LHCII is the most abundant membrane protein in the biosphere and comprises approximately half of the chlorophyll pool on Earth. All the pigment-protein complexes in the photosynthetic apparatus of plants are located within the lipid membranes called thylakoids.

The aim of the studies was examination the impact of additional exogenous carotenoid pigments (violaxanthin and zeaxanthin) on the molecular organization of the LHCII complex.

The objects of studies were monolayers LHCII and LHCII with additional violaxanthin and zeaxanthin. Monolayers were formed and analysed with application of the Langmuir technique.

The results of our studies showed that the xanthophylls pigments form LHCII-xanthophyll supramolecular structures. These structures ensure efficient Förster-type excitation energy transfer and prevent formation of aggregated structures characterized by thermal energy dissipation.

P.5.17. Heterologous expression of ZmCPK11 in Arabidopsis enhanced the salt tolerance and sensitivity to root growth inhibition by MeJA and ABA

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Keywords: Calcium Dependent Protein Kinases (CDPKs)

Calcium dependent protein kinases (CDPKs) are involved in the calcium signaling in response to endogenous and environmental stimuli in plants. For elucidation of the physiological role of ZmCPK11, a member of *Zea mays* CDPKs, transgenic *Arabidopsis thaliana* plants expressing c-Myc-ZmCPK11, were generated. Expression of ZmCPK11 enhanced the salt tolerance in Arabidopsis through the upregulation of some stress responsive genes and stabilization of photosystem PSII.

The phytohormones: jasmonic acid (JA) and abcisic acid (ABA) play an essential role in plant response to stresses and also mediate inhibition of root growth. Previously, it has been shown that ZmCPK11 participates in JA-dependent local and systemic response to wounding (Szczegielniak et al. 2012) and is involved in ABA-induced antioxidant defence (Ding et al. 2013). Presently, we have looked if ZmCPK11 participates in inhibition of roots growth regulated by methyl-ester of JA (MeJA) and ABA. The ZmCPK11 transgenic plants showed increased inhibition of root growth mediated by MeJA and ABA compared with control plants.

Above results indicate that ZmCPK11 confers tolerance to salinity in Arabidopsis and is a positive regulator of roots growth inhibition in MeJA- and ABA-dependent signaling pathways.

References:
Ding et al. (2013) J Exp Bot 64: 871-884
Szczegielniak et al. (2012) Physiol Plant 146: 1-14

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P.5.18. Hydropriming supplemented with melatonin modifies corn (*Zea mays* L.) embryo proteome during seed germination under chilling stress conditions

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Keywords: melatonin, priming, chilling, proteome analysis

Melatonin (N-acetyl-5-methoxytryptamine) plays an important role in plant stress defence. Various plant species rich in this indoleamine have shown higher capacity for stress tolerance. Moreover it has great potential for plant biostimulation, is biodegradable and non-toxic for environment. All this indicates that our concept of seed enrichment with exogenous melatonin is justified. The present work is a continuation of the previous one (see part I) and also concerns the effects of corn (*Zea mays* L.) seed pre-sowing treatments . Non-treated control seeds (nt), and those hydroprimed with water (H) or with melatonin water solutions 50 and 500 μM (HMel50 and HMel500) were compared. Seed priming positive effects are particularly apparent during germination under suboptimal conditions. The impact of melatonin applied by priming on seed protein profiles during imbibition/germination at low temperature conditions has not been discussed so far. In order to identify changes in corn seed proteome after applying hydropriming techniques purified protein extracts of chilling stressed seed embryos (14 d, 5 oC) were separated by two-dimensional electrophoresis. Then proteome maps were graphically and statistically compared using PDQuest software and selected protein spots were qualitatively analysed using mass spectrometry techniques and identified using the Mascot protein databases. This study aimed at analysing the priming-induced changes in maize embryo proteome and to identify priming-associated and melatonin-associated proteins in maize seeds subjected to chilling stress during germination.

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P.5.19. Identification of new Arabidopsis lines carrying T-DNA- insertion in the AtTOR gene

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Keywords: AtTOR, T-DNA mutants, Arabidopsis

TOR kinase is an evolutionary conserved serine-threonine kinase, that is responsible for integrating signals connected with environmental stress, cell energy status and availability of amino acids. Homologues of TOR kinases were also identified in plants. Due to embryo lethality of Arabidopsis *tor* (*attor*) mutants, our current knowledge about functioning and regulation of plant TOR kinase is very limited.

By PCR-based screening of MPIPZ (Köln) mutant collectionwe have identified four new T-DNA insertion mutations in the AtTOR gene. Subsequent phenotypic analysis revealed that three of these lines exhibited embryo lethal phenotype. By contrast, the fourth mutant line (named *tor-118*) carrying a T-DNA insertion close its 3'- end of the AtTOR gene, segregated viable homozygous attor mutant plants. The *tor-118* mutant line exhibited no obvious phenotypic changes, however showed altered response to reduced nitrogen availability and treatments with TOR- kinase inhibitors. This indicates that the *tor-118* mutation reduces partially AtTOR functionality but does not affect its functions necessary for plant survival.

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P.5.20. Improved salinity tolerance during osmoprimed *Brassica napus* seeds germination is an effect of proline accumulatio and PCSA gene induction

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Keywords: proline, P5CSA, osmopriming, salinity

Osmopriming is a pre-sowing treatment that enhances germination performance and stress tolerance of germinating seeds. In this study rape seeds were osmoprimed in PEG solution with -1.2 MPa osmotic potential for 7 days in darkness. *Brassica napus* seeds showed osmopriming-improved germination and seedling growth under salinity stress. To understand the molecular and biochemical mechanisms of osmopriming-induced salinity tolerance, the accumulation of proline, gene expression and activity of enzymes involved in proline metabolism and the level of endogenous hydrogen peroxide were investigated in rape seeds during osmopriming and post-priming germination under control (H2O) and stress conditions (100 mM NaCl). The relationship between gene expression and enzymatic activity of pyrroline-5-carboxylate synthetase (P5CS), ornithine-δ-aminotransferase (OAT) and proline dehydrogenase (PDH) was determined. The improved germination performance of osmoprimed seeds was accompanied by significant increase of proline content. The accumulation of proline during priming and post-priming germination was associated with high up-regulation of *P5CSA* gene, down-regulation of *PDH* gene and accumulation of hydrogen peroxide. Up-regulated transcript level of *P5CSA* was consistent with the increase in P5CS activity. These results showed that osmopriming improves *Brassica napus* seeds germination and salinity tolerance during post-priming germination and seedling establishment and this germination performance is linked with Pro accumulation as a result of hydrogen peroxide-induced *P5CSA* expression and P5CS activity.

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P.5.21. Influence of jasmonic acid on the growth and biochemism of cucumber (*Cucumis sativus* L.) treated with lead

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Keywords: cucumber seedlings, phytohormones, lead, toxicity

Hormones play an important role in the adaptation of plants to environmental stresses. Studies were conducted to determine the effect of jasmonic acid (JA) in the range of concentrations from 0,01 µM to 10 µM on the growth, biochemism and antioxidant processes occurring in cucumber (*Cucumis sativus* L.) seedlings treated with lead (Pb) at the concentration of 100 µM. Research focused on changes in length of shoots and roots, the content of photosynthetic pigments, proteins and monosaccharides, phytochelatins (PC), ascorbic acid, hydrogen peroxide (H2O2), ascorbate peroxidase (APX) activity and lipid peroxidation. Studies confirmed that Pb was toxic for cucumber seedlings, causing irreversible changes, i.e. decrease of shoots and roots length, proteins, monosaccharides and photosynthetic pigments content. Pb also induced oxidative damage as evidenced by increased lipid peroxidation, activity of APX and H2O2, ascorbic acid and PC level. JA in depending on the concentration had a stimulatory, inhibitory or neutral effect to the test parameters of cucumber treated with Pb. Studies have shown that plants treated JA in the range of concentration from 0,01 µM to 1 µM abolished the toxic effects of lead. Furthermore, phytohormone in the concentration of 0,1 µM in the presence of Pb had a neutral effect on the lipid peroxidation. Unfortunately, 10 µM JA didn't remove toxic effect of Pb on cucumber seedlings.

P.5.22. Involvement of brassinosteroids in modification of plasma membrane enzymes activities in cucumber plants

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Keywords: brassinosteroids, PM-H+-ATPase, NADPH oxidase

Brassinosteroids (BR), a class of steroidal phytohormones, regulate a broad range of physiological processes in plants including adaptive responses to environmental stresses. New findings on the molecular mechanism of BR perception indicate that BR binding triggers the activation of the plasma membrane (PM) H+-ATPase and modifies the membrane potential. Our previous study has shown, that PM-H+-ATPase and NADPH oxidase are the key enzymes in adaptation of plants to various stress conditions.

In the present study we have examined alteration of PM-H+-ATPase and NADPH oxidase activities in cucumber roots under 24-Epibrassinolide (EBL) treatment. The hydrolytic as well as transporting activities of H+-ATPase in the plasma membranes of cucumber root cells were increased in plants treated for 1 day with 10 nM EBL. The Western blot analysis with the antibody against phosphothreonine showed that modification of PM-H+-ATPase activity under EBL treatment could result from phosphorylation of the enzyme protein. Also the relative expression of *CsHA2*, *CsHA3* and *CsHA4* genes encoding tree isoforms of PM-H+-ATPase was affected by EBL. Moreover, the stimulation of H+-ATPase was correlated with the stimulation of NADPH oxidase activity in the same conditions. Alteration of this enzyme protein under EBL treatment could be due to the genetic level. Transcript levels of *CsRbohB* and *CsRbohF2* genes increased in plants treated with EBL.

Taken together, this data indicate that BR are important for understanding the mechanisms of modification of the PM-H+-ATPase and the NADPH oxidase activity, which are key enzymes in abiotic stress conditions.

P.5.23. Involvement of nitrate reductase in production of NO in cucumber plants subjected to salt stress

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Keywords: Nitrate reductase, NO, salt stress

Nitric oxide is an important signaling molecule in abiotic stresses. One of the possible NO source in plants is nitrate reductase (NR). NR reduces nitrate to nitrite. However, NO_2^- in the cytoplasm may be reduced to NO by cytoplasmic NR. The plasma membrane form of NR (PM-NR) functions as the donor of NO_2^- for nitrite NO/reductase (Ni-NOR), which is responsible for NO generation in apoplast. Moreover increase of nitrite level and the decrease of nitrite reductase (NiR) activity may promoted the production of NO by NR. The aim of our study was to examine the effect of salinity (50 mM NaCl) on production of NO in cucumber seedlings. Additionally we analyzed the possible sources of the NO.

It was shown, that the activity of cytoplasmic NR and PM-NR increased distinctly in roots treated with salt. The increase of NR activity was due to both posttranslational modifications and stimulation of expression of NR encoding genes. In salt stressed plant roots, the NO amount, detected by labeling with fluorescent dye (DAF-2D), was higher than in control roots. Moreover the level of nitrite measured by HPLC was also higher in stressed plants. The increase of nitrite level and the simultaneously decrease of NiR activity could promoted the production of NO by NR in salt treated cucumber roots.

Taken together, we can speculate that under salt stress condition modification of the NR, PM-NR and NiR activities simultaneously with higher level of NO_2^- may led to NO production in cucumber seedlings.

P.5.24. Local and systemic response induced by mechanical wounding in *Triticum aestivum* L.

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Keywords: wheat, non-lethal wounding, chlorophyll fluorescence

Winter wheat is one of the most widely cultivated cereals in Europe. Wheat plants are exposed to a number of abiotic stresses, like mechanical wounding (by intensive rain, snow or wind), which reduce plant growth as well as quality and quantity of harvests. The aim of study was to determine the local and systemic physiological responses of winter wheat seedlings (*Triticum aestivum* L., cv. Bamberka, 3-4 weeks old) to non-lethal wounding of leaves. It was investigated whether mechanical wounding (short-term and long-term) caused changes of wheat growth parameters, leaf cells anatomy (cell wall structure, plasma membrane integrity, plasmodesmata linkage), metabolite content, gas exchange and *in vivo* fast chlorophyll a fluorescence (0.5h, 24h, 48h and 1 week after leaves injury). In addition volatile organic compound emissions after leaf wounding was measured (by GC/MS). It was observed that winter wheat seedling are rather tolerant to non-lethal mechanical wounding of selected leaves. No significant changes of plant growth, chlorophyll content, photosynthesis and respiration rate between control and wounded plants were observed. Chlorophyll a fluorescence measurements indicate only slightly decrease of Fv/Fm and DI/RC and increase of ET0/RC and RE0/RC in wounded leaf. Volatile emission analysis after wounding confirm presence of 11 compounds (e. g. ethyl ether, dimethyl sulfide and limonene). Presented results suggest that winter wheat activate physiological and metabolic pathways of local and systemic response to minimalize negative effects of leaf mechanical wounding.

P.5.25. Mechanical wounding affects sugar metabolism in leaves of *Arabidopsis thaliana* hormonal mutants

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Keywords: response to wounding, stress, photosynthesis,sugars,phytohormones

Plants are constantly exposed to abiotic stresses in natural environment. Reaction to wounding involves various hormones e.g.: jasmonic acid (JA), abscisic acid (ABA), ethylene. Wounding induce defence mechanisms as a combination of biochemical and molecular processes which involve different enzymes and metabolites, although we are still far from understanding this complexity.

The aim of study was to determine the influence of mechanical wounding to gas exchange, fluorescence of chlorophyll a and carbohydrate metabolism in leaves of *Arabidopsis thaliana* L., wild type (wt) and hormonal mutants: *ein4* (ethylene insensitive), *aos* (deficit of JA, disrupted signal transduction of wounding), *rcd1-1* (reduced sensitivity to ABA, ethylene, MeJA). Measurements were made 2, 24 hours after injury of rosette leaves. It was observed that photosynthetic oxygen evolution decreased 2 hrs after injury of wt. Respiration rate increased in wt after wounding. Photosynthesis and respiration in mutants was lower according to wt. In spite of modification in gas exchange, chlorophyll a fluorescence did not change considerably after stress treatment, both in wt and mutants. It was observed that invertases (vacuolar, cell-wall and cytosolic) activity increased, especially 24 hrs after wounding. Activity of sucrose synthase did not change after stress treatment, however in mutants activity was significantly higher than in wt. Soluble sugars content increased 24 hrs after injury in wt, *aos* and *ein4*, probably as effect of higher invertases activity.

These results indicate that mechanical wounding and changes in hormones sensitivity and/or biosynthesis have an influence on Arabidopsis carbohydrate metabolism, especially on sugar hydrolyzing enzymes activity.

P.5.26. New data on hormesis mechanism in maize seedlings treated with Cd and Pb.

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Keywords: hormesis, maize, cadmium, lead

Effect of growth stimulation induced by low concentration of toxic substances like heavy metals is described as hormesis (Poschenrieder et al., 2013).

Uniform 4-days-old maize seedlings cv. 'Lokata' were introduced into hydroponic system filled with control medium and after 7 days were treated with $CdCl_2$ or $PbCl_2$ at concentrations of 0.25, 0.5, 1.0 , 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 µM. After 4 days of growth in the presence of the metal, lengths of roots and leaves were measured and content of IAA and H_2O_2 in roots and leaves was determined spectrophotometrically.

The toxic effect of Cd was larger than that of Pb in shoots. For Cd and Pb hormetic effect for shoots was observed at 5.0 µM and 10.0 µM respectively. In the case of roots, hormetic effect was observed only for Pb (2.5 µM), whereas there was no hormesis for Cd. Measurements conducted for plants treated with Cd or Pb revealed positive correlation between the content of IAA, H_2O_2 and elongation growth of shoots. It was found that Cd and Pb increased the IAA and H_2O_2 concentration in shoots, what could be correlated with hormetic effect. In roots treated with Pb there was also positive correlation between the concentration of IAA, H_2O_2 and elongation growth.

Interaction between H_2O_2 and IAA could be proposed as a possible mechanism of hormesis in plants.

P.5.27. NMR and SEM studies combined with aquaporins expression profiles in relation to water status in germinating osmoprimed *Brassica napus* seeds

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Keywords: aquaporins, Brassica, osmopriming, NMR spectroscopy,SEM

Osmopriming is a pre-sowing treatment that exposes seeds to a low water potential that allows partial hydration but prevents germination. Osmopriming improves seeds germination and stress tolerance of germinating seeds. In this study, *Brassica napus* seeds were osmoprimed with polyethylene glycol (PEG 6,000) solution with osmotic potential -1.2 MPa during 7 days in darkness. In order to examine water status in primed rape seeds, NMR and SEM studies and aquaporins expression profiles were investigated in seeds during osmopriming and post-priming germination.

Higher uptake of water was observed in primed seeds up to 12h of germination. The changes in water status were characterized by NMR spectroscopy. Analyses of T2 relaxation times revealed a four-component water proton system in rape seeds, each with a different magnetic environment. The intensities of two of them are predominant – the first one (the shortest T2 – 3 ms) is connected with bound water and the second one (~100 ms for dry seeds) is attributed to oil component. It is evident that during hydration of primed seeds the most significant changes in terms of its contribution in molecular dynamics occurred for the population of bound water. Changes in NMR relaxation parameters reflected structural changes of seed coat surface sculpturing pattern as revealed by SEM studies. The data point to a crucial role of aquaporins during germination of primed seeds due to accelerated water uptake and vacuole enlargement.

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P.5.28. Non-protein amino acids: canavanine and meta-tyrosine act as rapid inducers of oxidative stress in roots of tomato seedlings

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Keywords: canavanine, m-tyrosine, protein carbonylation, ROS

Non-protein amino acids (NPAAs) as other secondary metabolites act in plants as a weapon against herbivores, microbes and neighboring plants. Canavanine (Can) is found in legumes such as *Canavalia ensiformis* (L.) DC. mostly in seeds and is considered as a strong phytotoxin. Meta-tyrosine (m-Tyr) is identified as allelopathic compound of *Festuca rubra* L. ssp. *commutata* and *Euphorbia myrsinites* and known to exhibit harmful effect on growth of various plants species. Phytotoxic action of many chemical compounds secreted by plants or allelochemicals is linked to secondary oxidative stress.

The aim of the work was to investigate involvement of ROS in phytotoxicity of Can and m-Tyr on growth of roots. Tomato (*Solanum lycopersicum* L.) seedlings (developed from seeds imbibed for 3-4 days in water) were cultured for 24 h in Can or m-Tyr solution at concentration leading to 50 % inhibition of root growth or at concentration that entirely inhibited root elongation but were not lethal. Restriction in root growth by NPAAs was accompanied by enhancement in H2O2 concentration, alterations in tissue specific localization of H2O2 and superoxide anion (O2-•) detected by DAB or NBT staining. Increased activity of peroxidase (POx) suggested, action of POx in modulation of H2O2 concentration in roots exposed to m-Tyr and Can. Moreover, pattern of protein carbonyl groups in root extracts of NPAAs treated seedlings points at ROS as key agents of toxicity of non-proteinogenic amino acid, of main importance in short term experiments.

Acknowledgements:
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P.5.29. Novel Thylakoid Associated Chaperone 1 (TACH1) is a regulator of photosynthesis and abiotic stress responses in *Arabidopsis thaliana*

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Keywords: photosynthesis, oxidative-stress, chloroplast, chaperone, Arabidopsis

Thylakoid Associated Chaperone 1 (TACH1) has been previously identified as a component of thylakoid membrane proteome in *Arabidopsis thaliana*. Its N-terminal region contains a chloroplast targeting signal, while the C-terminal fragment is a chaperone-specific DnaJ domain, including Zn finger motif. Between these two domains there is additionally a single transmembrane helix, which anchors the protein in thylakoid membrane.

Our previous transcriptome profiling experiment demonstrated that *TACH1* expression is dependent on three well-described regulators of photosynthesis and programmed cell death (PCD): LESION SIMULATING DISEASE 1 (LSD1), ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4). This prompted us to investigate the role of TACH1 in photosynthetic reactions and abiotic stress responses.

Recently obtained results indicate that, compared to the wild-type Arabidopsis plants, the TACH1 mutant (*tach1*) demonstrates significant alterations in the photosynthetic pigments accumulation and photosynthetic efficiency. However, the structure of chloroplasts does not vary from the wild-type plants. Upon excess light (EL) stress, the expression of *TACH1* is markedly elevated. Moreover, the response of *tach1* mutant towards EL differs from the wild type, which is manifested by altered hydrogen peroxide (H2O2) concentration and distinct activities of antioxidant enzymes engaged in reactive oxygen species (ROS) scavenging. These results prove that TACH1 is involved in the regulation of photosynthesis, redox homeostasis and antioxidant signaling in response to abiotic stresses.

P.5.30. Oxidative stress in maize coleoptile cells incubated in the presence of juglone and lawsone

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Keywords: juglone, lawsone, maize, oxidative stress

Naturally occurring naphthoquinones, such as juglone (5-hydroxy-1,4-naphthoquinone) and lawsone (2-hydroxy-1,4-naphthoquinone), are referred as allelochemicals which may inhibit the growth of both plant shoots and roots as well as interfere with processes such as photosynthesis, respiration, transpiration and membrane transport. These harmful effects of both compounds, as it seems, are related with their strong redox activities.

While on the other hand, some studies have demonstrated protective role of juglone and lawsone by reduction of oxidative stress and inhibition of macromolecular oxidation.

Despite documented evidence of biological activity of juglone and lawsone, little is known about activity of antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX) under naphthoquinones induced stress.

The aim of the work was to determine the effect of juglone and lawsone on H2O2 concentration and antioxidant enzymes activity in maize (*Zea mays* L.) coleoptile cells .

Naphthoquinones mediated stimulation in activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) was assigned as an element of cellular response leading to its enhancement of oxidative stress tolerance. In contrast to juglone, lawsone was much more affective in elevation of both H2O2 concentration and antioxidant enzyme activity in maize coleoptile cells regardless of the presence of auxin (IAA) in the incubation medium of maize coleoptiles.

P.5.31. Pectins esterification in the apoplast of aluminum-treated pea root nodules

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Keywords: aluminum, apoplast, infection thread, nodule,pectin

The pea (*Pisum sativum* L.) root nodules were shown to respond to Al stress by thread growth inhibition and thickening of plant and infection thread (IT) walls. The hypothesis was tested that pectin content and methylation degree participate in regulation of wall mechanical properties and in this way may affect nodules and ITs growth under Al stress.

An immunolabeling technique with antibodies specific to homogalacturonan (HG) epitopes (JIM5, unesterified HG; 2F4, a blockwise de-esterified HG; JIM7, methyl-esterified HG) was used to visualize the distribution of different types of HG in cell walls of pea root nodules after short (2 and 24 hrs) Al treatment. After immunolabeling the samples were observed using a fluorescent and transmission electron microscope.

Immunolocalization of pectin epitopes indicated that the proportion of esterified and unesterified pectins change significantly under Al stress. In the absence of Al, JIM5 and 2F4 epitopes were located on the inner surface of the primary cell wall with higher intensity at cell corners lining the intercellular spaces and at inner ITs wall. By contrast, the JIM7 epitope was present throughout the plant and IT walls, and distributed more evenly along the walls. Al treatment increased de-esterified pectin concentrations of nodule apoplast, whereas esterified pectin content decreased.

These findings suggest that thread growth inhibition and wall stiffening under Al stress is associated with decrease of pectin esterification. The possible role of pectin in the plant-mediated control of infection thread growth under Al stress was investigated.

P.5.32. Phototropin1- blue light receptor in Arabidopsis interacts with different SUMO (small ubiquitin-related modifier) isoforms and with SUMO ligases

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Keywords: phototropin, *Arabidopsis thaliana*, SUMO

Phototropins are plasma membrane-localized photoreceptors in plants, which are activated upon blue light and UV-A absorption. In Arabidopsis two phototropins were identified, phototropin1 and 2. Phototropins mediate phototropism, leaf positioning, stomatal opening and chloroplast movements. Upon blue light absorption the C-terminal serine/threonine kinase domain of phototropin is activated resulting in receptor autophosphorylation. Following activation and autophosphorylation phototropin1 moves from the plasma membrane to the cytosol whereas phototropin2 moves from the plasma membrane to the Golgi complex.

SUMO (small ubiquitin-related modifier) is a type of the Ubiquitin Like Proteins (UBLs) that binds reversibly to target proteins. Sumoylation can influence many cellular processes, acting through several different pathways. It is a post-translational process that regulates plant signaling, development, and responses to hormonal and environmental cues. Sumoylation is similar to ubiquitination and requires similar types of enzymes. So far, two SUMO ligases were found – SIZ1 and MMS21. Unlike ubiquitination, sumoylation does not direct a protein for degradation, but can change its localization, activity or stability.

In this study we examined interactions of phototropin1 with different SUMO isoforms, and with SUMO ligases . The interactions were tested by Bimolecular Fluorescence Complementation (BiFC) method using transient transformation of *Nicotiana benthamiana* epidermis, and observed under a confocal microscope. We found out that phototropin1 interacts with isoforms of SUMO and with both SUMO ligases. This may suggest that phototropin1 function is regulated by sumoylation.

P.5.33. Pigment profiles of Arabidopsis wild-type and *nramp* mutants grown in different soil mixes at low temperature

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Keywords: NRAMP, environmental stress, pigments, cold

The family of NRAMP (Natural Resistance Associated Macrophage Protein) transporters plays an important role in the transport of various divalent metal ions (Mn+2, Fe+2, Co+2, Cd+2, Cu+2, Ni+2 and Zn+2). Homologues of this conserved family are involved in metal ion acquisition and homeostasis in all living organisms. We showed recently that NRAMP-specific highly conserved histidine is crucial for plant performance. Its mutation in the transmembrane domain 6 of AtNRAMP1 was associated with a severe leaf chlorosis, low chlorophyll content, decrease in photosynthetic performance and impaired plant growth under manganese deficiency at low temperatures. In this study, we tested the combined effect of different soil chemical properties and chilling stress on the pigment composition of wild-type control plants (Col-0) and five Arabidopsis *nramp* mutants (*nramp1, nramp2, nramp3, nramp4, nramp5*). We used spectroscopic analysis to measure chlorophylls content and Chla/b ratio indicating the photosynthetic capability of plants as well as anthocyanin, xanthophyll, carotene and total carotenoid levels depicting their antioxidant potential. All plants were grown at 4°C in selected soil mixes that differed in the pH, salinity and nutrient content. The only plants showing sign of chlorosis were *nramp1* mutants. In comparison to Col-0 plants they exhibited significant reduction in chlorophylls content and Chl/carotenoids ratio, lower xanthophyll content and major increase in anthocyanin level. Interestingly, *nramp5* mutants grown in optimal soil mix showed significant reduction in anthocyanin content associated with increased carotene level. To better understand the physiological status of *nramp* mutants we currently perform the measurements of oxidative stress.

P.5.34. Protective role of Nitric oxide and Salicylic acid vis-à-vis Arsenite Phytotoxicity in Rice (*Oryza sativa* L.)

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Keywords: Arsenic, Rice, Nitric oxide, Salicylicacid

Nitric oxide (NO) and salicylic acid (SA) are important signaling molecules in plant system. In present study they showed a protective role against arsenite (AsIII) stress in rice plants when supplied exogenously. Their application revert the plant growth hampered due to AsIII toxicity Nitric oxide supplementation to AsIII treated plants greatly reduced the As accumulation in root and the shoot/ root translocation factor as well. Arsenite exposure in plants decreased the endogenous levels of NO and SA which were partly recovered upon exogenous supplementation of NO and SA. Exogenously supplied SA enhanced the nitrate reductase activity while exogenously supplied NO reduced the NR activity. Arsenic accumulation was positively correlated with the increase expression level of its transporter Lsi1. Nitric oxide and SA were significantly positively correlated (R= 0.88) to each other weather AsIII was present or absent indicated that they functions in close harmony to modulate the signaling response in plants.

P.5.35. Proteome analysis of dehydration response in wheat (*Triticum aestivum* L) seedlings

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Keywords: dehydration, proteomics, wheat, 2DE

Ability of plants to tolerate dehydration is multigenic and multifactorial trait based upon mechanisms that protect and maintain the cellular integrity and repair during rehydration. Although there is impressive progress in improving our understanding of plant stress physiology and molecular biology there is a concomitant need for understanding responses of non-model systems such as agricultural crops because this knowledge will contribute to improve innovative plant breeding programmes. Wheat (*Triticum aestivum* L.) seedlings are a perfect plant model to study dehydration tolerance due to the transition from dehydration tolerant to intolerant state about 5th day of germination when seedlings are still in heterotrophic phase of growth. Therefore, experiments were done on 4-day-old seedlings, tolerant to dehydration and 6-day-old drought-sensitive seedlings.

To further investigate inducible responses in wheat seedlings to dehydration and gain more insight on drought tolerance strategies 2-D PAGE combined with high-throughput MS/MS 2-DE were used to identify protein expression changes triggered by dehydration equal to 65% WSD. Proteome profiles were compared and differential proteins were selected. In dehydration-tolerant 4-day-old seedlings 12 differential proteins were found, among them four were up-regulated and eight were down-regulated in response to dehydration. In dehydration-sensitive 6-day-old seedlings 13 differential proteins were found and five of them were up-regulated whereas eight were down-regulated. Proteins positively identified by searches across non-redundant protein database of NCBI and SwissProt using the Mascot search engine. Protein spots have been categorized into functional groups involved in photosynthesis, carbohydrate and energy metabolism, protein folding and degradation etc.

P.5.36. Relationships between root hairs and toxic effect of Cd on transpiration and photosynthesis in barley (*Hordeum vulgare* L.)

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Keywords: photosynthesis, transpiration, barley, cadmium stress

The aim of the present investigation was to find the relationship between root hairs' development and the toxic effect of Cd on photosynthesis and transpiration in barley.

Experiments were carried out with barley cv. Dema and its root hair mutants (*rhp* – root hair primordia only; *rhs* - root hairs significantly shorter) (Janiak, Szarejko 2007). Three-days-old barley seedlings were introduced into the hydroponic system with basal medium and after 3 weeks of growth were treated with 10 or 25 µM Cd for the next 5 days. On the fifth day of the cadmium treatment, transpiration rate, photosynthetic rate and stomatal conductance with LCpro+ (ADC BioScientific Ltd., England) were measured.

It was found, that all selected parameters (photosynthetic rate, transpiration rate and stomatal conductance) were higher for cv. Dema in comparison with the mutants in the control medium. The decrease of photosynthetic rate caused by Cd was least for rhp mutant, 99 and 85 % of the control for 10 and 25 µM Cd respectively. The toxic effect of Cd in concentration of 25 µM was the same for all genotypes in respect to transpiration rate (61 % of control). Stomatal conductance was less affected by Cd treatment in both mutants when compared with parent variety.

On the basis of the above data it is proposed that reduction of root hairs, and in consequence – of the root system absorption area, diminishes the toxic effect of cadmium on photosynthesis and transpiration in barley.

P.5.37. Selenium supplementation ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.)

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Keywords: Antioxidant, Arsenic, Oxidative

Arsenic (As) contamination of rice is a major problem for South-East Asia. In the present study, the effect of selenium (Se) on rice (*Oryza sativa* L.) plants exposed to As was studied in hydroponic culture. Arsenic accumulation, plant growth, thiolic ligands and antioxidative enzyme activities were assayed after single (As and Se) and simultaneous supplementations (As+Se). The results indicated that the presence of Se (25 µM) decreased As accumulation by 3-fold in roots and 2-fold in shoots as compared to single As (25 µM) exposed plants. Arsenic induced oxidative stress in roots and shoots was significantly ameliorated by Se supplementation. The observed positive response was found associated with the increased activities of ascorbate peroxidase (APX), catalase (CAT) and glutathione peroxidase (GPx) and induced levels of non-protein thiols (NPTs), glutathione (GSH) and phytochelatins (PCs) in As+Se exposed plants as compared to single As treatment. Selenium supplem entation modulated the thiol metabolism enzymes viz., γ-glutamylcysteine synthetase (γ-ECS), glutathione-S-transferase (GST) and phytochelatin synthase (PCS). Gene expression analysis of several metalloid responsive genes (*LOX, SOD* and *MATE*) showed upregulation during As stress, however, significant downregulation during As+Se exposure as compared to single As treatment. Gene expressions of enzymes of antioxidant and GSH and PC biosynthetic systems, such as APX, CAT, GPx, γ-ECS and PCS were found to be significantly positively correlated with their enzyme activities. The findings suggested that Se supplementation could be an effective strategy to reduce As accumulation and toxicity in rice plants.

P.5.38. "Single molecule" fluorescence analysis of photosynthetic antenna complexes

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Keywords: photosynthesis, single molecule, excitation quenching

Recent development in optical microscopy and electronics opens new possibilities for studying molecular assemblies at the single molecule (SM) level. SM technique exploited by our experimental group is fluorescence, widely used in biological research and medical/clinical diagnosis. In last decade, fluorescence detection has been developed to selectively address individual molecules. SM detection provides several crucial advantages over more conventional bulk methods, for biological measurements. By examining a separate systems, it is possible to avoid averaging effect or have a deeper insight into the individual properties of the measured sample.

Over the presentation it will be discussed several experiments performed on main photosynthetic antenna complex LHCII (Light Harvesting Complex of Photosystem II) studied in a single molecule regime. The different organization forms of LHCII (monomeric, trimeric and supramolecular) will be discussed in context of light stress conditions and adaptive regulatory mechanisms that operate at the molecular level of this pigment-protein complexes. Applying different excitations it has been noticed, that the photo-protecting mechanism is driven by blue light and operates in the trimeric but not in the monomeric complex of LHCII. The data regarding the intensity and lifetime changes will be presented based on confocal time-resolved microscopy system. The organization forms will be separated in regard to their` characteristic diffusion coefficients.

P.5.39. Sulfur reduces arsenic toxicity by efficient thiol metabolism and the antioxidant defense system in rice

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Keywords: Arsenic, Rice, Sulfur, Thiols

Arsenic (As) contamination is a global issue, with South Asia and South East Asia being worst affected. Rice is major crop in these regions and can potentially pose serious health risks due to its known As accumulation potential. Sulfur (S) is an essential macronutrient and a vital element to combat As toxicity. The aim of this study was to investigate the role of S with regard to As toxicity in rice under different S regimes (0.5 to 5 mM). High S treatment resulted in increased root As accumulation, likely due to As complexation through enhanced synthesis of thiolic metabolites, such as non-protein thiols and phytochelatins, which restricted As translocation to the shoots. Enzymes of S assimilatory pathways and downstream thiolic metabolites were up-regulated with increased S supplementation; however, to maintain optimum concentrations of S, transcript levels of sulfate transporters were up-regulated at lower S concentration. Oxidative stress generated due to As was counterbalanced in the high S treatment by reducing hydrogen peroxide concentration and enhancing antioxidant enzyme activities. The high S concentration resulted in reduced transcript levels of Lsi2 (a known transporter of As). This reduction in Lsi2 expression level is a potential reason for low shoot As accumulation, which has potential implications in reducing the risk of As in the food chain.

P.5.40. The accumulation of raffinose family oligosaccharides in seedlings of pea (*Pisum sativum* L.) in response to desiccation

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Keywords: pea, seedling, desiccation, raffinose

The accumulation of galactinol and raffinose family oligosaccharides (RFOs) in the vegetative tissues of different plant species under abiotic stresses seems to be a part of protective mechanisms leading to plants stress tolerance. In the present study, the ability of pea seedlings to restore the RFO's biosynthesis under desiccation was analyzed. Changes in the soluble carbohydrates were analyzed by the gas chromatography method in axis, cotyledons, roots and epicotyls during 7 days of germination of pea seeds and after desiccation.

In dry seeds, RFOs and sucrose were the main soluble carbohydrates in both axis and cotyledons. During seed germination and the seedling growth, the content of RFOs rapidly decreased, while the content of monosaccharides (mainly glucose), sucrose and myo-inositol increased. After 72 hours of seed germination, traces of raffinose and stachyose (but not verbascose) accumulated in the epicotyls and roots. Desiccation of seeds germinating for 2 days did not induce the accumulation of galactinol and RFOs. The ability of epicotyls and roots (but not cotyledons) to synthesis elevated amounts of RFOs in response to dehydration was detected even in 3-4-day-old seedlings. According to the progress in seedling growth and development (to the 7th day of germination), tissues accumulated increasing amounts of RFOs (up to 3.12 and 1.82 mg g-1 DW in the roots and epicotyls, respectively). In regard to the fact that tissues accumulated several-fold higher amounts of sucrose (up to 200-290 mg g-1 DW) than RFOs, the physiological significance of synthesis of RFOs in seedlings remains to be explained.

P.5.41. The Arabidopsis SWI/SNF ATP-dependent chromatin remodelling complex responds to environmental changes in temperature- dependent manner

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Keywords: Arabidopsis thaliana, cold, fertility, SWI/SNF

The SWI/SNF chromatin remodeling complexes (CRCs) have been shown to play important roles in regulation of gene expression throughout eukaryotes.

The Arabidopsis genome encodes four SWI2/SNF2 ATPases, four SWI3, a single SNF5 and two SWP73 subunits. Most of the genes encoding these core components of Arabidopsis SWI/SNF CRCs have critical but not fully overlapping roles during plant growth, including embryo- and sporophyte development.

During our study we found that genes encoding the SWI/SNF CRC subunits are ubiquitously expressed and that their expression levels depend on the temperature regime of growth. Furthermore, Arabidopsis mutants impaired in several of these genes growing at lower temperatures show partial alleviation of their phenotypic defects, including reduced fertility, root development, and others.

In summary, our data provide novel insight into potential regulatory role of SWI/SNF CRCs activity during plant growth at different temperature ranges.

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P.5.42. The effect of cytokinins on shoot proliferation, secondary metabolite production and antioxidant potential in the shoot cultures of *Scutellaria alpina*

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Keywords: Cytokinins, Scutellaria alpina, Shoot culture

The genus Scutellaria (Lamiaceae) comprises about 300 species, which are distributed in Europe, the United States and East Asia. Many of the plants belonging to the Scutellaria genus have been used for centuries in traditional Chinese medicine for the treatment of hyperlipidemia, arteriosclerosis, allergy, antibacterial and inflammatory diseases. The pharmacological properties of the plants are mainly due to the presence of flavonoids such as baicalein, baicalin, wogonin and wogonoside.

The present study evaluates the effects of various cytokinins on *Scutellaria alpina* shoot proliferation and production of polyphenolic metabolites (baicalin, wogonoside, luteolin, luteolin 7-O-glucoside, verbascoside).

Our results demonstrate that the type of cytokinin and its concentration significantly influence the accumulation of bioactive metabolites in the shoot cultures of *S. alpina* and their antioxidant activity. The shoots were induced from shoot tips on MS medium supplemented with IAA (0.57 µM) and various concentrations of BAP, kinetin, zeatin (1, 2, 4, 8 µM) or TDZ (0.2, 0.5, 1 µM). Among the cytokinins tested, BAP at concentration 2 and 4 µM was the most effective for shoot induction and number (about 25 per explant). Significantly higher baicalin, wogonoside and verbascoside contents were recorded in treatments containing cytokinins, when compared to cytokinin-free medium. TDZ at a concentration of 0.5 µM was the most effective for polyphenol production. In both antioxidant tests (ABTS, FRAP), the shoots from medium supplemented with 0.5 µM TDZ demonstrated the strongest activity.

P.5.43. The effect of lead on the biochemical and antioxidant response of green alga *Scenedesmus obliquus*

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Keywords: heavy metal, stress, toxicity

The biochemical and antioxidant response of unicellular green alga *Scenedesmus obliquus* (Turpin) Kützing (Chlorophyceae) (SAG Strain No.: 276-6) treated with lead (Pb) in the range of concentrations 1-1000 µM were studied. *S. obliquus* has been identified as good scavenger of heavy metal from aqueous solution. The accumulation of Pb was found to be increased in a concentration and duration dependent manner. However, the highest biosorption of this heavy metal was found in plants exposed to low levels (10 µM) of Pb in the nutrient medium. In response to Pb stress the inhibition of algal growth expressed as cell of number and the decrease in photosynthetic pigments, monosaccharides and proteins contents in the culture were observed. Pb induced also oxidative damage as evidenced by increased lipid peroxidation and hydrogen peroxide level. On the other hand, the deleterious effects resulting from the cellular oxidative state can be alleviated by enzymatic (superoxide di smutase, catalase, ascorbate peroxidase and glutathione reductase) and non-enzymatic (ascorbate, glutathione) antioxidant machinery activated in *S. obliquus* exposed to Pb, especially at 10 µM. These results suggest that *S. obliquus* is a promising bioindicator of heavy metal toxicity in aquatic environment.

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P.5.44. The effect of stress under natural and experimental conditions on nuclear genome size in Viola L. (Violaceae)

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Keywords: 2C-DNA, *in vitro*, pollution, Viola

Nuclear genome size varies tremendously in angiosperms, spanning nearly 2400-fold range (0.065 pg/1C in *Genlisea margaretae*; 152.23 pg/1C in *Paris japonica*) [1]. The evolutionary significance of this variation is still debated. According to the "Large Genome Constraint Hypothesis" by Knight et al. [2] species with large genomes are under evolutionary disadvantage under stressful environments and may become extinct locally or globally. Selective exclusion of large genomes in extreme environmental conditions was supported by studies conducted at sites polluted with heavy metals and/or at high-altitude habitats.

Using DNA flow cytometry we estimated nuclear genome sizes of selected *Viola* species from six sections *Chamaemelanium, Erpetion, Melanium, Nosphinium, Plagiostigma,* and *Viola*. We analyzed plants growing on both heavy metal polluted and non-polluted sites and *in vitro* regenerated individuals in order to address the effects of stressful environmental conditions on intraspecific variation in genome size. The estimated 2C-values ranged from ~1 pg to ~4 pg. There were no significant differences between *V. tricolor* plants from polluted and non-polluted sites (mean 2C=4.035 pg) or between metalliferous and non-metalliferous populations of *V. riviniana* (mean 2C=2.200 pg). *In vitro* conditions induced variation in nuclear genome size of regenerated plants as compared to maternal plants in *V. uliginosa* (2C=1.370 pg and 1.358-2.782 pg in maternal and regenerated individuals, respectively) and *V. epipsila* (2C=2.390 pg and 2.467-5.049) but not in *V. stagnina* (2C=1.316 pg and 1.297 pg).

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P.5.45. The impact of temperature on chloroplast movements in Arabidopsis

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Keywords: Arabidopsis, chloroplast movements, phototropin1, phototropin2

Chloroplast movements in *Arabidopsis thaliana* are controlled by two blue light photoreceptors, phototropin1 and phototropin2. Both phototropins redundantly regulate the chloroplast accumulation response under weak blue light. In weak light conditions chloroplasts accumulate at cell walls perpendicular to the direction of incident light. The avoidance response under strong blue light solely depends on phototropin2. Strong light stimulates chloroplasts to move to cell walls parallel to the direction of incident light. Temperature is an important factor which influences chloroplast relocations. At room temperature, chloroplasts under medium blue light start to move to cell walls parallel to the light direction and undergo a partial avoidance response. In the same conditions, but at low temperatures, a strong enhancement of the avoidance reaction is observed - chloroplasts behave as if they were responding to strong light. Higher sensitivity of the avoidance response is correlated with changes in phototropin expression. After cold treatment, the level of *phototropin1* is down-regulated, while the *phototropin2* level is up-regulated. Thus the motile system of chloroplasts in Arabidopsis is more sensitive to blue light at low temperatures, as observed in other species studied before.

P.5.46. The influence of cryopreservation on genetic differentiation and nuclear genome size of recovered plants of *Viola stagnina* (Violaceae)

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Keywords: cryopreservation, *in vitro*, ISSR, 2C-DNA

Cryopreservation is useful tool for plant *ex situ* preservation. Plant material e.g. adventitious shoots can be stored in liquid nitrogen (LN) for very long term without any loss of morphogenic potency. Immersion of plant material (LN) is always preceded by cryoprotection to adapt tissues to cryogenic conditions. Osmotic shock from improper cryoprotection frequently leads to tissues damage. Osmotic dehydration and abscisic acid supplementation to culture media used in cryoprotection and also post-thawing in vitro culture are stressful conditions for plant tissues often can cause genetic variability of recovered plants and disturbances in their generative reproduction. Genetic stability in recovered plants is important aspect of cryopreservation.

It this study we tested different cryoprotection techniques. Encapsulation/osmotic dehydration and encapsulation/vitrification proved to be the best for *Viola stagnina* – high percent of encapsulated young shoots was able to develop after LN treatment. Plants regenerated after cryopreservation were acclimatized and their genetic stability was analyzed using ISSR (inter-simple sequence repeat) molecular markers and flow cytometry (FCM). Most of recovered after cryopreservation individuals were genetically uniform with their initial (maternal) plants as confirmed by ISSR markers. Mean nuclear genome size of *V. stagnina* from natural sites used as initial material was 1.316 pg DNA/2C (range from 1.301 pg to 1.330 pg) whereas in plants regenerated after cryopreservation ranged from 1.292 pg – 2.614 pg indicating the effect of applied experimental conditions on genome multiplication by endopoliploidy.

P.5.47. The influence of induced oxidative and nitrosative stresses on production of secondary metabolites in *Dionaea muscipula in vitro* culture

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Keywords: oxidative, nitrosative, stress, naphtoquinones, flavonoids

Plants secondary metabolites are intensively studied due to their wide application in medicine. The antioxidant properties of flavonoids were examined as they can be used as potential cytostatic agents in anti-cancer therapies. The cytotoxic, antimicrobial, antiviral, antifungal and antimalaric properties of naphtoquinones are tested to evaluate their suitability as a new class of substances against multi-drug resistant pathogenes, i.e. *Staphylococcus aureus*. The present study was carried out to examine the influence of nitrosative and oxidative stresses on the accumulation of secondary metabolites in *Dionaea muscipula* L. in plant tissue cultures. Oxidative stress was induced by the addition of paraquat (PQT) herbicide to the culture media in the concetration of 0.5 µM. Nitrosative stress was induced by the replacement of nitrate by nitrite ions (39.4mM NO2-). Elicitation on *D. muscipula* was carried out on Murashige and Skoog medium in four variant: with a half concentration of macroelements (1/2MS; used as a control), 1/2MS with PQT, 1/2MS with nitrite ions and 1/2MS with both elicitors. Changes in the activities of antioxidant enzymes (nitric and nitrite reductases, superoxide dismutase and ascorbate peroxidase) were tested to evaluate the levels of nitrosative and oxidative stresses. Physiological condition of plants was determined by total proteins and chlorophylls content and photosynthetic activity. PQT appeared the best elicitor which gave the highest accumulation of plumbagin in planttissues (125% increase in comparison to the control). The medium, which induced nitrosative stress, almost doubled total flavonoids content inplant tissues (196% increase in comparison to the control).

P.5.48. The poison is in the dose: growth and electrophysiological responses of maize coleoptile cells to naphthoquinones

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Keywords: naphthoquinones, growth rate, membrane potential

Introduction: In accordance with generally known adage of Paracelsus “the dose makes the poison” we have tested couple of naturally occurring secondary metabolites, in most cases acting as allelochemicals. In general naphthoquinones are known for their cytotoxicity. However, it has been demonstrated that in some cases naphthoquinone could also act as a lifespan stimulator. Such experiments seems to be necessary due to the possibility of usage these substances as herbicides.

Methods: All experiments were performed on etiolated maize coleoptile segments. The measurements of elongation growth and pH of the incubation medium were carried out using an angular position transducer, allowing a precise record of the growth kinetics. In order to measure the electrophysiological response the standard electrophysiology technique was used in which the voltage between a reference electrode and a parenchymal cell interior was recorded.

Results: Four main naphthoquinones: 1,4-naphthoquinone, juglone, lawsone and naphthazarin in the chosen concentration were studied. It was found that all tested naphthoquinones in the lower concentration only slightly diminished the growth of the maize cells. However, in the higher concentration, the growth responses examined by us were highly differentiated. Similar differentiations in electrophysiological responses on naphthoquinones were observed. Some of them significantly decreased the membrane potential of maize parenchymal cells, while other demonstrated only a minor effect.

Conclusion: It could be concluded that particular naphthoquinones, in the same concentration, differentially affected the growth and electrophysiological responses of maize coleoptile cells. In some cases their effect was toxic; in others only slight influence was observed.

P.5.49. The possible role of PsbS protein in the energy quenching in photosystem I in *Echinochloa crus- galli*

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Keywords: CP22, energy quenching, phosphorylation, xanthophylls

Photoprotection of photosystems is essential to avoid the light-induced damage of the photosynthetic apparatus under excess light. Xanthophylls are involved in non- photochemical quenching (NPQ) of energy. In our experiments we examinated *Echinochloa crus- galli* (C4 plant, type NADP-ME), which is resistant to high light illumination. The HPLC analysis showed that the amount of zeaxanthin, a product of pH-dependent conversion of violaxanthin, after 1 hour of high light treatment (1600 µmol photons m-2 s-1) increased more than 4 times than in control light (300 µmol photons m-2 s-1). Separation of pigment-binding complexes by sucrose gradient ultracentrifugation of chloroplasts solubilized with β-DDM was performed. Each sucrose density fraction was analyzed by SDS-PAGE and immunoblotting. We found that the amount of PsbS (CP22) protein after 1 hour of high far red light (720 nm) treatment, drastically increased and protein is present in PSI fraction. In these conditions we also observed strong phosphorylation of D1 and LHCII antenna proteins contrary to the low intensity of far red light, where all proteins were dephosphorylated. The simultaneous phosphorylation of antenna and PSII core subunits may facilitate the dissociation of peripheral antenna. The CP22 protein was noted in the fraction where PsaA and P-Lhcb1 proteins are present. According to results we proposed that CP22 protein play a role in energy quenching from PSI through the interaction with phosphorylated LHCII and zeaxanthin in a similar way to qE mechanism in PSII.

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P.5.50. The response of TILLING barley mutants, *hvabi5* and *hvdrf1*, to abiotic stresses

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Keywords: ABA, drought, ABI5, DRF1, barley

Plants tolerance to abiotic stresses, such as drought and salinity, is mainly regulated by phytohormone ABA (abscisic acid). We analyzed physiological and molecular responses of newly identified barley mutants in HvABI5 (Abscisic Acid Insensitive 5) and HvDRF1 (Dehydratation Responsive Factor 1) genes encoding transcriptions factors in ABA-dependant regulatory pathway.

Homozygous mutants in HvABI5 and HvDRF1 genes were identified with the use of TILLING strategy (in HorTILLUS population developed in Department of Genetics, University of Silesia for spring barley cv. 'Sebastian'). Taking into account, that both genes are involved in abiotic stress response, we applied drought assay to seedlings of analyzed mutants in order to verify their reaction to water deficit. Both mutants, *hvabi5.d* and *hvdrf1.a* exhibited better response to drought when RWC and chlorophyll a fluorescence parameters were compared with their wild-type.

In order to investigate downstream target genes of HvABI5 and HvDRF1 or their partners in stress signalosome, the global profile of expression was analyzed using Agilent Barley Array 44K. We identified sets of genes related to stress response.

We performed analyses of response to ABA, salt and osmotic stress during seed germination and seedling development. Both mutants germinated in the presence of 300 µM ABA that inhibited germination of 'Sebastian' in 90%. We also observed changed response of both mutants to ABA and abiotic stress factors during seedling development. These results indicate, that HvABI5 and HvDRF1 regulate response to abiotic stress in ABA-dependant way during germination and seedling stage at physiological and molecular level.

P.5.51. Transcriptomic response to the salt stress in excised leaves of sea beet (*Beta vulgaris* ssp. *maritima*)

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Keywords: salt stres, Beta vulgaris, halophyte

Sea beet (*Beta vulgaris* ssp. *maritima*) is a halophytic relative of cultivated beets. The aim of the study was to characterize the transcriptomic response to acute salt stress imposed to excised leaves of sea beet. Subjecting plants to salt treatment consisted of adding NaCl directly to the transpiration stream by immersing the petioles of excised leaves into the salt solutions. To generate the leaf transcriptome, samples were collected from excised leaves incubated for 48 hours in Hoagland medium (control) or in the same medium but supplemented with 150mM NaCl (moderate stress) or 300mM NaCl (strong stress). Alterations in the leaf transcriptome due to the salt treatments were followed by RNASeq. To investigate differential gene expression, we mapped the reads from each sample (control or salt-treated) to the reference transcriptome assembled from pooled reads from all samples. The analysis revealed 684 transcripts being up-regulated and 633 transcripts which were down-regulated in salt-stressed leaves. The analysis of the distribution of DEGs according to Gene Ontology revealed several terms from “Biological process”, “Molecular function” and “Cellular compartment”. Within the “Biological process” category there were the considerable number of DEGs ascribed to the subcategories related to redox metabolism and protein synthesis. The distribution of DEGs according to “Molecular Function” shows the important participation of genes related to protein synthesis and ion binding activity. The distribution of DEGs among the “Cellular compartment” terms revealed that salt treatment significantly altered the expression of genes encoding proteins structurally and functionally associated with the system of cellular membranes.

P.5.52. UVB treatment changes the intranuclear localization of AtUVR3, a 6-4 PP-s photolyase

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Keywords: Arabidopsis, nucleus, photolyase, UVR3, UV-B

UVB irradiation leads to the formation of dimers between adjacent pyrimidines in the DNA strand. The predominant forms are cyclobutane pyrimidine dimers (CPDs) and, to a lesser extent, 6-4'-[pyrimidine-2'-one] pyrimidine photoproducts (6-4 PPs). In plants such photoproducts are repaired mainly by specialized enzymes, photolyases, which use blue light or UVA energy for splitting dimers. The *Arabidopsis thaliana* 6,4-PP photolyase, encoded by AtUVR3, is localized in cytoplasmic vesicles, chloroplasts and nuclei as shown using transient and stable transformation of Arabidopsis and Nicotiana with GFP-tagged protein. The intranuclear localization was changed upon UVB treatment. In control samples the uniform fluorescence in the nucleus with a stronger signal in the nucleoulus was visible. After irradiation with UVB AtUVR3 was translocated into the places where photoproducts had been formed. In result a 3-D structure could be observed inside the nucleus. Fluorescence recovery after photobleaching (FRAP) was performed to check the protein motility. In control samples UVR3 molecules localized in nucleus were moving constantly, with the time of recovery after photobleaching being around 300 ms. After 15 min of irradiation with 8 Wm-2 of UVB UVR3 which was bound to photoproducts became less mobile and the recovery time was over 80 times longer as compared with the control. After 2 hours of photoreactivation with 100 µmolm-2s-1 of white light, when all the dimers were repaired, UVR3 started to diffuse faster again reaching the recovery time of around 1 s.

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SESSION 6: SOCIAL AND COMMERCIAL ASPECTS OF CONTEMPORARY BIOTECHNOLOGY

Plenary Lectures

Don't be afraid about carnivorous plants, since they may help cure you

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**Equal contributions to this work*

Carnivorous plants are able to attract, trap, retain, kill, and digest prey (Juniper et al., 1989). They are found all around the world growing on nutrient-poor soils. They have established an original way to circumvent the shortage of mineral nitrogen resources: their leaves have evolved to form traps for catching prey. These preys (insects or small animals) are subsequently digested allowing acquiring substantial amounts of nitrogen. *Drosera* and *Nepenthes* are two genera of carnivorous plants able to produce and excrete out of their tissues a digestive fluid containing proteins which are mostly hydrolytic enzymes. *Drosera* leaves are covered on their upper face by stalked glands secreting sticky and viscous digestive mucilage. *Nepenthes* leaves are differentiated in pitchers, the lower internal part being covered by glands secreting a digestive liquid (Juniper et al., 1989).

The major problems related to the production of recombinant proteins from plants are related to the extraction, separation, and purification of the recombinant proteins. The downstream process costs can represent up to 80% of the total cost of production of a recombinant protein (Hellwig et al., 2004). To overcome this bottleneck, we aim to exploit the natural ability of carnivorous plants to secrete proteins in order to develop *Drosera* and *Nepenthes* plants as new plant recombinant protein platforms. These systems suppress two main latches: 1) extraction is made easier since the digestive fluid is readily accessible and 2) purification is simpler because the digestive fluid contains only a dozen of hydrolytic enzymes (Hatano and Hamada, 2008).

The proof of concept was done with *Drosera rotundifolia*, for which a protocol for genetic transformation was already available (Hirsikorpi et al., 2002). Our experimental studies clearly highlighted the presence of Green Fluorescent Protein (GFP) and β-glucuronidase (GUS) in the digestive secretions of transgenic plants (Biteau, 2009). These preliminary results were patented as the PAT Friday® technology in 2008 (Biteau et al., 2008). The technology was further extended to two additional plants: 1) *Drosera capensis*, which has longer leaves and hence higher quantities of mucilage available than *Drosera rotundifolia*, and 2) *Nepenthes mirabilis*, the digestive liquid of this latter species being harvestable just by pouring the pitchers. For both species, an original protocol for the genetic transformation has been established (Miguel, 2013; Nisse, 2014) and GFP could be detected in their digestive fluids.

A major drawback of this system relies on proteases naturally present in the secretions which could damage the recombinant proteins. Even if our ultimate results show that these hydrolytic enzymes can be inactivated by modulating the pH of the plant secretions, it is important to characterize them at the molecular level. Therefore, we are investigating the exact composition of the digestive liquids from *Drosera capensis* and *Nepenthes mirabilis* through transcriptomic and proteomic approaches. The knowledge of the corresponding coding sequences will help us to inactivate them, by gene silencing for example.

Our goal is to offer the PAT Friday® technology for the production of relevant recombinant therapeutic proteins. The production of a monoclonal antibody, of a cytokine, and a gastric protein among others are currently under way

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Treatment of acute and chronic inflammation by novel natural plant extracts with high biological activity

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Nutraceutics also known as functional food are natural bioactive compounds with potential applications in preventing and curing many human acute and chronic diseases and disorders. Due to incorporation of beneficial properties of nutrients and pharmaceuticals, nutraceutics bring variety of health benefits. Inflammation is the normal physiological immune response to tissue injury. During inflammation, macrophages play a central role in managing many different immunopathological phenomena, including the overproduction of pro-inflammatory mediators such as IL-6, IL-1β, NO, iNOS, COX-2, 5-Lox, TNF-α. Number of inflammatory stimuli such as LPS and pro-inflammatory cytokines activate immune cells to up regulate such inflammatory states and these are therefore useful targets in the development of new anti-inflammatory drugs/supplements. Biolevox inflox (BVC-07) is a proprietary combination of two natural components *Nigella sativa* and *Angelica archangelica* that together have a very potent anti-inflammatory effect in comparison to the commonly used anti-inflammatory drugs or to components by themselves. Active principles are prepared through innovative extraction process – carbon dioxide supercritical extraction (CSE) and ethanol extraction. Preclinical results show that BVC-07 exhibits strong anti-inflammatory properties both *in vitro* and *in vivo* providing significant alternative to traditionally used medicines. Along with strong anti-inflammatory activity, BVC-07 exhibits very good safety profile with MTD above 2000 mg/kg. Currently PK studies as well as clinical trials in humans are conducted to support strong *in vitro* and *in vivo* data for this combination.

Plant naphthoquinone derivatives as MAPK/ERK kinase inhibitors – potential application in breast cancer treatment

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Breast cancer is one of the most common malignancies diagnosed in women. It accounts for around 12% of all cancer incidents as is the leading cause of cancer-related deaths among women. Despite advances in diagnostics and treatment, resistance acquired towards chemotherapeutic agents still is an obstacle in effective treatment. Understanding the molecular mechanisms that govern cancer development has led to the identification of novel molecular targets responsible for cancer resistance. One of the key mechanisms involved in cancer resistance is the activation of the MAPK/ERK pathway. The MAPK/ERK is constitutively activated in many types of cancers including breast cancer. The activation of this pathway has been associated with the inhibition of apoptosis induction in cancer cells. Due to the association of MAPK signaling with chemoresistance, the identification of MAPK inhibitors and their use in combination therapy alongside chemotherapeutics could increase the efficacy of breast cancer treatment.

Recent research has shown the potential of plant-derived naphthoquinones in anticancer therapy. Naphthoquinones have been reported to display high anti-proliferative activity towards breast cancer cells in *in vitro* and *in vivo* studies. Moreover, research indicates that naphthoquinones are effective inhibitors of the MAPK/ERK signaling pathway. The potential application of naphthoquinones in breast cancer therapy and their potential in combination therapy will be presented.

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In vitro cultured carnivorous plants as a source of secondary metabolites with high antibacterial potential in combination with silver nanoparticles

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Carnivorous plants are a group of plants including more than a dozen genera, able to uptake nutrients from trapped and digested prey. The genus *Dionaea* and *Drosera* (family Droseraceae) are ornamental plants known from their medicinal potential. Carnivorous plants were used for centuries in folk medicine as a remedy for bronchitis, whooping cough or as treatment of burn wounds. The antimicrobial, anti-inflammatory or antitumor potential of infusions or extracts from carnivorous plant tissues are attributed to the presence of large quantities of secondary metabolites. Studies on the phytochemistry of *Dionaea* or *Drosera* revealed that the main group of secondary metabolites produced in their tissues are naphthoquinones and flavonoids. The introduction of these plants to *in vitro* allowed to multiply plant material regardless of natural climate conditions. The use of *in vitro* cultures also allowed to increase the levels of valuable secondary metabolites by changing culture conditions. Despite the antimicrobial potential of naphthoquinones, their medicinal use is limited due to their cytotoxicity toward human cells. Thus we studied the antimicrobial activity of extracts from carnivorous plants or secondary metabolites in combination with silver nanoparticles (AgNPs). We observed that the simultaneous use of AgNPs and extracts or pure naphthoquinones significantly reduced the bactericidal concentrations as well as cytotoxicity towards cultured human keratinocytes. The obtained results revealed the possibility of using plant derived naphthoquinones as an alternative antimicrobial therapy in the case of antibiotic resistance of microorganisms.

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The use of microalgae for the production of active compounds

Marcelina Jaros

Svanvid

Marine organisms are increasingly a source of interest to many scientists. Microalgae are one of them. They are unicellular species which exist individually, or in chains or groups. They can be found in clear, freshwater. The biodiversity of microalgae is enormous and they represent an almost untapped resource. Most of these microalgae species produce unique products like carotenoids, enzymes, polymers, peptides, toxins and sterols, fatty acids. While fish oil has become famous for its omega-3 fatty acid content, fish don't actually produce omega-3s, instead accumulating their omega-3 reserves by consuming microalgae. These omega-3 fatty acids can be obtained in the human diet directly from the microalgae that produce them. Culturing process, which consists of choosing the right strain, inoculum preparation, the actual process of fermentation, stabilization and subsequent isolation of biomass is so far quite time-consuming and costly. Research is still ongoing on optimizing costs. Oil extraction by supercritical fluid extraction using CO2 as the extraction solvent is applied. As a result of the high pressure and elevated temperature impacts, the cell comes to rupture, releasing the substance contained inside. That is why microalgae are a rich source of active ingredients, which use opens up new horizons.

LIST OF AUTHORS

Selected Talks

Poster Presentations