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## Session 3: Plant cell biology and biochemistry

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### Lectures

#### L3.1

##### A Medicago ABC transporter modulates the level of isoflavonoids during the defence response associated with de novo synthesis of phytoalexins

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Plant defense mechanisms against pathogen attack are based *inter alia* on *de novo* biosynthesis and secretion of biologically active compounds called phytoalexins. Various group of phytoalexins differ in chemical structures or biosynthesis pathways and can be distinctive for particular plant species. In legumes this role is attributed to products of phenylpropanoid pathway (PP) represented by isoflavonoid and flavonoid derivatives, for instance: glyceollin in soybean (*Glycine max*), pisatin in pea (*Pisum sativum*) or medicarpin in barrel medic (*Medicago truncatula*).

It has been previously shown that in *Medicago* roots, upon biotic stress, biosynthesis of medicarpin depends on a full size ABCG transporter (Banasiak *et al.*, 2013). Silencing of *MtABCG10* expression increases the plant susceptibility to the fungal pathogen infection and affect to the accumulation of medicarpin and its precursors in *Medicago* roots, as well as root exudates.

In order to show that *MtABCG10* might be involved in distribution of PP products we have conducted experiments of PP restoration in *MtABCG10*-silenced *Medicago* roots by exogenous application of early medicarpin precursors as well as transport experiments. The latter have been conducted in BY2 suspension cell cultures overexpressing the *MtABCG10* and/or membrane vesicles derived from them. To monitor the transport of phenolic molecules we have used HPLC/MS and/or radiolabeled substrates. Among tested compounds, p-coumaric acid and liquiritigenin have been shown to be transported in the *MtABCG10* dependent manner.

Identification of transporters participating in phenolics translocation within and between cells appear as another fundamental step in understanding of the regulatory process of PP metabolites biosynthesis. In view of the presented data, a new potential role for plant ABCGs as modulators of carbon flow in the phenylpropanoid pathway can be postulated.

#### Reference:

Banasiak J *et al* (2013) *J Exp Bot* **64**: 1005-1015.

#### L3.2

##### Intracellular trafficking of bacterial virulence factors – a hunt for host targets?

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Many bacterial pathogens deploy specific proteins, referred to as effectors, to manipulate host defense responses. Effector repertoires are shaped to target host immune signaling pathways at multiple levels. The bacteria use host cells as a source of eukaryotic cofactors that are indispensable for effector enzymatic activity or acquisition of their final conformation. They also co-opt host cellular machinery to activate pathogenic effectors *via* post-translational modifications and translocation to their site of action. A number of effectors possess organelle-targeting signals that control their subcellular distribution.

*Pseudomonas syringae* pv. *phaseolicola* is the causative agent of halo blight in common bean. Similar to other pathogenic Gram-negative bacteria, it delivers a set of type III effectors into host cells. HopQ1 is one of these effectors. We showed previously that HopQ1 specifically interacted with plant 14-3-3s, and this association affected its subcellular localization [1]. Our current studies demonstrate that the position of HopQ1 is regulated by several other host factors involved in the plant defense response. Intracellular calcium concentration and redox status, which change in response to pathogen attack, may control HopQ1 monomer/dimer equilibrium and thereby control HopQ1 nucleocytoplasmic partitioning. Plant kinases activated in response to pathogen infection change the HopQ1 phosphorylation status, which in turn determines its localization. The resulting cellular distribution patterns of HopQ1 change during the course of microbial infection. These results suggest that bacteria hijack host defense signaling components to dynamically tune the intracellular localization of their virulence factors. We hypothesize that this mechanism would enable effectors to perform systematic searches for their corresponding virulence targets.

#### Reference:

1. Giska F *et al* (2013) *Plant Physiol* **161**: 2049-2061.

## Oral presentations

### 03.1

#### Fraxetin synthase: a novel enzyme in the biosynthesis of coumarins involved in plant responses to iron deficiency

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Iron (Fe) deficiency represents a serious agricultural problem, particularly in the alkaline soils. Recently, it was reported that the secretion of phenolic compounds like coumarins by *Arabidopsis thaliana* roots is induced under Fe-deficiency conditions, but the mechanisms of action underlying this process has remained largely unknown. Therefore, a better understanding of coumarins biosynthesis and elucidating the precise role of their increased accumulation in response to Fe deficiency is important from the scientific and commercial point of view.

The objective of this study was to characterize an enzyme of unknown biological function that belongs to a Fe(II)- and 2-oxoglutarate-dependent dioxygenase (2OGD) family whose members are involved in various oxygenation/hydroxylation reactions, including biosynthetic pathway of coumarins. Importantly, gene encoding the selected enzyme is reported in the literature as being strongly induced under Fe deficiency.

We performed detailed enzymatic activities measurements by using heterologous and transient expression systems (*Escherichia coli* and *Nicotiana benthamiana*, respectively). This enzymatic characterization demonstrated that the studied dioxygenase is involved in the conversion of scopoletin (one of the predominant coumarin compound occurring in *Arabidopsis* roots) into fraxetin *via* hydroxylation at the C8-position. Consequently, the enzyme was named scopoletin 8-hydroxylase (S8H). Subsequently, we conducted the phenotypic characterization and metabolite profiling of *s8h* homozygous mutant lines grown together with control plants (Col-0) in different types of culture (soil mixes, hydroponic and *in vitro* cultures) under various regimes of Fe availability. The rosettes of *s8h* mutants grown in Fe-depleted conditions were paler than Col-0 plants and showed signs of chlorosis. It was associated with significantly higher accumulation of two coumarins (scopoletin and its glucoside scopolin) in mutant roots when compared with control plants. In parallel, no fraxetin was detected by LTQ LC/MS in *s8h* mutant line.

The above results indicate that the characterized enzyme designated as S8H is a fraxetin synthase involved in the last step of fraxetin biosynthesis and is a potential candidate for playing an important role in Fe acquisition by plants.

#### Acknowledgments:

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### 03.2

#### Cajal body functions in the post-transcriptional regulation of gene expression in plant cells under physiological and abiotic stress conditions

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CBs are considered a common element of somatic and generative cell nuclei in plants and animals. The best-known function of CBs is their participation in the process of snRNP but the list of processes in which these structures are involved appears to be far from complete.

We have recently shown the presence of poly(A) RNA in Cajal bodies (CBs) in root cells of three species (*Lupinus luteus*, *Allium cepa*, *Arabidopsis thaliana*). At least a portion of the poly(A) RNAs found in CBs are protein-coding RNAs. The cyclin B1, peroxidase, cytokinin-specific binding protein, and pectin methyltransferase mRNAs colocalise in Cajal bodies in *Lupinus luteus* cells. Next, we tested whether these structures are associated with the transcription process, or with subsequent stages of mRNA maturation. We have shown that in the CBs of lupine cells, there is a lack of active RNA polymerase II. However, *in situ* run-on transcription assays demonstrated small amounts of transcribed RNA in CBs, but only after a long incubation period when partial transport of a new form of RNA from the nucleus to the cytoplasm was observed. The splicing some pre-mRNAs occurs post-transcriptionally. The CBs of plants are not a direct site of splicing, as SR proteins are not observed in these nuclear bodies. Because the CBs are not the place of transcription or splicing, this indicates they participate in the later stages of post-transcriptional gene expression in plant cells.

Studies aimed at evaluation of the role of Cajal bodies in mRNA metabolism were continued in plants exposed to hypoxic stress. Progressive accumulation of poly(A) RNA was observed within the nucleus, particularly within the CBs, during consecutive hours of hypoxia. *In situ* hybridization to 7 different mRNAs indicates that the CB is a storage site for those of mRNAs which are not involved in the response to hypoxia for use by the plants after the hypoxic stress. Under hypoxia, *ncb-1* mutants of *Arabidopsis thaliana* with a complete absence of CBs died sooner than wild type (WT), accompanied by a strong reduction in the level of poly(A) RNA in the nucleus. These results suggest that the CBs not only participate in the storage of the nuclear RNA, but they also could take part in its stabilization under low-oxygen conditions.

### 03.3

#### The involvement of programmed cell death mechanisms in development of Zn-related pre-necrotic/necrotic regions in tobacco leaves

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Recent studies indicate that in the leaves of tobacco plants exposed to high Zn concentration in the medium pre-necrotic regions developed from mesophyll cells accumulating high amount of Zn [1]. This regions subsequently turn into the necrosis with high amount of Zn inside. Accumulation of Zn in the pre-necrotic/necrotic regions contributes to protecting the neighbouring cells from Zn toxicity. We proposed that formation of necrosis could be a mechanism of tolerance to Zn, rather than only the symptoms of Zn sensitivity. In this study the involvement of programmed cell death mechanisms in formation of Zn-related pre-necrotic/necrotic regions in tobacco leaves was investigated. Plants grown in hydroponic cultures were exposed to high Zn. On the fixed leaf cross-sectioned the TUNEL analysis was performed. In a plants leaves the programmed cell death marker genes (*NtSAG12*, *NtBAK1*, *NtBI-1*, *NtSIPK*, *Ntrboh*) expression was measured.

The TUNEL analysis showed the PCD-positive nuclei in a cross-sections of leaves from tobacco plants grown in the presence of high Zn in medium. They were not present in the control sections. The expression analysis of programmed cell death marker genes showed the increase in expression of genes in the leaves from high Zn-treated plants in comparison to the control plants (optimal Zn concentration in medium).

The presented results showed that Zn-related pre-necrotic/necrotic regions are most likely a form of plants Zn-tolerance strategy (a form of programmed cell death), rather than an accidentally cell dying as a result of Zn-toxicity.

#### Reference:

1. Siemianowski O *et al* (2013) *Plant, Cell and Environmental*, **36**: 1093-1104.

### Posters

#### P3.1

#### Effect of culture temperature on composition and fluidity of *Phaeodactylum tricornutum* thylakoid membranes

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*Phaeodactylum tricornutum* is diatom commonly used in laboratory studies. As in plants, diatoms photosynthesis takes place in thylakoids, however lipid protein and pigment composition of diatom thylakoid membranes is significantly different from vascular plants or even green algae. The diatom thylakoids have unique composition of unsaturated fatty acids and carotenoids. An one-step conversion of diadinoxanthin (Ddx) to diatoxanthin (Dtx) catalyzed by de-epoxidase (VDE) is also typical for diatoms. It is commonly known that fatty acids as well as carotenoids or proteins are responsible for regulation of membrane fluidity. However, the role of those components in temperature adaptation of diatom thylakoid membranes is unfamiliar. We present the comparative analysis of the two temperatures of culturing effect on fatty acids composition (estimated by GC) and proteins level (Lowry) in *P. tricornutum* thylakoid membranes. Role of diadinoxanthin cycle pigments (estimated by HPLC) in temperature-regulated thylakoid membranes fluidity (estimated by EPR) is also shown.

It was found that the level of Dtx/(Dtx+Ddx) after de-epoxidation in isolated thylakoids of the 8-day diatoms cultured at 12°C was about 25% higher than in thylakoids isolated from *Ph. tricornutum* growing at the same time, but at 20°C. Obtained results were well correlated with VDE expression (RT-PCR).

Membrane fluidity of thylakoid membranes was analyzed with two spin labels: 5-SASL (monitored changes in headgroup region of lipids) and 16-SASL (analyzed hydrophobic interior of membrane). It was shown that fluidity of hydrophobic interior of membranes was more sensitive both to culture temperature and Dtx/(Dtx+Ddx) level than fluidity of membrane headgroup region. 16-SASL order parameter ( $S_{1\phi}$ ) shown the thylakoid membranes isolated from diatoms growing at 12°C is slightly more rigid although the carotenoids content calculated per chlorophyll a was lower and proportion of unsaturated to saturated fatty acids higher at 12°C than 20°C. Additionally, at 70-90% of Ddx de-epoxidated, strong rigidifying effect of Dtx was observed. However, higher Dtx level was necessary for membrane rigidification in thylakoids isolated from diatoms adapted to 12°C than from those adapted to 20°C. Obtained results showed that Dtx is factor important for stabilization of diatom thylakoid membranes and together with fatty acids, carotenoids and proteins plays important role in regulation of diatom membranes fluidity.

## P3.2

### Comparing peroxidases from two waste products: potato pulp and mashed barley malt.

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Class III peroxidases (POX), abundant in all land plants, play in them diverse roles. Among others, they are engaged in auxin catabolism, defense against oxidative stress and cell wall lignification. Up to date, the most popular source of peroxidases is horseradish, and POX from this plant are used in enzymatic immunoassays, as a tool for bioremediation or for decolorization of industrial dyes.

Potato pulp and mashed barley malt are sources of peroxidases uncharacterized previously. They are both waste products, from starch and beer industry, respectively. In order to characterize these enzymes, kinetic studies are necessary. Also, they may differ in preferred substrates and susceptibility to known peroxidase inhibitors, such as potassium cyanide (KCN) or sodium azide (NaN<sub>3</sub>). The drawback of using enzymes commercially is often their instability, therefore an effect of adding stabilizing agent such as polyethylene glycol (PEG) also should be tested when characterizing enzymes from new sources. Better understanding of these properties may contribute to proposing new enzyme sources with applicable possibilities.

In presented studies, kinetic properties of peroxidases from both sources will be presented. The Michaelis-Menten constant and  $V_{max}$  of the reactions catalyzed by POX with different substrates will be analyzed, along with the inhibition constant ( $K_i$ ). Also, the protective effect of adding PEG will be tested.

#### References:

Kurnik K. *et al* (2015) *Water Air Soil Pollut* **226**: 254.  
Regalado C. *et al* (2004) *Phytochemistry Reviews* **3**: 243-256.

## P3.3

### Resistance to arsenic of plant callus tissue inoculated with arsenic resistant bacteria

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The aim of this study was to investigate the effect of arsenic on Caucasian fir (*Abies nordmanniana*) callus tissue infected with bacteria resistant to arsenic. Three different genotypes of callus tissue numbered 6, 11 and 22 were injected with three volumes of suspensions of three soil bacteria strains resistant to arsenic. The callus cultures were incubated for 3 weeks and analyzed. Then all samples were subcultured on fresh SH solid medium supplemented with different pure As concentrations of 0.01%, 0.01% with 1% EDTA, 1% (w/v) and without As as a control. The cultures were incubated for 3 weeks in dark, 24°C and analyzed on 7<sup>th</sup> and 21<sup>st</sup> day of incubation according to 1-3 scale. Following criteria were considered: color, cells hydration and somatic embryos presence. Therefore the scale included following characteristics: 1 – the best condition, visibly promising to proliferate, light color, somatic embryos present and no lysis observed; 2 – tissue cells hydrated in comparison to 1, color from beige to brownish/light brown, partly lysed, but able to proliferate; 3 – light to dark brown, tissue strongly hydrated or lysis observed, no somatic embryos present or dark brown not promising to proliferate observed.

The results demonstrated, that the infection of callus tissue with bacteria did not decrease its health condition. The condition of the tissue samples was determined as 1 of 1-3 scale. After 7 days incubation callus tissue on medium with 1% of arsenic resulted with hydration of cells for all 3 genotypes what indicates strong osmotic stress. In case 0.01% concentration of arsenic, after 3 weeks of incubation the condition of the tissue visibly differed. The strongest resistance was demonstrated by 22 genotype on medium supplemented with 0.01% + 1% EDTA and injected with As5, As6 and As9 strains. The condition of the all tissue samples was estimated as 1, whereas without bacteria as 2. In case of genotype 11 approximately 64% of samples were estimated as 1 or 2 (20% of samples as 1). Next results can indicate, that particularly As9 strain efficiently improves the callus resistance to arsenic as it ensured the lowest percentage of samples with 3 category in case of all concentrations (33% of samples characterized as 3).

### P3.4

#### The functional state of PSII in *AtEgy2 Arabidopsis thaliana* mutants

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Intramembrane proteolysis is an ubiquitous process occurring in all forms of life and involved in many important, often very distinct, processes like protein quality control, signaling or membrane remodeling. However, only a few intramembrane proteases have been characterized in plants. EGY2 is thylakoid membrane-associated protein belonging to site-2-proteases, an unusually-hydrophobic integral membrane proteins with a conserved zinc metalloprotease active site (HExxH) within a transmembrane domain. These proteases are generally thought to be involved in gene expression regulation through a process called regulated intramembrane proteolysis. Recently, the involvement of *AtEgy2* protein in hypocotyls development and fatty acids biosynthesis was demonstrated. It has been also suggested that *AtEgy2* may play a role in the regulation of expression nuclear and plastid genes, however deeper insight into the function of this protein is missing. According to our research the *Arabidopsis thaliana* mutants containing the T-DNA insertion onto *ATEGY2* gene sequence indeed display measurable changes in PsbA and PsbD accumulation level, indicating the potential role of *AtEgy2* protease in gene expression regulation of plastid genes. Since PsbA and PsbD form the core of the photosystem II reaction center we applied chlorophyll fluorescence analysis to investigate the functional state of photosystem II in *AtEgy2 Arabidopsis thaliana* mutant.

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### P3.5

#### Proportion of monogalactosyldiacylglycerol to violaxanthin influences violaxanthin de-epoxidase activity and its binding constant to violaxanthin.

#### A microscale thermophoresis study

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Violaxanthin cycle plays a key role in photoprotection of vascular plants and some algae. Under high light conditions enzyme violaxanthin de-epoxidase (VDE) catalyzes rapid conversion of the diepoxide violaxanthin (Vx) via the intermediate antheraxanthin (Ax) to the epoxide-free zeaxanthin (Zx). The activity of VDE is pH-dependent with the optimum about 5. At low pH, due to conformational changes, the enzyme binds to the thylakoid membrane. It is known that VDE activity is strongly lipid-dependent and can be observed only in presence of special group of lipids which create inverted hexagonal structures. One of these lipids essential for VDE activity is monogalactosyldiacylglycerol (MGDG), the most abundant lipid in thylakoid membranes.

In this study the role of MGDG – Vx proportion for VDE activity was tested by microscale thermophoresis, a new technology for the molecular interaction studies. Four tryptophan residues of VDE provide excellent fluorescence signal for thermophoresis therefore no exogenous fluorescent markers are needed for the experiments.

VDE to Vx binding curves in presence of MGDG were analyzed at pH 5, with Vx as a ligand. Three different binding curves of VDE to Vx were obtained, depending on MGDG concentration. The measured binding constant was inversely proportional to the concentration of MGDG. Subsequently, three different points on each curve were selected, for the following MGDG:Vx proportions: 246; 30; 0.24 The VDE activity was also analyzed at the selected MGDG:Vx proportions. Good correlation was observed between shapes of binding curves obtained in microscale thermophoresis and VDE activity.

### P3.6

#### AtFTSH4 Regulates mitochondrial proteostasis by controlling turnover of the essential protein import component

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Mitochondria are multifunctional organelles that play a central role in energy metabolism. In addition to their role in energy conversion, these double-membrane bound compartments are fulfilling multiple vital tasks, such as iron-sulfur cluster and heme synthesis or metabolism of amino acids or lipids. Mitochondria are placed on the crossroad of many signalling pathways and are considered as central regulators of cell death and survival. Due to the essential functions of these organelles, mitochondrial content, quality, and dynamics are tightly controlled. Across the species, highly conserved ATP-dependent proteases are preventing malfunction of mitochondria. These intriguing, ATP-fuelled protein machineries exert versatile activities. This study focuses on an *Arabidopsis thaliana* ATP-dependent protease, FTSH4, which is anchored in the inner mitochondrial membrane. Lack of FTSH4 is associated with severe morphological and developmental abnormalities in plants grown under stress conditions. This is accompanied by decreased levels and activities of specific respiratory complexes, anomalies in mitochondrial morphology and membrane lipid composition and increased oxidative stress. Yet, the molecular mechanism that explains how FTSH4 participates in the maintenance of functional mitochondria in *A. thaliana* is unknown. The goal of this work is to identify *in vivo* substrates and binding partners of FTSH4. Interestingly, using distinct approaches we found that the essential component of the TIM23 translocase, Tim17-2, constitutes a potential *in vivo* target of FTSH4. TIM23 complex is responsible for the transport of approximately two-third of the mitochondrial preproteins, which are synthesized in cytosol on free ribosomes, across the inner mitochondrial membrane. Accurate regulation of the level and composition of TIM23 is critical for mitochondrial functioning. Our data indicate that FTSH4 protease prevents accumulation of Tim17-2 in the inner mitochondrial membrane that is not assembled into TIM23 complex.

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### P3.7

#### Changes in sugar content in leaves of two potato cultivars Desiree and Russet Burbank in response to cold

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Sugar accumulation is a widely known way of regulating osmotic potential and cryoprotection in plants exposed to cold. Results presented here demonstrate differences in early response to cold between two studied cultivars. Determination of glucose, fructose, sucrose and starch concentrations in the leaves of plants grown under optimal conditions revealed that the examined cultivars differ in the natural distribution of photoassimilates between synthesis of starch and sucrose. Desiree accumulates energy substrates largely in the form of starch while Russet Burbank collects more soluble sugars within the cell. As soon as three hours after cold exposure, Desiree leaves accumulated more glucose and fructose when compared to the control plants. It is in agreement with our previous study which showed increased amylolytic activity in Desiree leaves after corresponding time of cold treatment that can lead to sugar accumulation (Sitnicka D & Orzechowski S (2014) *Biol Plant* **58**: 659-666). In turn, in the Russet Burbank leaves increased level of starch persisted up to the sixth hour of the cold treatment. Higher content of this polysaccharide suggests that the starch is not utilized as efficiently as under optimal conditions. Availability of soluble sugars at constitutively high levels (independent of the cold treatment) in the leaves of Russet Burbank, may delay the response to cold involving starch degradation. Soluble sugars present within the cell may act as osmoprotectants during short-term cold stress. At the end of the twelve hour experiment, decreased sucrose content was observed in both cultivars. Sucrose may be hydrolyzed in order to supply precursors required for synthesis of specific compounds limiting negative effects of cold. Additionally, decreased content of starch to the level measured in the control plants and accumulation of fructose and glucose observed after twelfth hour of cold in Russet Burbank are probably caused by the increased activity of  $\alpha$ -amylase which was shown in our previous report (Sitnicka D & Orzechowski S (2014) *Biol Plant* **58**: 659-666). Altogether, our data indicates that despite the discrepancies in the early stage of cold response, the further stage after twelfth hour of cold involves metabolism of sucrose as well as starch degradation in both studied cultivars.

## P3.8

### Recombinant indole-3-acetyl-amido synthetase PsGH3 catalyzes formation of high-molecular weight amide conjugates in immature seeds of pea

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Indole-3-acetyl-amino acid synthetase (IAA-amino acid synthetase) is encoded by PsGH3 gene that belongs to the one of early-auxin responsive genes. Gretchen Hagen 3 (GH3) genes encode acyl-adenylate/thioester-forming synthetases that catalyze ATP-dependent conjugation of some phytohormones with amino acids. This enzyme activity modulates concentration of signaling molecules (auxin, jasmonic acid, salicylic acid). Previously, we purified IAA-Asp synthetase from immature pea seeds. Recently a novel IAA-amido synthetase from pea has been obtained in bacterial cells as a recombinant (His)<sub>6</sub> PsGH3 fusion protein. The purified enzyme prefers IAA and L-aspartic acid as substrates for conjugation. In contrast to previously characterized IAA-Asp synthetase, the novel pea enzyme is able to synthesis of IAA-Met, IAA-Phe, IAA-Tyr, and IAA-Trp conjugates. Diadenosine pentaphosphate (Ap<sub>5</sub>A) that is an inhibitor of ATP involving enzymes acts as a competitive inhibitor of PsGH3 activity. L-Tryptophan competes with L-aspartate for catalytic site in PsGH3 enzyme but does not interfere with IAA for indole ring-binding site of the enzyme. Taking into account that IAA-amino acid synthetase requires hydrolysis ATP during first step of an acyl-adenylation, the effect of exogenous inorganic pyrophosphatase (PPase) on PsGH3 activity was examined. PPase strongly induces the IAA-Asp conjugating activity. Additionally, this report describes synthesis of high-molecular weight amide conjugates with IAA in immature seeds of pea. Protein fraction was isolated from homogenate of immature seeds by Sephadex G-10 gel filtration. Formation of IAA-protein conjugates was analyzed by radioactivity assay and Western blot using [<sup>14</sup>C]-IAA radiolabeling and anti-IAA antibody, respectively. The effects of inhibitors (L-Trp, Ap<sub>5</sub>A) and activator (PPase) of PsGH3 activity on synthesis IAA- protein have been studied.

#### References:

Ostrowski M *et al* (2016b) *Plant Physiol Biochem* **107**: 9-20.

## P3.9

### Various nucleoside 5'-phosphoramidates can induce biosynthesis of stilbenes (*trans*-resveratrol and *trans*-piceid) in grape suspension cultured cells

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Adenosine 5'-phosphoramidate (NH<sub>2</sub>-pA) was detected among cellular nucleotides purified from the green alga *Chlorella pyrenoidosa* [1]. It is known that in cells this nucleotide can be synthesized [2, 3] and degraded by various proteins [4, 5, 6]. Also in higher plants NH<sub>2</sub>-pA can be a product of the reaction catalysed by Fhit/adenylylsulphate:ammonia adenylyltransferase [3]. Our recent studies showed that exogenous NH<sub>2</sub>-pA added to *Arabidopsis* seedlings induced expression of several genes of phenylpropanoid pathway and caused accumulation of lignins, anthocyanins and salicylic acid, which protect plant cells against various stresses [7]. Therefore this nucleotide should be considered as a novel signal molecule that participate in signal transduction in response to stress. Another group of phenylpropanoid involved in stress response are stilbenes. Their particularly intensive metabolism has been observed in grape cells.

We wondered if induction of phenylpropanoid pathway by NH<sub>2</sub>-pA is a common phenomenon in plants and if nucleoside 5'-phosphoramidates other than NH<sub>2</sub>-pA can also enhance the biosynthesis of the stilbenes, such as *trans*-resveratrol (*t*-R) and its glucoside, *trans*-piceid (*t*-P). We studied the effect of 5 mM NH<sub>2</sub>-pA, NH<sub>2</sub>-pG, NH<sub>2</sub>-pU or NH<sub>2</sub>-pC on the phenylpropanoid pathway in *Vitis vinifera* cv. Monastrell cells.

Among tested nucleotides, NH<sub>2</sub>-pU and NH<sub>2</sub>-pC evoked quick accumulation of *t*-R. We observed the highest concentration of *t*-R, reaching 7 mM, in the medium after 6 and 12 h. However, in the presence of NH<sub>2</sub>-pA, the accumulation of *t*-R in the medium increased gradually reaching 7 mM after 48 h. NH<sub>2</sub>-pG and NH<sub>2</sub>-pC were the best as concerns the induction of *t*-P synthesis. In contrast to aforementioned growth media, the concentration of stilbenes in the cells in each experimental point was practically the same as in the controls, *i.e.* in the cells which grew on the medium without tested nucleotides.

#### References:

1. Frankhauser H *et al* (1981) *Biochem Biophys Res Commun* **101**: 524-532.
2. Frankhauser H *et al* (1981) *Biochem J* **195**: 545-560.
3. Wojdyła-Mamoń AM, Guranowski A (2015) *Biosci Rep* **35**:e00235.
4. Guranowski A *et al* (2008) *FEBS Lett* **582**: 3152-3158.
5. Bretes E *et al* (2013) *Acta Biochim. Pol* **60**: 249-254.
6. Guranowski A *et al* (2010) *New J Chem* **34**: 888-893.
7. Pietrowska-Borek M *et al* (2015) *Plant Physiol Biochem.* **94**: 144-152.

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### P3.10

#### Photosynthetic responses of the NAD-ME and PEP-CK subtype C<sub>4</sub> grasses to light intensity during growth

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Plants in the fluctuating environment, are able to sense changes in light intensities varying from very low to excess. Then, they developed a series of strategies which allow for efficient photosynthesis. These strategies include both the rapid responses or the long-term adjustments of photosynthetic apparatus. Appearance of diverse photosynthetic pathways was also the consequence of a better adaptation to changing environment. Under optimal conditions, characterized by high temperatures and high light intensities, C<sub>4</sub> plants have higher photosynthetic rates than C<sub>3</sub> plants. The aim of this study was to investigate whether C<sub>4</sub> grasses of NAD-ME and PEP-CK subtype can easily adjust their rate of photosynthesis to wide range of light intensities and what are their strategies of the acclimation. Chlorophyll content, level of plastoquinone, selected pool of metabolites and photochemical efficiency of the PSII were determined in the leaves of *Panicum miliaceum* (NAD-ME) and *Megathyrus maximus* (PEP-CK) plants grown under high, moderate and low light. Additionally, the activities of both photosystems, Lhc polypeptide compositions in mesophyll and bundle sheath chloroplasts (which differ morphologically and biochemically), and chloroplast ultrastructure were investigated as well. Acclimation of both species to different light conditions did not cause marked changes in photosynthetic pigment content, Fv/Fm value, nor in the size of light-harvesting antenna. Surprisingly the moderate and low light irradiances generated differences only in chloroplast ultrastructure. Bigger mesophyll chloroplasts with enlarged and more abundant grana were present in plants grown under low light. On the other hand, the changes in photosynthetic apparatus in plants acclimated to high light, compared to these grown in moderate and low light, resulted in a higher activity of PSII in both types of chloroplast and higher plastoquinone content in the leaves. The differences in influence of light intensity were noticed in both species. The examined plants are well adapted to high light because they show considerable capacity to adjust their photosynthetic characteristics to their growth light conditions. Moreover, they did not develop efficient mechanisms, allowing for better utilization of limited light, since under low light, their photosynthesis and growth are significantly lower.

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### P3.11

#### SWP73A and SWP73B subunits of Arabidopsis SWI/SNF chromatin remodeling complexes modulate leaf and flower development

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Highly organized chromatin structures are dynamically affected by ATP-dependent chromatin remodeling complexes in order to genes expression regulation. Among these complexes SWI/SNF is critical for controlling various developmental pathways. Arabidopsis SWP73A and SWP73B are important class of SWI/SNF chromatin remodeling complex subunits.

So far, there is no data about the function of SWP73A in *Arabidopsis*. Plants with RNAi silenced *SWP73B* gene show dwarf phenotype and *SWP73B* is involved in flowering time regulation by directly control of *FLC* and leaf growth through interaction with AN3. The mechanistic bases underlying SWI/SNF recruitment to specific loci are still unexplored and is focus of our research.

We found that *swp73a* mutant does not show significant morphological defects under normal growth conditions, while *swp73b* mutants are characterized by retarded growth, severe defects in leaf and flower development, delayed flowering and male sterility.

To better understand the biological processes in which SWP73A and SWP73B participate we used MNase-seq and ChIP-seq methods. Interestingly, inactivation of either *SWP73A* or *SWP73B* doesn't modify global nucleosome occupancy, however the lack of SWP73B alters positioning of particular nucleosomes on genes loci crucial for leaf and flower development. We identified, using chromatin immunoprecipitation, that SWP73B occupies promoters of these genes controlling leaf (e.g. AS1, AS2, KAN1) and flower development (e.g. AGL24, SOC1, LFY, AP1, AP3). This findings is consistent with altered expression of this genes in *swp73b* plants.

Our study showed that SWP73B appears to act as important modulator of major developmental pathways, while SWP73A functions in flowering time control. Our findings illustrate a differential contribution of SWP73A and SWP73B containing SWI/SNF complexes to regulation of transcription networks directing Arabidopsis development.



## P3.12

### Transport in woody plants – exploring the intercellular communication in xylem region

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The efficient growth of trees requires precise coordination of various developmental and physiological processes by an effective communication within the wood (secondary xylem). Wood is a heterogeneous tissue composed of both dead and living elements and is responsible for long-distance transport of water, ions, hormones and other signalling molecules. The long-living xylem parenchyma cells are known to form a three-dimensional cell system for the continuous, symplasmic transport via plasmodesmata, and maintain the integrity within the secondary xylem.

The main aim of the presented project is to decipher the role of still largely underestimated cells of the xylem parenchyma in the intercellular transport in the wood. We will visualize transporting pathways on the cellular and subcellular levels within the secondary xylem by the application of the various fluorescent tracers to three selected tree species: *Acer pseudoplatanus* (maple tree), *Fraxinus excelsior* (ash tree) or *Populus tremula x tremuloides* (poplar tree). Importantly, our analyses will focus on the seasonal changes within the cell-to-cell communication in wood 1) between radial and axial parenchyma cells and 2) between the vessel elements and the adjoining axial parenchyma cells.

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## P3.13

### Synthetic seeds from flax (*Linum usitatissimum* L.) plants

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Nowadays, technology of synthetic seeds has been developed for many species of plants. This method is especially important for elite plant material and genetically modified plants or plants, which produce recalcitrant seeds. The aim of the present study was to develop and apply encapsulation technology for flax plants. Thus apical parts of shoots and axillary buds from tissue-cultured flax were encapsulated in 3% calcium alginate. Resulted synthetic seeds showed the ability to regenerate to plants. The different composition (sucrose content, MS medium presence) of alginate beads were studied during 5 months of storage. It was observed that sucrose concentrations have different impact on germination and conversion of synthetic seeds and plant material (percentage of shoot and root formation) after short and long time of storage. The best results after 5 months storage were obtained for the artificial seeds with the highest analyzed sucrose level (50 g/l), this concentration of sucrose had the positive effect on seed germination, conversion to flax plants and amounts of resulted plants. While negative correlation between high sucrose level in alginate beads and amount of obtained rooted shoots in short time of storage (1 month) was noticed. Because alginate is biomedical material, the possible application of flax tissue encapsulated in alginate is discussed.