Session 20: Plant Biochemistry and Metabolomics

Lectures

L20.1

Plant 'Metabolic Caravans': from Anti-nutritional to anti-Parkinson alkaloids

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The regulation of metabolic pathways in plants is constantly tuned in order to suit the needs of development and fitness. Our main research objective is to unravel networks of genes and proteins which coordinate the activity of metabolic pathways, predominantly secondary metabolism, during plant development and stress response. An integrated investigation of several members of the Solanacea family (mainly tomato, potato and eggplant), rather than studying a single plant, provided us with unprecedented insights to metabolic biology in these species. Most if not all processes characterized, impact to a certain degree key quality, nutritional and post-harvest traits of these crop plants. Integrating cutting-edge transcriptomics, proteomics and metabolomics tools together with genes co-expression assays were of great value in making several key discoveries. In a recent example, combined co-expression analysis and metabolic profiling in tomato and potato led to the discovery of the multi-step, core pathway leading to the formation of the renowned Solanum alkaloids including the biosynthesis of their precursor, cholesterol. This class of molecules represent important anti-nutritional compounds in these crop plants. In the presentation, I will highlight several advanced technologies and genetic research tools and the invaluable knowledge on core metabolic traits obtained through combining them in a single study. Most if not all could be applied in the coming years to the study of key traits in other, less studied plant species.

L20.2

Genetic regulation of isoprenoid accumulation in plants – what have we learned from analysis of biochemical and genetic data

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Polyisoprenoids are found in all living organisms from bacteria to mammals and plants. They modulate the physicochemical properties of lipid membranes. Two subgroups of polyisoprenoids, dolichols and prenols are differently distributed within plant organs and play distinct biological functions: dolichols are critical for protein glycosylation indispensable for their activity and stability, while prenols affect plant photosynthetic performance and increase environmental fitness of plants. Plant metabolism undergoes re-orchestration in response to environmental stimuli and consequently content of numerous metabolites is changed. Polyisoprenoids are representatives of such stress-responsive compounds still the regulatory mechanisms underlying their accumulation remain elusive. Here, we focused on elucidation of genetic variation in polyisoprenoid content and dissection of metabolic network leading to accumulation of dolichols and prenols in plant cells by taking advantage of contemporary genetic and biochemical approaches available for a model plant *Arabidopsis thaliana*. A considerable variation in the content of tested compounds was found among tested Arabidopsis ecotypes. Subsequently, we used quantitative genetics approaches that resulted in identification of several candidate genes underlying natural variation in polyisoprenoid accumulation. Importantly, this lead us to an identification of two genes encoding key-regulators of polyisoprenoid biosynthesis and accumulation.

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Oral presentations

O20.1

Bionanohybrids between phycobilisomes and colloidal quantum dots – studies of energy transfer in complicated systems

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Phycobilisomes (PBS) are huge protein complexes, playing an antennae role for cyanobacterial photosystems. The main component of PBS is blue protein, phycocyanin (PC). Its color and high-yield fluorescence come from its covalently bound phycocyanobilin pigment. Colloidal quantum dots (QDs) are semiconductor nanoparticles, characterized by broad absorption range and narrow, size-tunable emission spectrum. It is known that energy transfer between CdSe-QDs and PC occurs after covalent coupling [1]. Here we are showing that it is possible to obtain self-assembled QD:PC and QD:PBS structures, with efficient energy transfer of Förster mechanism features. We used CdTe QDs with the negatively charged surface and PC/PBS isolated from Synechocystis sp. Self-assembly between QDs and PC/PBS was induced by simple aminothiols, as cysteine. The mechanism of that process is not fully understood yet but involves electrostatic interactions. QDs with different diameters and different emission maxima were tested. Depending on QD size, the final hybrid may contain one to ten nanoparticles per one PC functional unit. Size and organization of hybrid structures, the efficiency of energy transfer as well as possible applications of such clusters will be discussed.

Refernecs:
1. Karpulevich *et al.* (2016) *J Photochem Photobiol* **160**: 96-101.

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O20.2

Acyl-CoA:lysophosphatidylethanolamine acyltransferase (LPEAT) activity regulate plant growth and autophagy level

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Autophagy is an evolutionarily conserved process, observed in plant, yeast and animal cells. The significance of autophagy in plant response to the environment has been acknowledged for many years. However, gaps in our knowledge remain as to the precise mechanisms regulating autophagy level. Recently phosphatidylethanolamine (PE) was found to regulate the autophagy intensity in yeast *Saccharomyces cerevisiae*. Nevertheless PE involvement in regulation of autophagy intensity in plants still needs to be elucidated. PE can be synthesized, in between, in the reaction catalysed by LPEATs *via* acylating lysophosphatidylethanolamine LPE with use of acyl-coenzyme A.
In the presented research we used Arabidopsis mutants both with reduced (T-DNA insertion mutants) and elevated (mutants with overexpression of LPEAT encoding genes) activity of these enzymes. Our results show that the plants lacking LPEAT activity had a dwarfed appearance, smaller leaves, produced fewer seeds, and their root growth was inhibited. Conversely, to what was observed in the deletion mutants, plants that overexpressed LPEAT genes were larger and produced more seeds than wild type plants. We postulate that regulatory role of LPEATs on Arabidopsis growth can be realised, at least partially by regulation of the intensity of autophagy. This assumption was confirmed in our research.

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Posters

P20.1

Occurrence of HpLV and ArMV in hop gardens in Poland

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Viruses cause losses in hop production. They are very difficult to eliminate, and infected plants become a source of infection for other plants. Hop cones contain lupulin glands producing alpha acids that give the beer a bitter taste and essential oils that contribute flavor and aroma. Viral hop infection can lead to a reduction in the cones yield and cause adverse chemical changes. The hop latent virus (HpLV) belongs to the genus *Carlavirus*. Usually, the infection is asymptomatic, but sometimes it can cause chlorotic spots on the leaves. The virus is transmitted by the hop aphid (*Phorodon humuli*) in a non-persistent manner. Due to the latent nature of the infection, the exact occurrence area of HpLV is unknown. So far, it has been detected in hop gardens in Europe, the United States, New Zealand, Australia, China, South Africa and Japan. The Arabis mosaic virus (ArMV) belongs to the genus *Nepovirus*. It has a large group of natural hosts including many species of weeds, crop plants such as: hop, cucumber, pumpkin and many trees and shrubs. ArMV has been detected in many hop gardens in Europe – including: Great Britain, France and Germany. The aim of the study was to determine the occurrence of HpLV and ArMV viruses in Polish hop gardens.

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P20.2

Profiling DGAT acyl donor specificity in castor bean and soybean seed development

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Castor bean (*Ricinus communis*) and soybean (*Glycine max*) are two both economically important and scientifically interesting oilseed plants. Castor seeds contain an unusual, hydroxy fatty acid known as ricinoleic acid, unique among the *Planta* kingdom. Soybean is both protein-rich and a crucial oil crop.

Diacylglycerol:acyl-CoA acyltransferase (DGAT) is an enzyme responsible for the last step of the synthesis of triacylglycerol – main plant storage lipid. DGAT catalyses the acylation of diacylglycerol acceptor from acyl-CoA donor. A triacylglycerol molecule consists of three fatty acids, therefore DGAT acyl-CoA substrate specificity influences plant's fatty acid composition.

DGAT acyl-CoA specificity was investigated in three stages of castor and soybean seed development. In castor, DGAT's activity with various acyl-CoA was consistently increasing throughout seed growth. In correlation with mature seeds fatty acid composition, DGAT activity was the highest with ricinoyl-CoA.

Contrary, in soybean we found four varying profiles of DGAT substrate specificity towards various acyl-CoA. Depending on the acyl group donor, DGAT activity was highest and lowest in different stages of seed development. This can be explained by the existence of different DGAT isoforms with varying acyl-CoA specificity. The isoforms may have changing importance depending on the soybean seed development stage.

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P20.3

The effect of drought on the content and degradation of soluble sugars and starch in *Solanum tuberosum* L. leaves

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Water deficiency is a disadvantageous phenomenon influencing crop productivity. In the coming years climate warming is forecasted, which in combination with no changes in the amount of rainfall, may contribute to the increasingly common drought. In a pot experiment made in a greenhouse, the experimental material were the leaves of potato variety Desiree. Samples were collected at weekly intervals, starting from the day the experiment commenced. The drought indicator, which was the ratio of relative water content (RWC), reached 50% in the third week of the experiment. This was mainly due to the more than 30-fold decrease in the content of transitory starch in the leaves. There were also observed statistically significant, several times reduced contents of glucose, fructose and sucrose in leaves of plants subjected to drought stress, as compared with control plants. Additionally, drought caused increased activities of enzymes involved in starch degradation including hydrolases: alpha-amylases (EC 3.2.1.1) and beta-amylases (EC 3.2.1.2), as well as glucan phosphorylases (EC 2.4.1.1). Activities of acid and cytosolic invertases (EC 3.2.1.26), which hydrolase sucrose in potato leaves, were increasing during drought in comparison with properly watered plants. Water deficiency in leaves led to limited photosynthesis, that is disability of CO_2 accumulation and of transitory starch synthesis. Soluble carbohydrates and starch were more intensively degraded in leaves subjected to drought stress as compared with control plants, contents of these compounds per dry weight were significantly reduced.

P20.4

Plant polyphenols prevent the deleterious effects of Shiga-like enterotoxins

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Shiga-like toxin (STX) belongs to the AB_5 family of enterotoxins produced by *Escherichia coli* (STEC) and are responsible for fatal infections causing thousands of death every year worldwide. The STX consists of toxic subunit A and pentameric subunit B responsible for toxin binding to glycosphingolipids - Gb_3 and Gb_4 - located at the plasma membrane of host cells. The main problem with the STEC infection is lack of treatment inactivating STX. To find a solution to this problem we employed plants extracts used in traditional medicine to treat diarrhea. We focused on the ability of plant extracts and their polyphenols to block toxin endocytosis into host cells. We investigated if the interaction between STX and plant extracts can stop the toxins from binding to Gb_3 immobilized or/and located on cell plasma membranes of Vero cells. Our results allowed us to identify several plant species, including meadowsweet, agrimony and blackcurrant leaves, which aqueous extracts can simultaneously inhibit bacteria growth and toxin binding to their receptors. We suggest that likely mechanism of inhibition the toxin binding to the host cell receptors is related to the ability of plant extracts enriched in polyphenols to induce toxins aggregation. We hope this finding will be useful to create new tools in fighting diarrheal diseases caused by STEC infection in the future.

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P20.5

Indole-3-acetyl-*myo*-inositol biosynthesis pathway is regulated during germination of maize seeds

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Maize (*Zea mays* L.) seeds contain majority of auxin pool (97-99% of total auxin pool) as ester conjugates of IAA. About 15% of these conjugates exist as indole-3-acetyl-myo-inositol (IAInos) that is synthesized in a two-step pathway. First reaction is catalyzed by UDP-glucosyltransferase (IAGlc synthase. ZmIAGlu) that glucosylates IAA to 1-*O*-indole-3-acetyl-β-D-glucose (IAGlc). In the second step, IAGlc as an energy-rich donor of indole-3-acetyl moiety is converted to IAInos by the IAGlc: myo-inositol acyltransferase (IAInos synthase, ZmIAIn). These reactions follow according to the equations:
IAA + UDPG ↔ IAGlc + UDP
IAGlc + *myo*-inositol → IAInos + glucose
Both purified enzymes (IAGlc synthase and IAInos synthase) from maize liquid endosperm have been characterized (Leznicki & Bandurski, 1988; Kowalczyk & Bandurski, 1991; Kowalczyk *et al*., 2003), however physiological significance of IAInos biosynthesis remains unknown. Taking into account that IAA conjugation is a part of auxin homeostasis in various physiological processes, we focus on regulation of IAInos formation during development of maize seeds. In this study, we selected four stages of maize seeds development. Both enzymes, IAGlc synthase and IAInos synthase displayed the highest activity in the second stage of seed development. This finding is consistent with IAA concentration that was the highest in seeds of 1st stage and gradually diminished during next developmental stages. This result may suggest that high concentration of IAA is required for initiation of IAGlc synthase gene (*ZmIAGlu)* transcription. Despite the fact that expression level of *ZmIAIn* mRNA indicate differences between tested developmental stages, Western blot analysis revealed the identical intensity of ZmIAInos protein band in all tested stages. We cannot rule out the possibility that protein level of ZmIAInos synthase is similar during development of maize seeds, but *ZmIAIn* mRNA expression as well IAInos activity display differences. On the other hand, immunofluorescence microscope analysis show that both IAA and IAInos synthase differ in distribution patterns during development of seeds. Interestingly, UDPG pyrophosphorylase (UGPase) activity that synthesizes UDPG for IAA glucosylation, slightly increased in 2nd step of seed development (32 nmol min^{-1} mg^{-1} protein). The highest activity of UGPase (265 nmol min^{-1} mg^{-1} protein) was observed in seeds at 4th stage and probably inhibits the IAGlc synthase (about 3-fold decrease of IAGlc synthase activity in comparison to 2nd stage). These studies confirm that IAInos biosynthesis pathway is involved in maintenance of auxin homeostasis during seed germination and strongly suggest that this pathway is regulated by various effectors such as auxin and UDPG concentrations.

P20.6

Antioxidant properties of plant oils: tocopherols and beyond

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Vitamin E is a complex of naturally occurring compounds that possess strong antioxidant properties. This group includes α, β, γ and δ-tocopherols, analogous tocotrienols and plastochromanol-8, known as tocochromanols. Plant oils are one of the main sources of these compounds.
The aim of this study was the quantitative and qualitative analysis of vitamin E complex in oils of selected plant species. Separation and identification was performed using high-performance liquid chromatography with fluorescence detection. Moreover, the total antioxidant activity of plant oils and antioxidant properties of each tocochromanol have been evaluated. In order to determine the antioxidant status of plant oils a DPPH assay was used. The antioxidant activity of tocochromanols was measured in liposomes. Lipid peroxidation was evolved by azo-initiator in water phase as well as in lipid bilayer.
The analysis showed that plant oils are a rich source of vitamin E, including less widespread and known compounds – tocotrienols and plastochromanol-8. The qualitative and quantitative contribution of vitamin E homologues in particular oils is different. The results obtained on DPPH radical scavenging activity showed that there is a strong correlation between the total content of tocochromanols and the antioxidant power of plant oils. The study also showed that individual tocochromanols differ in their inhibition potential of lipid peroxidation in model systems.

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P20.7

Generation of *Arabidopsis thaliana* lines with reduced *CPT3* expression

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Polyisoprenoids are linear polymers of isoprenoid units found in the cells of all living organisms which play a crucial role in the posttranslational modification of proteins, e.g. glycosylation and prenylation. Among enzymes involved in polyisoprenoid synthesis are *cis*-prenyltransferases (CPTs) responsible for elongation of their hydrocarbon chain. So far, nine putative *CPTs* (1-9) have been identified in *Arabidopsis thaliana* genome but only three of them have been characterized (CPT1, CPT6, and CPT7). Our previous results suggest that AtCPT3 might be involved in the biosynthesis of the main family of dolichols (with dominating Dol-16) supposed to serve as cofactor of protein glycosylation. Moreover, plants overexpressing At*CPT3* have considerably higher content of this family of Dols in comparison with the wild type plants.
The goal of the presented research was to generate *A. thaliana* lines with reduced At*CPT3* expression using a small interfering RNA (siRNA) or micro RNA (miRNA) mediated knockdown. These methods of gene silencing enable generation of plants with the variable level of target gene expression. Two types of constructs, with constitutive or inducible promoter, were used in parallel to silence At*CPT3*. Such approach gives the chance to generate viable plants with decreased expression of At*CPT3* despite of the putative lethality of the *CPT3* knockout. Silenced lines will be used for the analysis of the role At*CPT3* plays in Arabidopsis cells and for further studies on the role of dolichol in plant cell metabolism.

P20.8

Effects of disrupted vesicular transport on plant sterol biosynthesis and localization

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All stages of vesicular transport in cells are controlled by Rab proteins, which belong to the family of small G proteins. The proper functioning of Rab proteins is possible due to their geranylgeranylation. This reaction is catalyzed by the enzyme called Rabgeranylgeranyltransferaze (Rab GGTase). In *Arabidopsis thaliana* two copies of each of the genes encoding for the α and β subunits of a catalytic core and one copy of a gene that encodes for an accessory REP protein were identified. Sterols, which are transported from the site of their synthesis – endoplasmic reticulum (ER) - to a cell membrane, are translocated *via* the vesicular transport system. The main plant sterols are campesterol, sitosterol, stigmasterol and cholesterol. Dedicated biosynthetic pathway of brassinosteroids – plant hormones – starts from campesterol.
The aim of this study was to determine the role of vesicular transport in plant sterol homeostasis. To this end qualitative and quantitative composition of sterols in *A. thaliana* insertion mutants that are homozygous for mutations in *rgtb1* encoding for β subunit of Rab GGTase as well as mutant with disturbed cellular response to brassinosteroids was evaluated. In parallel, the analysis of the transcription of selected genes encoding for enzymes of the sterol biosynthesis pathway was also performed using the real-time qPCR. Finally, microscopic observations of *rgtb1*plants morphology and subcellular localization of sterols (filipin staining)were performed too using fluorescent and confocal microscopy.

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P20.9

Functional analysis of scopoletin UDP-glucosyltransferase in plant responses to environmental stresses

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Previous research performed in our group revealed that a gene encoding scopoletin UDP-glucosyltransferase (UGT) is a strong candidate to be involved in differentiation of scopolin to scopoletin ratio in *Arabidopsis thaliana*. Scopoletin and its glucoside scopolin belong to plant secondary metabolites called coumarins, which have various biological activities and are beneficial for both plants and humans. In this study, we characterized the responses of plants with different scopoletin and scopolin content (Col-0, Est-1 and ugt mutant in Col-0 background) grown under selected abiotic stresses, as well as started the comparative analysis of the activity of UGTs derived from two A. thaliana accessions (Col-0 and Est-1).Previous research performed in our group revealed that a gene encoding scopoletin UDP-glucosyltransferase (UGT) is a strong candidate to be involved in differentiation of scopolin to scopoletin ratio in Arabidopsis thaliana. Scopoletin and its glucoside scopolin belong to plant secondary metabolites called coumarins, which have various biological activities and are beneficial for both plants and humans. In this study, we characterized the responses of plants with different scopoletin and scopolin content (Col-0, Est-1 and ugt mutant in Col-0 background) grown under selected abiotic stresses, as well as started the comparative analysis of the activity of UGTs derived from two A. thaliana accessions (Col-0 and Est-1). In silico analysis of UGT gene expression led to the identification of environmental stresses that possibly influenced the growth of plants with various scopoletin/scoplin ratio. The most interesting plant phenotypic variation was observed under salinity stress. Metabolic profiling results indicated the presence of interesting differences in scopoletin and scopolin accumulation between tested Col-0, Est-1 accessions and ugt mutant line. In order to check the possible impact of detected polymorphisms on the Col-0 and Est-1 UGT activities, we started *in vivo* analysis based on transient transformation of Nicotiana benthamiana leaves and in vitro enzymatic characterization using heterologous expression system of *Escherichia coli*.

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P20.10

Lysophospholipid acyltransferases (LPLATs) from *Camelina sativa* – activity, biochemical characteristics and substrate specificity

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C. sativa is an oil crop that arouses growing interest due to its unique properties. Because of prospective possibility to use *C. sativa* oil in industry and its genetic similarity to well-studied model species *A. thaliana*, it becomes an increasingly important subject of research. LPLATs are enzymes participating in synthesis of both storage and membrane lipids. They are able to utilise a broad spectrum of lysophospholipids and acyl-CoAs, which leads to production of different types of phospholipids. In forward and reverse reactions they can also remodel fatty acid composition of phospholipids.
The aim of the study was to determine activity, specificity and biochemical properties of LPLATs of *C. sativa*. In the analysis different [^{14}C]acyl-CoAs and different none-labelled lysophospholipids were used. Variables conditions were tested to determine biochemical properties and the best parameters for *in vitro* enzymatic reactions.
Detailed study showed that each of the tested LPLAT enzyme groups (LPCATs, LPEATs, LPAATs) reach their optimal activity in quite different conditions. Optimal values of parameters like reaction time, pH, temperature, concentration of microsomal fraction and ions (Mg^{2+}, Ca^{2+}, K^{+}) were established for them. Substrate specificity and activity, which differed significantly between analysed enzyme classes and between tested microsomal fractions, were also set up.

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