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of the Polish Society of Experimental Plant Biology,
9–12 September 2019, Toruń

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New trends in plant reproduction and growth regulation

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CONFERENCE OPENING

Jan Kopcewicz

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Mechanisms conferring seasonal flowering responses in annual and perennial plants

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Keywords: flowering; environmental control; meristem development; perennials; Crucifers

The plant life-cycle varies tremendously among species. We study how flowering is controlled by seasonal cues in the context of annual and perennial life cycles. Annuals reproduce once in their life time producing high seed yields, whereas perennials live for many years but limit the extent of reproduction each year. We focus on the *Brassicaceae* family, in which annual species have diverged from perennials many times. We use annual *Arabidopsis thaliana* to decipher regulatory networks controlling seasonal responses and exploit perennial *Arabis alpina* to determine how these networks change during evolution to confer ecologically significant differences in flowering behaviour. Recently, we showed how a network of microRNAs and transcription factors act in the shoot apex to control the age at which the plant becomes sensitive to winter cold. The activity of this network differs between annuals and perennials, effectively delaying flowering of perennials. The talk will describe our recent progress in understanding flowering in the perennial system and how it differs from the annual.

Session 1

SEXUAL PLANT REPRODUCTION

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Tip growth in pollen tubes: a role for ions and actin

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Keywords: actin; calcium; KCN; pollen tube; secretion

Sexual reproduction in flowering plants involves a rapid and polarized growth of the pollen tube, which delivers the two sperm cells to the egg apparatus. Because of its central role the growing pollen tube has attracted considerable attention that has focused on understanding the mechanism of growth. Thus it has long been known that calcium ions are essential for pollen tube growth. Protons are also important, given that acid pHs are necessary. More recently it has been established that the actin cytoskeleton is essential because it drives cytoplasmic streaming and transports the numerous cell wall containing vesicles from their site of formation in the shank of the tube to the apex where the fuse and deliver their contents to the expanding cell wall. It is the thrust of this study to explain how these events are orchestrated, both in time and space, to effect the polarized growth that occurs. Initial studies exploited the oscillatory nature of growth as well as of the expression of calcium, pH and secretion. Phase analyses of these data show that both secretion and the increase in the alkaline band precede growth, whereas the increase in the apical calcium follows, suggesting that pH and secretion may be initiators of growth. To examine these issues further we applied KCN, as a reversible inhibitor of pollen tube growth. Thus the application of 200 μM KCN elicits a rapid inhibition of growth and concomitant reduction in the apical calcium gradient, the pH gradient, secretion and the expression of the apical actin fringe. Simply washing out the KCN allows growth to restart, with the alkaline band being the most anticipatory event, followed very closely by secretion. The apical calcium gradient also precedes growth, but definitely follows both pH and secretion. Our temporal data for actin changes are not as precise as they are for calcium, pH and secretion. Nevertheless, they show that KCN inhibition of growth causes a rapid dissipation of the actin fringe, which then returns concomitantly with the re-emergence of growth. Further observations indicate that during KCN inhibition, some secretion continues, but is delocalized, leading to a momentary phase of global expansion at the apex. But once the fringe is re-established, secretion becomes polarized. Taken together our results implicate the apical alkaline band as a prime player in polarized pollen tube growth. Calcium may be more involved in limiting growth. The apical actin fringe, which may be localized by the pH gradient, appears to dictate where secretion occurs.

Here, there, and everywhere: The importance of storage lipids in pollen performance

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Keywords: pollen; pollen tube; oil bodies; lipid droplets; caleosin

Lipids are major and vital cellular constituents. In plants, stored lipids reserves play an important role in the life cycle by providing carbon and energy equivalents for periods of active metabolism. For most eukaryotes, the preferred storage compounds are lipids in the form of triacylglycerols (TAGs). Plants accumulate the storage lipids in specialized organelles called lipid droplets (LD), oil bodies (OBs) or oil globules. These organelles have been found in different sporophytic plant organs and tissues, like oil seeds and oleaginous fruits as well as in cells of both, male and female gametophyte. However, despite the obvious presence of LDs in pollen grains of different species little is known about their behaviour, breakdown mechanisms and their role in pollen development, germination and pollen-pistil interactions.

This lecture will explore our current knowledge on behaviour and mobilization of pollen LDs and the high importance of storage lipids during sexual processes in higher plants. Special emphasis will be placed on LD-associated proteins and their role during pollen development and germination.

The genetics behind being „Not Like Daddy": new insights in double fertilization thanks to maize haploid inducer lines

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Keywords: maize; fertilization; haploid; pollen; phospholipase

Mixing male and female genetic information during sexual reproduction is considered as key to the evolutionary success of higher eukaryotes and is the basis of plant breeding. Sexual reproduction in flowering plants involves a unique process named double fertilization. It is characterized by two separate fusion events between the male and female gametes. A maize line first reported in the 60s deviates from this classic pattern: Crosses using pollen from this so-called haploid inducer line, trigger the development of the egg cell into a haploid embryo with only the maternal genome, a process known as *in vivo* gynogenesis. Derivatives of this maize haploid inducer line have now become the preferred tool of numerous maize breeding companies, because it can produce perfectly homozygous plants in only 2 generations instead of 5 to 8 in classical breeding schemes.

More than 50 years after the discovery of haploid inducer lines, our work identified the major causal gene responsible for gynogenesis in maize. Our map based cloning restricted the QTL to a zone containing a single gene coding for a patatin-like phospholipase, which was named Not like Dad (NLD) because haploid embryos do not have paternal contribution. In all surveyed haploid inducer lines, NLD carries a 4 pb insertion leading to a predicted truncated protein that does not localized to the sperm cell plasma membrane, contrary to wild-type full-length protein. This frameshift mutation is responsible for haploid induction as complementation with wild-type NLD abolishes the haploid induction capacity, and creation of knock-out mutations by CRISPR/Cas9 within wild-type *NLD* lead to haploid induction. To sum-up, we demonstrated that an intact sperm-specific phospholipase is required for successful sexual reproduction. On the applied side, targeted disruption of *NLD* orthologous genes may allow establishing powerful haploid breeding tools in numerous crops. We are now aiming at solving the mystery by which a sperm cell membrane bound NLD protein is needed to maintain paternal genome integrity in embryo. We thus aim to provide new insights into how paternal genome integrity is maintained during normal plants double fertilization.

Exploring the molecular and cellular mechanisms of promising genes involved in Arabidopsis embryogenesis and seed maturation

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Keywords: Arabidopsis; embryogenesis; seed formation; no homozygote progeny mutants

Seeds and grains constitute the major source of food supply around the world, and their production depends on successful double fertilization and subsequent embryogenesis. Exploring the early embryogenetic events is important, because proper embryo development significantly affects the quality and quantity of seeds. We have conducted an *in silico* forward genetic screen for *Arabidopsis* T-DNA insertional mutant pools and identified several mutations of genes involved in embryogenesis and seed maturation. These T-DNA-insertional mutants lacked homozygote progenies were called *no homozygote progeny* mutants, *nhp*s. We have intensively characterized these *nhp* mutants and their corresponding genes are critical for cell division, organellar and nuclear RNA metabolisms, and endomembrane trafficking. For example, NHP36 and NHP44 are essential for mitochondrial transcripts processing; *NHP148* encodes an *Arabidopsis* nucleolus-localized SAS10/C1D family protein required for rRNA post-transcriptional processes; *NHP78* encodes an *Arabidopsis* one ESCRT-II component, VPS36, and is critical for multivesicular body formation and vacuolar biogenesis during *Arabidopsis* embryogenesis. Recently, we found that mutation of *NHP247* showed a severe disturbance in the distribution and transport of auxin to result in embryo lethal. *NHP247* encoding an unknown protein is critical for cell plate formation where NHP247 localized to the proximal region of the developing cell plate during cytokinesis. Additionally, NHP247 shows both microfilaments and microtubulin bundling activity *in vitro*. Our study showed that NHP247 is a cytoskeletal bundling protein and is essential for phragmoplast formation during cytokinesis in developing *Arabidopsis* embryo.

Insights into the machinery that mobilizes pollen lipid droplets

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Keywords: pollen; lipid droplet; phospholipase A; lipase; lipoxygenase

In numerous plants, pollen grains accumulate storage lipids that serve as energy supply during germination. They are stored in in specialized organelles called lipid droplets (LDs). While the great majority of data on the biochemical machinery and the metabolic pathways involved in mobilization of LDs came from studies in seeds, our knowledge on mobilization of storage lipids in pollen is poor. Here, I will characterize the key enzymes involved in early steps of oil body mobilization: phospholipase A, lipase, and lipoxygenase in the male gametophyte of olive (*Olea europaea* L.). The most current model of their functional and cellular organization as well as their importance for a proper pollen germination will also be discussed.

Expression of the CNX/CRT chaperones during pollen tube growth

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Keywords: actin cytoskeleton; FISH; *in vitro* studies; siRNA; tip-focused Ca^{2+} gradient

Pollen tube growth requires precise regulation of calcium ions level (Ca^{2+}) in its cytoplasm, which determines the interaction of basic cellular processes such as exo/ /endocytosis and intracellular transport of organelles and vesicles carried out with the actin cytoskeleton. The molecular mechanism of stabilizing a tip-focused Ca^{2+} gradient in growing pollen tube has not been clarified to this day. However, some results of our studies indicate that an important role in Ca^{2+} homeostasis during pollen tube elongation is played by calreticulin (CRT) – a Ca^{2+}-binding/buffering chaperone that resides in the endoplasmic reticulum (ER) of eukaryotic cells. Post-transcriptional *CRT* gene silencing in *Petunia hybrida* (*Ph*) pollen tubes growing *in vitro* causes destabilization of the Ca^{2+} gradient and significant disturbances in the organization and function of the actin cytoskeleton, and consequently the inhibition of pollen tube growth (Suwińska *et al*. 2017). Since CRT interacts closely with the other Ca^{2+}-binding chaperone – calnexin (CNX) – creating a quality-control system of polypeptides in the ER (so-called CNX/CRT cycle), the role of CNX in pollen germination and pollen tube elongation can be equally important. To address this possibility, we cloned the full-length cDNA of a new *CNX* gene from *Petunia* (*PhCNX*) and generated a species-specific molecular probe for fluorescence *in situ* hybridization (FISH) to study the CNX expression in *Petunia* germinating pollen and growing pollen tubes. Our *in vitro* studies clearly showed localization of the *PhCNX* transcripts to all apertures of germinating pollen and their accumulation in the germinal aperture. During dynamic growth of the pollen tube, presence of *Ph*CNX mRNAs was observed in the cytoplasm of the shank and subapical zones, both in the shorter and highly elongated pollen tubes. These results prove that the *PhCNX* gene is expressed in germinating pollen and elongating tubes. Because distribution patterns of the *PhCNX* and *PhCRT* transcripts are similar, we argue that transmembrane CNX together with lumenal CRT cooperate during pollen tube growth by modulating Ca^{2+} storage/mobilization and molecular chaperoning within the ER.

■■■

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■ Suwińska A, Wasąg P, Zakrzewski P, Lenartowska M, Lenartowski R (2017) *Planta* 245:909–926

Could photosynthate deficiencies and sensitivity to thermal stress lead to low seed set in common buckwheat (*Fagopyrum esculentum* Moench)?

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Keywords: seed set; embryo sac degeneration; embryo abortion; pollen viability and germination

Common buckwheat (*Fagopyrum esculentum* Moench) of nutritive value is a serious worry of breeders due to its low fruit (commonly termed seed) yield sometimes even not exceeding 15% despite an abundant formation of flowers through a long period of time. As the main reasons, sensitivity to ground frost, high temperature, drought, and competition for the photosynthates between flowers and seeds, are given. Here, using Polish two cultivars (Kora and Panda) and four strains (PA13, PA14, PA15 and PA16), we focus on the explanation: (1) the reasons of low seed set; (2) the influence of (a) thermal stress and (b) nutrient deficiency on seed set in *in vitro* and *in planta* conditions.

In plants cultivated outside pollen viability and germination were very high (>90%), while 49–59% of the ovules exhibited signs of degeneration or abortion (mature embryo sacs, proembryos, embryos). The highest share of mature embryo sac abortions resulted from degeneration of synergids or the whole egg apparatus. The significant deterioration of embryo sac and ovule qualities was observed in flowers grown on media containing one third of sugar, vitamins, macro- and microelements compared to the control. In plants cultivated under 30°C pollen viability was unaffected whereas embryo sac development was clearly disturbed. Of the abnormal development patterns of female gametophyte improper position of the nucleus in relation to the vacuole in the egg cell, degeneration of the entire embryo sac, and degeneration of the cells of the embryo sac and of entire ovule were the most frequent.

The high temperature affecting plants of Polish genotypes of *F. esculentum* during flowering is the main reason of their low seed set however photosynthate deficiencies could also not be excluded. To explain it we apply removal inflorescences treatments to find if female gametophyte degeneration is associated to a strong sink restriction.

■■■

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Mixing under control: genetic factors that regulate crossover frequency in plants

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Keywords: meiosis; recombination; crossover; Arabidopsis

Sexual reproduction is possible only thanks to meiosis, a unique type of cell division, where genetic information from parents is mixed and then reduced by half to form gametes. This depends on meiotic recombination, crossover, in which fragments of homologous chromosomes are reciprocally exchanged. As this process is crucial for proper chromosome segregation, frequency and spatial distribution of crossovers along the chromosome are tightly controlled. I will discuss the current progress on our understanding of crossover regulation in plants. Specifically, I will show our recent data on identification of *trans*-acting factors responsible for crossover control at the genome-wide scale in *Arabidopsis thaliana*. Interestingly, one of the identified factors limits crossover numbers: providing extra copies of the corresponding gene leads to a spectacular increase in recombination. I will also present our unpublished data on identification and initial characterization of another modifier of crossover frequency. Finally, I will discuss the perspective of application of this knowledge for the development of new plant breeding strategies.

■■■

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Structure and evolution of the non-recombining Renner complexes in the permanent translocation heterozygote *Tradescantia spathacea* (syn. *Rhoeo spathacea*)

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Keywords: chromosomes; Rhoeo; permanent translocation heterozygosity (PTH); Tradescantia

In *Tradescantia spathacea* (syn. *Rhoeo spathacea*, 2n=12), as a result of multiple reciprocal translocations, a large meiotic ring of twelve is formed instead of bivalents during meiosis (Golczyk 2013). The ring is preserved by acquisition of permanent translocation heterozygosity (PTH). Within the ring, maternal and paternal chromosomes are alternately arranged, forming two non-recombining genomes: a and b Renner complexes. The already proposed scenario (Golczyk *et al.* 2010, Golczyk 2011a, b) is that subtelomeric 45S rDNA and pericentromeric chromatin may have served as putative breakpoint sites, generating whole-arm translocations and/or whole-arm inversions. Such rearrangements are expected to be accompanied by DNA sequence flow/invasion from subtelomeric regions into pericentromeres and *vice versa*, allowing sequence spreading and homogenization. This type of genome-wide sequence homogenization should have been enhanced by pericentromere clustering and fusion of terminal NORs. However, segmental rearrangements cannot be excluded (Golczyk *et al.* 2010, Golczyk 2011). Here we tested these possibilities based on FISH-comparative physical mapping using an array of molecular probes, e.g.: semi-tandem repeat, *copia*-like repeat, synthetic microsatellites, *Arabidopsis* telomeric motif, 45S rDNA, 5S rDNA. Our results indicate that both whole-arm and segmental rearrangements occurred, however the frequency of the latter is inferred by us to be much higher than previously envisaged. Surprisingly, the mutual chromosomal distribution of the semi-tandem repeat, rDNAs and telomeric motif indicates that significant amount of these sequences have spread independently of one another and points to a severe non-random restriction in the postulated subtelomere-pericentromere sequence flow. Our findings are discussed in relation to Rabl-polarized architecture of interphase nuclei, nucleolar fusion and a pronounced tendency for chromocenter associations observed in this species (Golczyk *et al.* 2005).

■ ■ ■

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Spatiotemporal expression of calnexin during *Petunia* pollen development and maturation

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Keywords: anther; calcium homeostasis; FISH; microsporogenesis; molecular chaperoning

In angiosperms, pollen development consists of three main stages: (1) differentiation of sporogenous cells and meiosis (microsporogenesis), (2) post-meiotic development of haploid microspores, and (3) microgametogenesis. Finally, mature pollen grains (bicellular in *Petunia*) serve as a functional male gametophyte containing a vegetative cell (which will form a pollen tube) and a generative cell (which will produce two male gametes – sperm cells). This multistep process is associated with tissue/cell-specific gene expression that control cell division, chromatin remodeling, cytoskeleton functions, biogenesis of the sporoderm, and Ca^{2+}-dependent cell signaling pathway. Because a high rate of protein synthesis and intracellular Ca^{2+} homeostasis are strictly required during pollen development and maturation, we hypothesize that two highly conserved Ca^{2+}-binding/buffering endoplasmic reticulum (ER) chaperones – lumenal calreticulin (CRT) and transmembrane calnexin (CNX) – may be involved in the male gametophyte formation within the anther. We previously showed that *CRT* gene is highly expressed in *Petunia* developing anther (Wasąg *et al*. 2018). Using northern hybridization and western blotting as well as immunocytochemistry and fluorescent *in situ* hybridization (FISH), we revealed variable levels and distributions of *CRT* mRNA and the protein at the successive stages of the pollen grains formation. Here we show, for the first time, variable expression level of the *CNX* gene during *Petunia* male gametophyte development using FISH with the species-specific molecular probe complementary to the *CNX* mRNA. We revealed spatiotemporal distributions of *CNX* transcripts in functionally different anther tissues, including the male germline and the active tapetum cells. Based on our results, we propose that CNX (probably together with CRT) is involved in (1) modulating Ca^{2+} storage/mobilization and (2) molecular chaperoning during the key events of microsporogenesis within the anther.

■■■

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Immunocytochemistry of the ovules of *Pilosella* and *Taraxacum* (*Asteraceae*) with emphasis with integument mucilage cells

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Keywords: mucilage; *Pilosella*; *Taraxacum*

Occurrence of mucilage (slime) has been reported in the seeds and fruits in some families of angiosperms, including *Asteraceae*. In both *Pilosella* and *Taraxacum* ovule mucilage cells belong to type in which mucilage accumulation occurs between the plasmalemma and the cell wall (Płachno *et al*. 2016, 2017). The aim of our study was to analyse the chemical composition of the mucilage cells checked for the presence of arabinogalactan proteins (AGPs), hemicelulose, some pectic epitopes, and extensinsin the ovule and young seeds of apomictic *Pilosella* as well as *Taraxacum*. We used similar immunocytochemistry methods as Gawecki *et al*. (2017). The results point to that in mucilage occurred accumulation of pectins including both low and high esterified pectins and hemicelluloses. Differences between genera are shown.

■ ■ ■

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Expression of calnexin in *Petunia* germinating pollen and growing pollen tubes

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Keywords: Ca^{2+} storage/mobilization; ER chaperones; FISH; *in vitro* studies; *PhCNX* gene

In double fertilization, a reproductive system unique to flowering plants, two immotile sperm cells are delivered to the female gametophyte by a tip-growing pollen tube. The male gametes are released into the embryo sac where one of the sperm cells fuses with the egg cell to generate a zygote/embryo, whereas the other one fuses with the central cell to form the nutritive endosperm. 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 specific gradient of cytoplasmic calcium (Ca^{2+}) in distinct pollen tube zones. However, the molecular mechanism of stabilizing tip-focused Ca^{2+} gradient in growing pollen tube has not been explained. Several pieces of evidence point to calreticulin (CRT) and calnexin (CNX) as the key Ca^{2+}-binding/buffering proteins involved in pollen germination and tube growth. Both these highly conserved lectin-like molecular chaperones participate in protein folding and quality control in the endoplasmic reticulum (ER) and regulates Ca^{2+} homeostasis. We previously showed that in *Petunia hybrida* (*Ph*) germinating pollen and growing tubes the ER-lumenal chaperone CRT is translated on the ribosomes associated with the ER and that post-transcriptional *PhCRT* gene silencing with the specific siRNA selectively degrading *PhCRT* mRNA disrupts pollen tube growth (Suwińska *et al*. 2015, 2017). It has been also suggested that the ER-transmembrane chaperone CNX may play a role in pollen development, pollen germination and the tube elongation (Vu *et al*. 2017). To begin to address this possibility, we cloned and characterized the full-length cDNA of a new *CNX* gene (*PhCNX*) from *Petunia*. The deduced amino-acid sequence of *Ph*CNX shares homology with other known plant CNXs. Next, using fluorescent *in situ* hybridization (FISH) with the species-specific molecular probe complementary to the *PhCNX* mRNA, we revealed distribution of these transcripts in *in vitro* germinating pollen and growing pollen tubes. Our results support the idea that CNX (probably together with CRT) plays an important role in (1) modulating Ca^{2+}storage/mobilization and (2) molecular chaperoning during pollen germination and the tube elongation.

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Raman spectroscopy as useful method analysis of spatial distribution of chemical compounds in *Platycerium bifurcatum* leaves during the course of sporogenesis

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Keywords: FT-Raman specrtroscopy; 2D Raman spectroscopy; *Platycerium bifurcatum*; carotenoids

Vibrational spectroscopic techniques together with computational methods are especially useful for *in vivo* plant analysis (Lukaszuk *et al*. 2017; Rys et *al*. 2015; Saja *et al*. 2016). An undoubted advantage of Raman spectroscopy used in presented results is that it does not required any preparation of the sample (or the sample preparation is minimal e.g. liophylization) and it does not need any reagents for experimental procedure. That means the technique is environmental friendly.

The study was carried out on several years' sporophytes of fern *Platycerium bifurcatum* (Cav.) C. Chr., from the collection of the Pedagogical University in Cracow. Plants were grown under a natural photoperiod in a greenhouse. The analyses were conducted on the leaves representing different stages of the development of sporangia. In presented research the Raman method was used not only to identify of various chemical compounds but also to analyze of the carotenoids in *P. bifurcatum* leaves in their subsequent phases of growth. The presence of strong carotenoid signals in the FT-Raman spectra of leaves allow to apply mapping techniques (2D Raman spectroscopy measurements) to explore the spatial distribution of carotenoids in plant tissue (Schulz *et al*. 2005).

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Potential markers of oilseed rape (*Brassica napus* L.) seed deterioration advancement

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Keywords: seed aging; metabolites; antioxidant enzymes; RelA/SpoT homolog; (p)ppGpp/alarmones

Vast number of plants, especially of agricultural importance, reproduce by seeds. Thus, high seed vigour maintenance through the time of their storage is important for progeny distribution. One of the factors affecting the vigour is seed aging, which delays germination and reduces its rate and causes even total loss of seed viability. The purpose of the research was to find markers of seed deterioration advancement, mainly of oilseed rape (*Brassica napus* L.). In this study, it is shown that low seed vigour is characterized by low intensity of DNA synthesis, which can quickly be measured by means of flow cytometry, changes in RNA quality, low sugar levels, changes in proline content and antioxidant enzymes activity, as well as by decreased abscisic acid content. Furthermore, it is demonstrated that the expression of *BnRSH1* and *BnRSH3*, encoding for enzymes responsible for metabolism of the effectors of the stringent response – (p)ppGpp (guanosine tetra- and pentaphosphate) – that function in chloroplasts to regulate transcription, translation and metabolites levels, is downregulated in seeds of low vigour. Last but not least, we show a potential marker that can be used to distinguish seeds that are dormant from those that are aged.

Molecular cloning and transcriptional activity of a new *PhCRT3* gene in germinating pollen and growing pollen tubes

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Keywords: Ca^{2+}-binding protein; calreticulin 3; ER chaperone; FISH; molecular phylogenetics

Calreticulin (CRT) is a multifunctional endoplasmic reticulum (ER)-lumenal protein implicated in regulating a variety of cellular processes, including Ca^{2+} storage/mobilization and molecular chaperoning. The CRT family consists of three members in higher plants (CRT1, 2 and 3) which are encoded by three different genes classified into two distinct subgroups: *CRT1/2* and *CRT3* (Waśąg *et al*. 2019). Based on expression of *CRT* genes, it has been proposed that CRT1/2 are the main CRT isoforms, whereas CRT3 may function in specialized tissue/cell types suggesting functional specialization for diverse plant CRTs.

CRT has long been suggested to play a role in plant sexual reproduction. We previously cloned the full-length cDNA of a *CRT* gene from *Petunia hybrida* belonging to the *CRT1/2* subclass (*PhCRT1/2*) and demonstrated that siRNA-mediated post-transcriptional silencing of this gene expression strongly affects pollen tube tip growth *in vitro* (Lenartowski *et al*. 2014; Suwińska *et al*. 2017). Our data clearly showed an interplay between expression of the *PhCRT1/2* and the tip-focused Ca^{2+} gradient, actin-dependent cytoplasmic streaming, organelle positioning, and vesicle trafficking during pollen tube elongation. Thus we argue that CRT1/2 isoforms are involved in Ca^{2+} homeostasis and the actin cytoskeleton arrangement and function that are required for several key processes driving pollen tube tip growth.

Here we show that CRT3 may be also involved in pollen germination and the tube elongation. We have cloned and characterized the cDNA of a new *PhCRT* gene. Phylogenetic analysis indicates that this *PhCRT* cDNA clone belongs to the *CRT3* subclass and the deduced amino-acid sequence of *Ph*CRT shares homology with other known plant CRT3 isoforms. Next, using fluorescent *in situ* hybridization (FISH) with the species-specific molecular probe complementary to the *PhCRT3* mRNA, we revealed localization of these transcripts in *in vitro* germinating pollen and growing pollen tubes. Thus our present studies confirms that the *PhCRT3* is expressed in the male gametophyte developing into pollen tube. From these results, we postulate that different CRT isoforms are involved in pollen germination and pollen tube growth in angiosperms, but further studies are necessary to verify the functional specialization of different CRTs in these key processes for plant reproduction.

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Session 2

PLANT OMICS

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Germplasm meets systems biology – the next green revolution in agroecology

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Keywords: *Chlamydomonas reinhardtii*; COVAIN; MASS WESTERN; PANOMICS; phosphoproteomics

Genomics, transcriptomics, proteomics, phosphoproteomics, metabolomics and mathematical and statistical computer modelling (PANOMICS) are revolutionizing biology and life sciences (1). However, agroecological questions addressed with systems biology approaches are rather underrepresented. Here an integrated PANOMICS platform and the Vienna Metabolomics Center are introduced. We apply these PANOMICS platforms to biomedical up to environmental sciences. A special focus is on the investigation of environmental adaptation of crop plant germplasm collections as well as plant model systems to changing climates and ecosystem analysis. Germplasm collections in combination with a PANOMICS platform allow for the systematic investigation of intra- and interspecific genetic and epigenetic variation and consequences for stress physiology, plant productivity and quality. We apply the PANOMICS platform to crop plants potato, tomato, legumes, grapevine, lotus, cacao, millet, wheat, barley, model systems for third generation biofuels Chlamydomonas reinhardtii, and other model systems tobacco and *Arabidopsis thaliana* strategies within this platform include MAPA (mass accuracy precursor alignment), MASS WESTERN and different informatic toolboxes such as COVAIN for functional data integration, machine learning, modelling and interpretation GroupAnalyzer is a toolbox for structural elucidation of complex secondary metabolites from non-targeted metabolomics profiling. COVAIN can be utilized for the integration of metabolite-protein- transcript data with phenotypic data such as physiology and morphometry as well as phosphoproteomics data. Further novel algorithms for data driven inverse modelling from untargeted GC-MS and LC-MS based metabolomics data are presented. I will have a specific focus on stress signaling networks in plants and algae which cannot be predicted with any genomic tools. Stress perception in algae and plants is transduced by posttranslational modification of proteins in highly complex signaling networks. These signaling networks have direct consequences on cellular processes and overall physiology of the plants and algae. Recently, we have investigated the AMPK-dependent signaling network in *Arabidopsis thaliana* and revealed an intimate connection with TOR signaling. The antagonistic AMPK-TOR signaling pathways are highly conserved from animals to plants and are crucial for energy and stress perception. These pathways seem also to be interwoven in nitrogen starvation and recovery experiments in *Chlamydomonas reinhardtii* as we demonstrated in recent studies where we integrated physiological with metabolomics, proteomics and phosphoproteomics data. We provide evolutionary and metabolic modelling analysis demonstrating how efficient sugar and energy homeostasis is regulated by these regulatory key players. I will review our studies on these signaling pathways and further discuss consequences for plant and algae stress physiology and systems biology.

Plant temperature acclimation and growth rely on cytosolic ribosome biogenesis factor homologs

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Keywords: metabolome; transcriptome; proteome; ribosome biogenesis; Arabidopsis REIL proteins

REIL1 and REIL2 are Arabidopsis homologs of a yeast ribosome biogenesis factor (RBF). Inhibited growth of the *reil1-1 reil2-1* mutant at 10°C can be rescued by expression of FLUORESCENT PROTEIN (FP)-REIL fusions driven by the *UBIQUITIN 10* promoter. Arabidopsis REIL1 appears to be functionally conserved, based on the cytosolic localization of FP-REIL1 and the interaction of REIL1 with the 60S subunit. In contrast to its yeast homologs, REIL1 was also present in translating ribosome fractions. Integrated multi-omics systems analysis at metabolome, transcriptome and proteome levels revealed that wild-type Arabidopsis remodels the cytosolic translation machinery at 10°C by accumulating cytosolic ribosome subunits and inducing the expression of cytosolic rRNA, ribosomal genes, RBFs, and translation factors. In the *reil1-1 reil2-1* mutant, all processes associated with inhibited growth were delayed, but the plants maintained cellular integrity and acquired freezing tolerance. Non-acclimated reil1-1reil2-1 exhibited cold-acclimation responses including activation of the DREB/CBF regulon. In addition, acclimated *reil1-1 reil2-1* plants failed to activate *FLOWERING LOCUS T* expression. REIL function may therefore also contribute to temperature perception. Arabidopsis REIL proteins apparently influence ribosome remodelling and enhance accumulation of cytosolic ribosome subunits after cold-shift either by de novo synthesis or by recycling them from the translating ribosome fraction.

Multiple posttranslational control mechanisms for the proteome of tetrapyrrole biosynthesis

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Keywords: tetrapyrrole biosynthesis; chloroplast biogenesis; posttranslational modification; chlorophyll; photosynthesis

The tetrapyrrole biosynthetic pathway provides the vital cofactors and pigments for photoautotrophic growth (chlorophyll), several essential redox reactions in electron transport chains (heme), the N- and S-assimilation (siroheme) and photomorphogenic processes (phytochromobilin). While the biochemistry of the pathway is well understood and almost all genes encoding enzymes of tetrapyrrole biosynthesis have been identified in plants, the posttranslational control and organization of the pathway is currently under intensive exploration. Posttranslational mechanisms controlling the metabolic activities of tetrapyrrole of synthesis are of particular importance, since this pathway needs a tight adaptation to environmental challenges to ensure adequate synthesis of end-products and the avoidance of accumulation of photodynamic metabolic intermediates at any time of development and environmental condition. Hence, the tight control of the entire tetrapyrroles-synthesizing pathway is indispensable to ensure a fast and precise response on changing environmental conditions. While transcription and translation of genes involved in tetrapyrrole biosynthesis are expected to be responsible for long-term control, the posttranslational modification of proteins adds another layer of control, which is responsible for short-term modulations of protein expression and function. Our group contributed to the rapidly increasing number of studies on posttranslational control of enzymes in tetrapyrrole biosynthesis. Multiple posttranslational mechanisms and new factors will be presented that control activity, stability, protein-protein interactions and subcompartmental localization of the proteins involved in tetrapyrrole biosynthesis.

EGY2-dependent intramembrane proteolysis may regulate expression some of PEP – transcribed chloroplast genes in *Arabidopsis thaliana*

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Keywords: EGY2 protease; chloroplast; *Arabidopsis thaliana*; regulated intramembrane proteolysis

The proteases are generally consider as enzymes participating in the protein turnover and quality control. Recent research indicate, however, that proteases participate also in regulation of gene expression through releasing membrane anchored transcription factors. The process was named regulated intramembrane proteolysis (RIP). The proteases involved in RIP are highly hydrophobic, integral membrane proteins comprising several transmembrane domains with catalytic centres buried within the membrane. So far four families of proteases that may be involved in RIP were identified. One of them, site-2-proteases family (S2P), is formed by zinc- containing intramembrane metalloproteases. In *Arabidopsis thaliana* the S2P family comprise of 6 proteins, including EGY2 protease. The proteolytic activity of EGY2 as well as its chloroplast localization were confirmed experimentally, however substrates for this protease remain unknown. Our analysis indicate that in thylakoid membranes of *Arabidopsis thaliana* mutants devoid of EGY2 protease increased abundance of three proteins pTAC10, pTAC16 and FLN1 is observed. This proteins were proven to interact with the core complex of plastid-encoded RNA polymerase (PEP) and participate in regulation of genes expression. Simultaneously the changes in the expression level two of PEP – regulated chloroplast operons, namely PsbA and PsbC/PsbD, were observed in *egy2* mutants suggesting the existence of EGY2- dependent pathway of their regulation. Further analysis with use of yeast two hybrid system revealed interactions between pTAC16 and EGY2 protease, indicating pTAC16 as potential substrate for EGY2.

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How high-throughput sequencing techniques allow researchers to gain a deeper understanding of plant processes, on the example of transcriptome analysis of yellow lupine (*Lupinus luteus*)

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Keywords: RNA Sequencing; transcriptome; yellow lupine

The advances of modern biology techniques, especially in the fields of genetics, molecular biology, as well as new and ever improving methods for data analysis contributed to massive increase of data in the past 20 years [1].

The genetic transformation of many plant species, especially *Arabidopsis thaliana* which is a staple of genetically modified organism (GMO) used for research, allowed investigators to test many hypotheses with clear and easily distinguishable results. However, the main advantage of A. thalina which allowed this plant to be easily modified, which is small genome, can in some cases turn out to be a disadvantage. In many cases the results obtained from A. thaliana could not be accurately extrapolated to other plant species, and in some cases they were even contradictory, especially in case of plants with much larger, and by estimation, more complex genome [3]. The whole genome sequencing is also an expensive and time consuming errand, and the transformation success rate for many plant species is very low, which leads to a conclusion that other techniques should be also advocated for research.

One of the relatively new techniques used to research plant response to different stimuli, as well as to observe differences in selected plant tissues or developmental stages is transcriptome sequencing [3].

Despite the fact that yellow lupine's genome has not been sequenced, and poor transformation success rates render the genetically modification of this plant near-impossible, the analysis of its transcriptome was able to provide us with a massive data pool, which upon further investigation provided insightful information on the molecular basis of pod abscission. It was well established, that pod abscission is occurring in the pedicel area called abscission zone (AZ) and is mainly caused by changes of phytohormone levels [4], however the more intricate details of this process remained unclear. In our study we aimed to reach a new level of complexity and shed a new light into subject of molecular control of pod abscission.

■■■

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Proteomic analysis of tree seeds storability

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Keywords: chilling stress; germination; longevity; proteomics; seed viability; seed vigour

Understanding seed storage behaviour is a key factor for effective seed preservation and conservation. In seed conservation, it is very important to use optimal temperatures and water content. Seed longevity, the period over which seeds remains viable, is an important trait for plant adaptation to changing environments and conservation of biodiversity. Estimation of optimal preservation conditions is crucial for seed longevity. For such purposes, viability tests are usually employed; however, there is a lack of more sophisticated methods. Proteomic approaches enable the possibility for evaluation of protein markers which can be useful in determination of optimal storage conditions. Protein markers can give information about physiological changes occurring during seed conservation, and about the condition of stored seeds. Changes in the proteome can show otherwise invisible signs of seed deterioration, what can be useful in predication of loss of seed vigour. Such a loss of vigour precedes the loss of the ability to germinate. Proteomic research has mainly concentrated on the negative influence of external and internal factors causing loss of seed viability, such as drying, high temperature and humidity. Proteomic data has shown that reduction of seed longevity is often associated with oxidation of cellular macromolecules such as nucleic acids, proteins and lipids. These data concerned generally seeds exposed to natural or artificial aging protocols which were based on high humidity and high temperature storage conditions. The present study illustrates the influence of low temperatures on seeds viability. The aim of this study was to determine, and functionally characterise, the proteins associated with storability of recalcitrant tree seeds. Based on protein identification, the physiological, biochemical and molecular processes related to seed viability can be characterized. These results showed that low seed moisture content, low temperature, or cryopreservation result in an increase in storage life span. Loss in seed vigour was accounted for by the largest changes in protein abundance. As storage temperature decreased, proteins became less variable. It is interesting that the identified proteins are highly involved in response to stimulus, which indicates that they may play a potential role in stress tolerance during seed storage. Good storability of seeds in freezing conditions may be associated with: inhibition of metabolism and protein turnover activity, and maintaining long-term stability of dehydrated tissue. Optimal storage conditions preserves metabolic functionality until the end of storage.

Changes in lipid compounds content during an enhancing autophagy in cells of embryo axes of lupin germinating seeds

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Keywords: asparagine; peroxisome; sugar starvation; transcriptomic analysis

During carbon or nitrogen starvation many storage compounds are intensively degraded to sustain respiration and growth. However, we observed that in sugar-starved lupin embryo axes after 96-hour growth under *in vitro* condition, the level of storage lipid is significantly higher than in not starved axes. We also observed that advanced autophagy occurred in cells of sugar-starved axes. It was reflected in massive vacuolization of cells and in lower phosphatidylcholine content. Based on such data we suppose that under sugar-starvation conditions peroxisomes (organelles involved in storage lipid breakdown) are degraded during autophagy (pexophagy) and this caused that storage lipid is slowly used, resulting in higher content of this storage compound in starved axes (Borek et al. 2015). Currently, we investigate changes in content of total lipid as well as phosphatidylcholine and phosphorylcholine (metabolic indicators of autophagy) during the 96-h period of *in vitro* culture. We also investigate an effect of asparagine (a central amino acid in lupin seed metabolism) on lipid compounds content. We used asparagine because we discovered that this amino acid slows down decomposition of autophagic bodies in cells of sugar-starved lupin embryo axes (Borek et al. 2017). Results concerning a content of lipid compounds we will compare to changes in transcriptome of lupin embryo axes cultured *in vitro*.

Embryo axes were isolated from imbibed seeds of white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet) and were cultured for 96 h *in vitro* on a mineral medium supplemented with 60 mM sucrose, without the sugar, and on both above mentioned media enriched in 35 mM asparagine. Total lipid content was measured by the gravimetric method. Content of phosphatidylcholine was made using thin layer chromatography, the level of phosphorylcholine was measured spectrophotometrically. RNA was isolated and transcriptomic libraries were prepared. The quality of the libraries was verified by Sanger sequencing method. After positive qualification of the libraries we have performed large-scale transcriptomic sequencing using Illumina HiSeq Next Generation Sequencing technology (NGS). The obtained sequence reads were aligned to reference transcriptome and counted in the aim to find differentially expressed genes.

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Proteomic analysis of endogenous nitrotryptophan-containing proteins in leaves of *Arabidopsis thaliana*

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Keywords: tryptophan nitration; reactive nitrogen species; senescence; *Arabidopsis thaliana*

Nitric oxide (NO) is a well-known signaling molecule involved in many aspects of plant physiology. NO exerts its biological function mainly through the induction of post-translational modifications (PTMs) on target proteins, which may lead to changes in their structure and function. One of the major PTMs induced by NO-derived peroxynitrite ($ONOO^-$) and nitric dioxide (NO_2) is the nitration of proteins consisting in the addition of a nitro group ($-NO_2$) to the aromatic residues. Moreover, recent literature sources indicate that this phenomenon is not only a marker of nitro-oxidative stress, but also seems to be a highly specific element of the NO signaling in living organisms. Heretofore, research on protein nitration in plants has focused only on the tyrosine residues. However, in animal systems there is growing evidence that nitrotryptophan (NO_2-Trp) may also play a significant role in the regulation of cellular signaling during both physiological and pathophysiological processes.

Therefore, in the present study immunological and proteomic approaches were combined to verify whether nitrotryptophan-containing proteins are accumulated in *Arabidopsis thaliana* plants growing under physiological conditions. Since NO-derived molecules are engaged in senescence regulation, NO_2-Trp-containing proteins were also detected during dark-induced leaf senescence. To monitor senescence, green and senescing leaves were tested for chlorophyll *a* fluorescence kinetics and leaf nitrogen status.

Preliminary research, using Western blot analysis with a 6-nitrotryptophan-specific monoclonal antibody, confirmed the presence of proteins containing NO_2-Trp in *Arabidopsis* leaves. What is more, the obtained data revealed the differences in nitroproteome patterns between the control and dark-induced senescent leaves. These endogenous nitrotryptophan-containing proteins were subsequently subjected to trypsin digestion and MS analysis.

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Expression of *LlABI3* and *LlFUS3* genes regulating the transcriptional activity of genes encoding beta conglutin in subsequent days of development of yellow lupine seeds (*Lupinus luteus* L.)

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Keywords: ABI3; FUS3; conglutin; Seed Storage Protein; *Lupinus luteus*; yellow lupine; RNA-seq

Stable yielding of native legumes and obtaining proteins with optimal nutrition for amino acid composition is an important challenge in modern agriculture. This is particularly important given the significant dependence of the feed market on the import of soy meal and its use as a rich source of vegetable protein. The lack of diversification of sources of this nutrient and its homogeneous composition may have a significant impact on the economy and society in the future. Therefore, to reduce the amount of imported soybeans, it becomes necessary to search for more, alternative sources of protein. The native lupine species, cultivated in Poland for several decades, may be the solution.

The process of accumulating seeds' storage proteins (SSP) has already been known in several species of model plants. The results of the research conducted in the last several years show clearly that the greatest amount this kind of protein is accumulated in the final stages of seed development. These processes are precisely controlled by many factors at multiple levels of regulation. The best-known regulators of genes which encode seed storage proteins are peptides, created from the expression of *LEC1*, *LEC2 (LEAFY COTYLEDON1/2*), *ABI3*(*ABSCISIC ACID-INSENSITIVE3*) and *FUS3* (*FUSCA3*) genes, as well as phytohormones and some metabolites such as sugars. ABI3, FUS3 and LEC2 belong to the family of transcription factors that contain the B3 domain, binding nucleic acids. Thus, they may bind to RY motifs in the promoters of genes encoding SSP, inducing their expression.

The aim of the study was to determine the level of expression of the *LlABI3* and *LlFUS3* genes as well as genes encoding conglutin beta in subsequent days of development of yellow lupine seeds. The results were obtained using the RNA-seq technique. RNA was isolated from seeds at 10, 20 and 30 DAA (Day After Anthesis). The STATS_logFC value over 7,5 and about 10 was found for the *LlABI3* gene in DAA 20 and 30 DAA respectively. For the *LlFUS3* gene the STATS_logFC also increases to value about 8,6 in 20DAA, and then decreases in 30 DAA to value 8,02. In the case of genes encoding beta conglutins, the STATS_logFC value was 1,58 in 20 DAA and increases to value about 12,2 in 30 DAA. The obtained results may indicate the functioning of similar relationships between the examined genes to those described in other plant species.

■■■

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Role of Cajal bodies in nuclear retention and posttranscriptional modyfications of mRNA in plants

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Keywords: cb; nuclear retention of mRNAs; intron retention; poly(A) RNA

Similarly to mammalian cells, the genes in larch microsporocytes are transcribed in bursts (Kołowerzo-Lubnau et al. 2015). During diplotene there were five such bursts of transcription. In this research we have shown that a large quantity of poly(A) RNA in the nuclei of microsporocytes appears with the launch of transcription. These transcripts are retained in the nucleus for a long time and are stored within the nucleoplasm and CBs. Pool of transcripts within the CBs gradually increases and reaches a maximum preceding the release of large quantities of poly(A) RNAs to the cytoplasm. Cajal bodies are the compartment in which polyadenylated transcripts are retained for a very long period of time (of the order of days). In the stages which end poly(A) RNAs flow cycles in microsporocytes, the nuclear pool of these transcripts is stored mainly in Cajal bodies. Therefore it seems that CBs have a significant effect on the nuclear retention of mRNA, as well as the subsequent export of these transcripts to the cytoplasm.

We performed the analysis of the transcriptome from the cytoplasmic fraction of microsporocytes to check what mRNAs are present in the cytoplasm and whether there are transcripts that undergo nuclear retention. As a result of sequencing 7,001 transcript sequences were obtained. 3 283 sequences (44%) were assigned a total of 307 714 GO terms. Of these sequences, we chose 15 to which molecular probes were designed for their location by FISH. *In situ* localisation the showed that the vast majority of these transcripts are nuclear retaining mRNAs. On the example of mRNAs encoding Sm proteins, we showed that these retentive transcripts are stored in a not-fully matured form with one or several introns retained. Such pre-mRNAs was observed both nucleoplasm and CBs, however, fully mature mRNAs were never present in CBs. This indicates the relationship of these nuclear domains with pre-mRNAs retention and / or the process of their release into the cytoplasm. The detailed role of CBs in this mechanism must still be examined.

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Trade-offs between activity of RubisCO forms and energy demands for their biosynthesis. Theoretical and comparative studies with CO_2-reductases

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Keywords: evolution; optimization; photosynthesis

Most carbon dioxide (CO_2) on Earth is fixed into organic matter via carboxylation reactions catalysed by enzymes collectively called carboxylases. Carboxylases dependent CO_2-fixation occurs in Calvin-Benson-Bassham (CBB) cycle, and the crucial role in this CBB cycle plays RubisCO (D-ribulose 1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39). CO_2 can also be fixed in a pathway, where a reduction of CO_2 to formate or carbon monoxide (CO) occurs. The latter reactions are performed by so-called CO_2-reductases e.g. formate dehydrogenase (FDH, EC 1.17.99.7) and carbon-monooxide (CO) dehydrogenase (CODH, EC 1.2.7.4). In most cases a simple model of enzymatic activity based only on a turnover rate of an enzyme for an appropriate substrate (k_{cat}) is insufficient. Based on estimated metabolic costs of each amino acid biosynthesis in bacteria and *A. thaliana*, energetic demands for various RubisCO forms and CO_2-reductases were calculated. Average energetic costs of amino acid biosynthesis for selected RubisCOs and CO_2-reductases were analyzed in relation to their CO_2 specificity factor (S_{rel}) and/or k_{cat} for CO_2 (k_C). The obtained results showed that there exists an evolutionary conserved trade-off between kinetic properties of analyzed CO_2-fixing enzymes and energetic demands needed for their biosynthesis.

Using of quantitative genomics for recognition of genes involved in starch accumulation in potato tubers

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Keywords: ADP-glucose pyrophosphorylase; eQTL mapping; starch content; Solanum

Starch is the major storage carbohydrate of potato tubers. The biochemistry of potato starch metabolism is well documented, but knowledge on genes regulating starch content in potato tubers still remains poor. The major challenge in the field of genetics of starch accumulation is the identification of natural variation in genes involved in tuber starch metabolism and understanding, on the molecular level, function and regulation mechanism of genes pivotal for carbohydrate metabolism. In our recent study, we have mapped quantitative trait loci (QTL) for tuber starch content (TSC) on seven potato chromosomes: I, II, III, VIII, X, XI and XII using in the diploid potato population 12–3. The most important QTL spanned a wide region of chromosome I (42.0–104.6 cM) with peaks at 63 cM and 84 cM, which explained 17.6% and 19.2% of the phenotypic variation, respectively.

ADP-glucose pyrophosphorylase (AGPase) is the key enzyme of starch biosynthesis in potato. We have mapped a marker of the large subunit of AGPase, AGPaseS-a. The marker was localized on chromosome I at 102.3 cM and accounted for 15.2 % of the variance in tuber starch content (Śliwka et al. 2015, TAG 129:131–140).

In the present study we used RNA-seq technology for selection of genes displaying differential expression between RNA pools prepared from tubers of the parental clones and F1 individuals of the population 12–3.

Bulked segregants approach was applied. The expression level (RT-qPCR) of the marker AGPaseS-a was determined in all individuals of the population 12–3, and compared with starch content. Two bulks consisting of six individuals were prepared. Bulks were characterized by high starch content (19%-23.4%) and high AGPaseS-a expression (A) or low starch content (11.5%-15%) and high AGPaseS-a expression (B). Based on RNA-seq data ten candidate genes were selected, for which mapping of expression QTL (eQTL) was applied. This research strategy, known as "quantitative genomics", gives a chance to find genetic factors located within QTL for starch content (*cis*-eQTL) or indirectly from a distant location on the genome (*trans*-eQTL).

Additionally, AGPaseS-a eQTL was mapped and compared with QTL for starch content. The eQTL overlapped with QTL for starch content, had a peak at AGPaseS-a locus (102.3 cM) and 40.7% of variance in the *AGPaseS-a* gene expression could be ascribed to this peak (LOD=6.6).

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The research was supported by The National Science Centre in Poland, Grant UMO-2015/19/B/NZ9/00776.

Regulatory role of asparagine in autophagy in cells of embryo axes of lupin germinating seeds – a transcriptomic approach

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Keywords: ATG proteins; autophagic bodies; hydrolases; sugar starvation

In our research we focused on the role of asparagine (a central amino acid in lupin seed metabolism) in a course of autophagy, which we induced by sugar starvation in cells of lupin embryo axes. Previously we found that sugar starvation enhances autophagy in cells of lupin isolated embryo axes, but one of the final stages of autophagy, namely the decomposition of autophagic bodies inside vacuole, is clearly inhibited by asparagine (Borek et al. 2017). Trying to describe the role of asparagine in autophagy, especially in a mechanism of autophagic bodies degradation, we performed transcriptomic analyses of lupin embryo axes. The experiments were performed on embryo axes isolated from seeds of white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet). Embryo axes were isolated from imbibed seeds and cultured for 96 h *in vitro* on a mineral medium supplemented with 60 mM sucrose, without the sugar, and on both above mentioned media enriched in 35 mM asparagine. RNA was isolated and transcriptomic libraries were prepared. The quality of the libraries was verified by Sanger sequencing method. After positive qualification of the libraries we have performed large-scale transcriptomic sequencing using Illumina HiSeq Next Generation Sequencing technology (NGS). The obtained sequence reads were aligned to reference transcriptome and counted in the aim to find differentially expressed genes. Bioinformatic analysis was performed on two replicates of the experiment. Our goal was to analyze the effect of asparagine on the expression of genes encoding proteins involved in autophagy.

■ ■ ■

Acknowledgments: This work was financed by the National Science Centre, Poland (Grant no. 2016/23/B/NZ3/00735).

■ Borek S, Paluch-Lubawa E, Pukacka S, Pietrowska-Borek M, Ratajczak L (2017) Asparagine slows down the breakdown of storage lipid and degradation of autophagic bodies in sugar-starved embryo axes of germinating lupin seeds. Journal of Plant Physiology, 209: 51–67

Assessment of potato (*Solanum* sp.) phytotoxic potential using metabolomic and transcriptomic approaches

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Keywords: potato; allelopathic potential; phytotoxcity; glycoalkaloids

The cultivated potato (*Solanum tuberosum* L.) is the fourth most important food crop after corn, rice and wheat. Allelopathic potential of the cultivated potato can be recognized as impaired growth of following plants. This phenomenon is also known as soil sickness caused by accumulation of allelopathic compounds. Among wild *Solanum* species, one favorites individual growth while other can form carpets. The absence of neighboring plants around potato may indicate allelopathic potential. Nowadays, studies on allelopathy are rather conducted in laboratories than in natural environment. The term "phytotoxicity" is used to distinguish allelopathy (as phenomenon occurring in natural environment), from laboratory studies on phytotoxic compounds. Our recent study (Sołtys-Kalina et al. 2019) on potato leaf extracts suggests a significant role of glycoalkaloids in potato phytotoxicity especially among diploid potato hybrids and wild potato species.

To evaluate the role of glycoalkaloids in this phenomenon, we obtained segregating potato population 15-1 from a cross between seed parent DG 88-89 (potato diploid hybrid) and pollen parent *S. chacoense*. Based on the strength of phytotoxic potential against a test plant, mustard (*Sinapis alba* L.) and glycoalkaloids concentration in potato leaf extracts, four bulks consist of individuals obtained from the population 15-1 were selected. We used RNA-seq technology and gene ontology (GO) for selection of the genes displaying differential expression between RNA pools prepared from leafs of the individuals and indicate the physiological processes involved in potato phytotoxicity. We also performed analysis of secondary metabolites presented in the potato leaf extracts to indicate the differences between qualitative composition of leaf extracts from individuals of the population 15-1.

Bulks characterized by various phytotoxic potential differ between each other with α–solanine:α-chaconine proportion. Analysis of transcriptome and GO between RNA pools confirms significant role of genes involved in steroid biosynthetic processes.

■■■

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Proteomic analysis of barley mapping population subjected to drought identifies proteins with genotype×environment interaction

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Keywords: drought response; cereals; barley; mapping population; large-scale proteomics; proteomic quantitative trait loci – pQTL; 2D electrophoresis; mass spectrometry

Drought is one of the major abiotic stresses negatively influencing crop yield and is a serious issue in modern agriculture. To achieve further substantial crop improvements in terms of drought resistance it is necessary to incorporate scientific results into breeding strategies. However, most of the data on plant drought responses arises mostly from small-scale studies and, therefore, its use in breeding programs is very limited. Here, we present the results of the large-scale proteomic analysis performed on barley recombinant inbred lines (RILs) and their parental genotypes subjected to drought, applied shortly before tillering. The conducted proteomic analyses enabled us to monitor drought-induced proteome changes in leaf and root tissue, and to identify proteins that responded to drought in a genotype-specific manner, for instance Rubisco activase, luminal binding protein, phosphoglycerate mutase, glutathione S-transferase, heat shock proteins as well as enzymes involved in phenylpropanoid biosynthesis. We also demonstrated feasibility of incorporating proteomic data resulting from large-scale study into genetic linkage analysis, which constitutes a fundament in biotechnology-driven breeding strategies.

The amount of data from large-scale proteomic studies enables differentiation of proteins with genotype-specific reaction to drought and identification of proteomic QTLs in barley mapping population.

IAA-aspartate modulates thiol redox status of proteins and protein S-nitrosylation in pea tissues

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Keywords: IAA-aspartate; S-nitrosylation; *Pisum sativum*

IAA-aspartate (IAA-Asp) is an amide auxin conjugate that predominates in pea tissues. IAA-Asp acts as an intermediate during auxin oxidation, but some evidences indicate physiological signficance of its. IAA-Asp is synthesized by ATP-dependent reaction using IAA-amido synthetase that belongs to acyl-adenylate/amido-synthetase of Gretchen Hagen 3 (GH3) family. Our previous study revealed that IAA-Asp affects protein carbonylation and catalase/peroxidase activity during pea responses to salinity. In this study we verify the hypothesis that IAA-Asp modulates protein thiol redox status and protein S-nitrosylation (SNP) in 7-day-old pea stems. For verification of this hypothesis, the effect of IAA-Asp on glutathione peroxidase, (GP), glutathione reductase (GR), nad glutathione S-transferase (GST) was investigated. Moreover,GSH/GSSG ratio and total thiol group were determined. To study protein S-nitrosylation, one-dimensional (1D: SDS-PAGE) as well two-dimensional (2D:IEF/SDS-PAGE) electrophoretic methods were used. SNPs were detected by Western blot using anti-iodo-TMT antibody. Our results suggest that IAA-Asp diminishes protein S-nitrosylation.

Session 3

EPIGENETIC AND EPITRANSCRIPTOMIC REGULATION OF GENE EXPRESSION

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The Arabidopsis m6A Epitranscriptome

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Keywords: RNA; epitranscriptome; sequencing

Patterns of pre-mRNA processing and base modifications determine eukaryotic mRNA coding potential and fate. Consequently, revealing RNA modifications and processing combinations is essential to understand gene expression and what genomes really encode. Arabidopsis is a pathfinder model in plant biology, and its genome annotation strongly influences the annotation and understanding of what other plant genomes encode. Viable Arabidopsis mutants defective in RNA processing and modification have helped establish roles for RNA regulation in controlling fundamental features of plant biology. We asked if long read direct RNA sequencing with nanopores might provide new insight into understanding the complexity of *Arabidopsis thaliana* RNA processing and modifications. We applied nanopore direct RNA sequencing to wild-type Arabidopsis and multiple mutants defective in RNA processing. I will present our results that reveal the complexity of mRNA processing in full-length transcript reads and the unanticipated consequences of disrupting RNA processing.

Epitranscriptome-mediated reprogramming of the plant transcriptome

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Keywords: RNA biology; epitranscriptome; post-transcriptional regulation; plant stress response

N^6-methyladenosine (m^{6}A) is a dynamic and reversible, covalently-modified ribonucleotide that occurs predominantly towards 3' ends of eukaryotic messenger RNAs (mRNAs), and is essential for their proper function and regulation. In *Arabidopsis thaliana*, many RNAs contain at least one m^{6}A site, and we have recently demonstrated that many m^{6}A-modified mRNAs in *Arabidopsis* have reduced abundance in the absence of this mark. This decreased abundance is due to transcript destabilization caused by cleavage occurring 4–5 nucleotides directly upstream of unmodified m^{6}A sites. We also find that upon agriculturally-relevant salt treatment, m^{6}A is dynamically deposited on and stabilizes transcripts encoding proteins required for salt and osmotic stress response. These initial findings reveal that m^{6}A generally acts as a stabilizing mark through inhibition of site-specific cleavage in plant transcriptomes, and this mechanism is required for proper regulation of the salt stress-responsive transcriptome. However, the importance of this epitranscriptome-mediated transcriptome reprogramming in other stress responses is still unknown. I will present our latest findings addressing this important research question.

mRNA methylation complex components and methylation outcomes in a model plant system

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Keywords: N6 methyladenosine; epitranscriptome

N6-methyladenosine (m6A) is a ubiquitous base modification found internally in the mRNA of most Eukaryotes. In the model plant *Arabidopsis thaliana*, levels of methylation equivalent to at least 50% of transcripts carrying m6A are common, but methylation levels vary between transcripts, tissue types and developmental stage. Methylation is not distributed evenly across mRNAs – most m6A is found close to the stop codon.

We have identified a core set of mRNA m6A writer proteins. The components required for m6A in Arabidopsis include the methylases MTA and MTB and their interacting partners FIP37, VIRILIZER and the E3 ubiquitin ligase HAKAI. With the exception of the E3 ligase, these m6A writer components are essential during embryonic development, and appear to be well conserved between plants and mammals. Whilst complete knockout of the plant methylase is embryonic-lethal, a set of hypomorphic mutants with reduced m6A levels has been generated. These plants show altered growth patterns, changed cell identities and perturbed hormone responses. Using these mutants and the wild type plants we are using various approaches to study the role of m6A in plant development.

Arabidopsis thaliana mRNA Adenosine Methylase (MTA) is a new player in miRNA biogenesis regulatory pathway

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Keywords: m6A methylation; adenosine methylation; microRNA biogenesis; microRNA

Methylation of adenosine at N6 position (m^6A) is one of the most abundant mRNA modifications. In this study we uncovered the role of MTA and m^6A methylation in plant (*Arabidopsis thaliana)* miRNA biogenesis. We used NGS to show that miRNA levels are downregulated in *mta* hypomorphic mutant whereas pri-miRNA levels are upregulated in such plants. We then identified a set of 11 pri-miRNAs that are m^6A methylated using m^6A-IP seq. Furthermore, RNA-IP using MTA-GFP tagged *Arabidopsis* plants showed enrichment of pri-miRNAs in the MTA-GFP line (including 8 pri-miRNAs found in m6A IP), further proving that pri-miRNAs are also substrates for m^6A methylation by MTA. We also report that MTA interacts with RNA Pol II and TGH (known miRNA biogenesis related player) indicating that MTA acts in early stages of miRNA biogenesis. Lastly, we show that MTA modulates auxin response in plants via methylation of pri-miR393b. Our data indicate that MTA is an important player in the biogenesis of a set of *Arabidopsis* miRNAs.

m6A methylation of mRNA is required for auxin mediated processes in Arabidopsis

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Keywords: auxin; m^{6}A; epitranscriptome; vascular development

Increasing number of evidences demonstrates that regulation of various steps of mRNA maturation mediates auxin response. *N6*-adenosine methylation of mRNA (m^{6}A) is important posttranscriptional modification, which receives increased attention in past years. We have recently identified components required for m^{6}A in *Arabidopsis* and characterized phenotypes associated with this modification. In our further work, we found that depletion of m^{6}A is associated also with a range of auxin related defects. Moreover, mutants with low m^{6}A levels show strong resistance to exogenously applied auxins. We systematically compared the phenotypic defects of these mutants with collection of other known lines carrying both defects in mRNA maturation and auxin dependent processes and revealed that these mutants show particular resistance to exogenous auxins. Possible targets related to this process are discussed.

Dormancy and drought – One antisense to rule them all?

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Keywords: seed dormancy; drought; antisense transcription

Dormant seeds are able to ignore favorable condition and postpone the germination. This phenomenon helps plants to survive in changing environment as well as allow us to store seeds forming the basis of agriculture. Seed biology has been extensively explored by many groups however how seed dormancy is established at the molecular level is largely unknown. One particular aspect of seed biology that has not been investigated is the variability among genetically identical seeds. In Arabidopsis, the major Quantitative Trait Locus controlling dormancy variability among accessions has been identified as *Delay of Germination 1* (*DOG1*).

DOG1 gene expression is extensively regulated. For example, *DOG1* alternative splicing is controlled by DNA dependent RNA polymerase II (PolII) elongation (Dolata, EMBO 2017). Recently this phenomenon was shown by us and others to serve as light sensing mechanism in Arabidopsis (Herz, Mol Cell 2019). *DOG1* is also a subject of alternative polyadenylation and our work revealed that the selection of the proximal polyadenylation site leads to production of *short DOG1* transcript that codes for evolutionary conserved and functional DOG1 protein (Cyrek, Plant Phys 2016). We have also shown that *DOG1* expression is suppressed by a *cis* acting *DOG1* antisense transcript (*asDOG1*) (Fedak, PNAS 2016). *DOG1* antisense promoter is a key response element required for *DOG1*response to plant phytohormone ABA, an observation that allowed us to characterize a novel role of DOG1 protein in drought response (Yatusevich, EMBO Rep 2017).

I will present our current understanding of the seed dormancy and *DOG1* regulation. Including insights into PolII elongation we learned from the recently develop antibody independent method to analyze PolII elongation at a given locus. I will also describe our insight into a fascinating aspect of biological variability observed among individual seeds.

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

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Keywords: cold stress; SWI/SNF; chromatin remodeling

The SWI/SNF class of ATP-dependent chromatin remodeling complexes (CRCs) plays important role in regulation of gene expression. SWI/SNF CRCs are evolutionary conserved multiprotein complexes thatcontrol important biological processes, such as transcription, cell cycle, replication and hormone signalling.The core of yeast SWI/SNF complex is capable to perform chromatin remodeling *in vitro*and is composed of a central bromodomain-containing SWI2/SNF2 ATPase, one SNF5 and two SWI3-type subunits. In *Arabidopsis thaliana*, the SWI/SNF core subunits are encoded by multiple diversified genes. Thus, in the core of plant SWI/SNF complex SNF5 (BSH) is associated with one of the four ATPases (BRM, SYD, CHR12 and CHR23) and two of the four SWI3 proteins (SWI3A, SWI3B, SWI3C and SWI3D). Genes encoding core components of Arabidopsis SWI/SNF CRCs play critical but not fully overlapping roles during plant growth, including embryo and sporophyte development. In present study, we show that some of the Arabidopsis SWI/SNF subunit mutants when grown at lower temperature exhibit partial reversion of their phenotypic defects, including reduced fertility, defective embryo and root development, and improved freezing tolerance. Our work demonstrates that SWI/SNF CRCs are involved in the control of cold-responsive genes expression. Furthermore, we found that the location of SWI3C-containing SWI/SNF CRCs and nucleosome positioning on selected target loci depends on temperature conditions. These findings provide a novel insight into potential regulatory roles of SWI/SNF CRCs during plant growth under different temperature regimes.

■■■

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Targeted histone acetylation by the plant NuA4 complex supports growth, chloroplast development and reproduction in Arabidopsis

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Keywords: NuA4; histone acetylation; chloroplasts

Acetylation of nucleosomal histones in transcriptionally active loci is a common feature of eukaryotic chromatin. In plants, a three-way positive relationship between light, chromatin acetylation and transcriptional activity of light-inducible genes can be observed. Accordingly, reverse genetic studies in Arabidopsis showed that control over transcript levels of genes involved in photosynthesis requires active acetylation and deacetylation of histones.

The results I am going to present introduce a multisubunit histone acetyltransferase NuA4 as a pivotal element of transcriptional regulation in plants. Taking advantage of high level of evolutionary conservation between the plant NuA4 and its well characterized yeast counterpart, we were able to formulate and test several hypotheses explaining the role of NuA4 in plants. As expected, we found that mutants lacking the scaffold subunits EAF1 and EPL1 display stronger phenotypic effects than mutants lacking chromatin reader subunits, characterized by others. *Eaf1* and *epl1* mutants grow slowly, contain smaller mesophyll chloroplasts than WT plants and are sterile. H4 acetylation levels are decreased in the chromatin of *epl1* mutant, concomitant with reduced transcript level of photosynthesis-related genes and increased expression of stress-inducible genes. Surprisingly, we found that in contrast to yeast, complete loss of EPL1, which is necessary for basic non-targeted activity of the complex, is non-lethal in Arabidopsis. The phenotype of *eaf1* mutants, on the other hand, is only slightly less severe than that of *epl1* which suggests that genomic targeting, mediated by non-essential EAF1 subunit in yeast, is a vital aspect of the plant NuA4 complex. Our results also suggest that plant NuA4 may be supported by a previously uncharacterised group of transcription factors in this process.

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Is there a crosstalk between transcriptional machinery and DRB1 protein?

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Keywords: microRNA; RNA binding protein; posttranscriptional regulation

DRB1 (Double-Stranded RNA Binding Protein 1, also known as Hyponastic Leaves 1 (HYL1)) protein was shown to play an important role in the efficient recognition of primary transcripts of microRNA (pri-miRNAs) as well as in further steps of their maturation. DRB1 interacts with a DCL1 (Dicer-Like 1) protein, which is the main RNAse enzyme that releases mature microRNAs from their precursors. However, recently it was demonstrated that DRB1 has to be dephosphorylated by a CPL1 (C-Terminal Domain Phosphatase-Like 1) protein for its optimal activity. On the other hand, CPL1 also dephosphorylates a CTD domain of RNA Pol II. These observations suggest that DRB1 may be involved already in the very early steps of microRNA biogenesis, probably in the initiation of transcription of *MIR* genes. To test this hypothesis, two reporter gene constructs (GUS cDNA under the control of either *MIR393A* or *MIR393B* gene promoters) were introduced via crossing into *hyl1-2* mutant background. Results showed that expression of both reporter genes in the *hyl1-2* mutant is downregulated when compared to the expression of the same constructs in wild type plant background. Additionally, ChIP-seq experiments showed higher occupancy of the total RNA Pol II at several genes (including *MIR* genes) in *hyl1-2* mutant background. Presented data suggest the existence of crosstalk between DRB1 and the transcriptional machinery in plant cells.

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Immunohistochemical approach to study histone acetylation in the epigenetic regulation of somatic embryogenesis in Arabidopsis

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Keywords: histone acetylation; histone H3; immunohistochemistry; somatic embryogenesis

Epigenetic modifications of chromatin structure that control transcriptome activity involve DNA methylation and posttranslational modifications of histones. The acetylation of histones is associated with an open state of chromatin and is believed to play a key role in regulation of plant development. However, experimental evidences on the involvement of this process in *in vitro* induced morphogenic processes are limited. Thus, we focused our research on the role of histone acetylation in somatic embryogenesis (SE) induction in Arabidopsis, a model system to study plant cell reprogramming *in vitro*. In most of plants, including Arabidopsis, auxin is routinely used to release the embryogenic potential in differentiated explant cells that are cultured *in vitro*. However, we found that instead of auxin treatment trichostatin A (TSA), an inhibitor of histone deacetylase activity, might be used to induce SE in Arabidopsis explants. This observation implied a role of histone acetylation in regulation of the embryogenic response and motivated us to study a pattern of histone acetylation in the explants induced toward embryogenic response. The Arabidopsis explants induced by auxin (2,4-D) versus TSA were analysed with the immunohistochemical method. To this end, polyclonal antibody against acetylated isoforms of H3 histone was used and a level of fluorescence signal of Alexa 488 conjugated with secondary antibody was measured. The spatio-temporal changes of histone acetylation was monitored in the freshly isolated (0 day) and SE-induced for 5 days explants. In order to induce SE, the explants were cultured on E0 medium supplemented with TSA (E0 + 1 μM TSA) and 2,4-D (E0 + 5 μM 2,4-D). In addition, the analysis involved the explants that were cultured on a control, non-SE inductive E0 medium.

The results demonstrate that reprogramming of plant somatic cells into embryogenic ones is accompanied by distinct changes in histone H3 acetylation level. To get further insights into a role of histone acetylation in epigenetic regulation of SE, histone acetylation in chromatin associated with the genes of regulatory function in embryogenic transition need to be studied.

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Integrated analysis small RNA and degradome sequencing reveal the role of miRNA, phased siRNAs and their target genes in flower development and abscission in yellow lupine (*Lupinus luteus* L.)

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Keywords: miRNA; siRNA; yellow lupine; flower development; RNA-seq

Yellow lupin (*Lupinus luteus* L., Taper c.) is an important legume crop, however, development of its flowers and formation of pods is often affected by abscission of these organs. Excessive abscission of *L. luteus* flowers represents the important economical drawback. Shedding primarily concerns flowers formed at the upper part of the inflorescence. Moreover, the fate of upper flowers seems to be determined during organ development before anthesis. Mechanisms underlying organ development and abscission use complex network of molecular interactions, and our understanding of this field still remains uncompleted. By controlling the activity of their target genes, small non-coding RNAs (microRNAs and siRNAs) orchestrate almost all developmental processes in plants, however, their role in the regulation of generative organ development and abscission in yellow lupine has not been studied. The aim of the study was to type the differentially expressed small ncRNAs (mi- and phased siRNA) by comparison of small RNA-seq libraries generated from developing flowers from upper and lower parts of raceme, and abscising and non-abscising flower pedicels. Additionally, we identified their target genes by transcriptome and degradome sequencing. Our results indicate that miRNAs and siRNAs may influence flower development and consequently its fate, via repression of their target genes related to homeostasis of phytohormones, mainly auxin.

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Function of m6A in the localization and the degradation of mRNA stress responsive and housekeeping genes in plants subjected hypoxia stress

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Keywords: N6 methyladenosine; hypoxia; stress granules

We observed the accumulation of mRNA during hypoxia in cytoplasmic bodies, which correspond to stress granules (SGs). There are two hypothesis concern function of SGs: (i) the participation in RNA degradation (ii) or storage of transcripts until stress condition are removed. Currently the participation of m6A in mRNA degradation and the regulation of mRNA triaging to SGs are intensively studied.

We studied function of m6A in the localization and the stabilization of mRNAs stress responsive and housekeeping genes in roots of *Lupinus angustifolius* during normoxia, hypoxia and reoxygenation. Differences in the localization mRNA of ADH1 (stress responsive gene) and L37(60S ribosomal protein L37-3-like), L44 (60S ribosomal protein L44), RPB1 (DNA-directed RNA polymerase II subunit rpb1) were observed. Only mRNA of ADH1 mostly occurs outside SGs in the cytoplasm and no colocalised with rRNA while other mRNAs were present in the SGs. Next, the quantity of m6A and poly(A) RNA in the nuclei, cytoplasm and stress granules were measured. The level of m6A and poly(A) RNA slightly decreased during hypoxia. In spite the poly(A) RNA which strongly accumulates in the SGs, the quantity and the concentration of m6A in SGs was the same as in the surrounding the cytoplasm. The results indicate that m6A doesn't participate in the accumulation of mRNAs in SGs in plant hypoxia treatment.

Subsequently the levels of studied genes expression were measured by qPCR. During hypoxia, an expected quantity of mRNA ADH1 increased and after reoxygenation decreased. By contrast levels of housekeeping genes strongly decreased during hypoxia and increased in reoxygenation. Next we studied the function of m6A in the degradation / stabilization of mRNA in depraved oxygen condition and after the stress removed in the plants using RIP (RNA immunoprecipitation). Preliminary data suggest that m6A prevents degradation of mRNA.

DXO1 links nuclear gene expression and chloroplast function in *Arabidopsis thaliana*

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Keywords: RNA metabolism in Arabidopsis; DXO proteins; NAD cap; siRNA; anterograde and retrograde signaling

DXO/Rai1 proteins participate in mRNA 5'-end quality control (5'QC), removal of non-canonical NAD^+ caps (deNADding) and maturation of fungal ribosomal RNA (rRNA). This work describes the biochemical activity and physiological significance of the *Arabidopsis thaliana* DXO1. Detailed enzymatic assays showed that DXO1 has strong deNADding and weak 5'-3' exoribonuclease activities. Other biochemical properties characteristic of DXO/Rai1 homologs are inhibited in plants due to the presence of a single amino acid modification within the active site. DXO1 also contains a large, plant-specific N-terminal extension (NTE) that negatively affects its biochemical properties, participates in RNA biding and confers the potential for posttranslational modifications. In addition, enzymatic activity of DXO1 is inhibited by adenosine 3', 5'-bisphosphate (PAP), which is a component of chloroplast-to-nucleus retrograde signaling and an inhibitor of XRN 5'-3' exoribonucleases.

This work demonstrates that the most prominent function of DXO1 relies on NTE and is independent of the catalytic activity. DXO1 knockdown leads to a NTE-dependent morphological and molecular aberrations that include severe growth retardation, pale-green coloration, accumulation of RDR6-dependent RNA quality control siRNAs (rqc-siRNAs) and defects in chloroplast rRNA maturation. DXO1 also co-purifies with multiple nuclear-encoded proteins that are further transported to chloroplast to function in photosynthesis and redox homeostasis. FRY1 that regulates PAP levels was present among the DXO1-associated proteins, so DXO1 biochemical activity could be autoregulated through retrograde signaling. RNA sequencing data also revealed that DXO1 deficiency phenocopies chloroplast-driven transcriptomic changes.

Functional implications of DXO1 biochemical activity remain unknown. Presumably, DXO1 parallelly acts in multiple processes, but its biochemical activity is redundant with other enzymes and eludes detection. Altogether, this work shows that DXO1 retains certain features of its fungal and mammalian homologs, but most prominently it evolved to perform plant-specific role that is associated to chloroplast, but also affects nuclear gene expression, possibly through modulation of chloroplast-to-nucleus retrograde signaling.

Expression of metallothionein genes in seeds and seedlings of *Brassica napus* L.

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Keywords: metallothionein; expression of metallothionein; *Brassica napus*

Metallothioneins (MTs) are low-molecular-weight, cysteine-rich proteins common in various prokaryotic and eukaryotic organisms. Plant metallothioneins have been divided into four types by the arrangement of cysteine residues (MT1–MT4). The subfamily MT4 is particular for only being present in seeds and during germination.

The aim of the study was to determine the expression level of metallothionein genes (*BnMT*) in seeds and seedlings of rape (*Brassica napus* L.) of the variety "Harry".

The expression of the metallothionein type-4 gene was determined in dry and imbibed rape seeds, irrespective of the year the seeds were harvested. In dry seeds, the highest level of expression was observed in seeds stored for two years (from 2017), as compared to seeds that had been stored for three, four and five years. In the 24th hour of seed germination, it was found that there were more *BnMT4* transcripts in seeds of a longer storage period than in seeds collected in 2017.

The presence of regulatory elements responsible for reaction to the presence of fungal elicitors was found in the promoters of rape metallothionein genes, so the influence of five *Trichodermaviride* strains on the mRNA level of genes *BnMT1–BnMT3* was analysed. The three analysed genes were found to be expressed in rape seedlings not inoculated with saprophytic fungi. Expression was increased by inoculation of seeds with a *T. viride*spore suspension, especially the expression of genes *BnMT2* and *BnMT3*. All analysed fungi increased the level of *BnMT* transcripts in six-day-old seedlings. The highest expression was found in the presence of *T. viride* V. The research needs to be continued in order to establish the involvement of *BnMT* in plant–fungi interaction.

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Nitric oxide as an epigenetic mediator of effector-triggered immunity in potato

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Keywords: nitric oxide; late blight; epigenetics

Late blight caused by Phytophthora infestans is the most devastating crop disease in the world. Moreover, in view of the annual considerable economic losses caused by potato late blight, any effort to fill the existing gaps in knowledge on the potential role of NO in the epigenetic control of host resistance after challenge inoculation with P. infestans is of great value. One important question that arises from the presented study is whether and how nitric oxide (NO) influences the expression of resistant genes (R-genes) implicated in the RNA-directed DNA methylation (RdDM) pathway. Plants have evolved hundreds of innate immune receptor genes against a broad range of various pathogen effectors. Precise regulation of R-genes is pivotal to prevent fitness cost and autoimmune responses in the absence of the pathogen. Non-coding miRNAs block a wide range of R-genes causing posttranscriptional gene silencing and keeping transcriptionally silent chromatin. In turn, early and rapid overexpression of R-genes is necessary to improve resistance under biotic stress. The boosted NO generation also occurs during the first minutes after pathogen recognition and the NO signal is rapidly translated into redox sensing targets, effective in triggering plant resistance (Floryszak-Wieczorek i in. 2012). It is reasonable to speculate that NO could also function in the precise fine-tuning R-gene co-activation during the host-pathogen interaction. The research is focused on the identification of direct or indirect targets of NO which provide a link with intranuclear signals influencing chromatin remodeling and immunity-specific gene expression. The plant material used in experiments involves potato genotype Sarpo Mira possessing R-3a gene with the corresponding avirulent Phytophthora infestans isolate giving the hypersensitive response (HR) type of resistance. DNA and histone methylation governed by specific methyltransferases affects chromatin structure and its function in transcriptional regulation (Meller i in. 2018; Kuźnicki i in. 2019). Typically, pathogen ingress involves potato genome hypomethylation, which interferes biogenesis of miR482 by suppression of R-gene silencing and protects potato against late blight disease. However we are still searching for connection between NO and epigenetic marks making chromatin more accessible for transcription factors in the promoter regions of the R-gene.

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The chromatin organization and the distribution of 5-methylcytosine in mature female gametophyte cells of *Arabidopsis thaliana*

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Keywords: 5-methylcytosine (5mC); DNA methylation; egg cell; central cell

During the sexual reproduction of flowering plants, the epigenetic control of gene expression and the genome integrity by DNA methylation and histone modifications plays an important role in female gametophyte gametogenesis. Our previous reports of nuclear metabolism, including the chromatin organization, the total transcriptional activity and the distribution of hypo- (initiation) and hyperphosphorylated (elongation) forms of RNA Pol II in *Hyacinthus orientalis* (monocots) mature embryo sac cells indicate that in the egg cell and central cell, whose activity is silenced, the chromatin is largely dispersed compared to the somatic cell (Niedojadło et al., 2011). In turn, during anthesis in the female gametophyte cells of *Arabidopsis thaliana* (dicotyledons) we didn't observe a characteristic pattern of transcriptional activity which probably related with the maturation of the ovule in the ovary. To determine whether the different patterns of the transcriptional activity of the egg cell and the central cell observed in *Arabidopsis* are linked with differences in chromatin states and DNA methylation we analyzed the chromatin ultrastructure and the distribution of 5-methylcytosine (5mC). The obtained results have shown that the egg cell and the central cell which participate in the process of double fertilization have the decondensed chromatin and DNA methylation marks are present. However, the different levels of 5mC reflect the chromatin dimorphism in female gametes which is associated with a different status DNA methylation and probably their transcriptional activity.

Identification of evolutionarily conserved interplay between the SWI/SNF-type chromatin remodeling complex and BRCA1 in *Arabidopsis thaliana*

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Keywords: chromatin; SWI/SNF complex; BRCA1

Within the nucleus of all eukaryotes, DNA is tightly packaged into a nucleoprotein complex called chromatin. This compaction allows the storage of large amount of DNA, but on the other hand nucleosomes inhibit transcription, DNA repair, and other chromosome transactions. One of the best studied complexes, responsible for chromatin structure changes is evolutionary conserved, multiprotein SWI/SNF complex, able to relieve this inhibition by sliding or disassembling nucleosomes, substituting histones with histone variants, or interactions with transcription factors. It is know that hSWI/SNF subunits, with the ATPase activity (BRG1, hBRM), directly interact with BRCA1 protein. The BRCA1 protein is a product of the suppressor gene, that is involved in genome stability maintenance by e.g. participating in DNA double strand breaks repair (DSB), cell cycle control, regulation of genes involved in DSB response as well as control of higher chromatin structure. The aim of presented study was to check, whether interactions between BRCA1 and SWI/SNF complex are evolutionarily conserved in *Arabidopsis thaliana* model plant and assess how the aberration of BRCA1 influence the SWI/SNF complex function. We also checked other interacting partners for overexpressed recombinant BRCA1 protein, using combined pull-down method followed by mass spectrometry protein identification. We identified Arabidopsis mutant lines carrying T-DNA insertions in the *BRCA1* gene and line with overexpression of *BRCA1*. Subsequently we constructed double Arabidopsis lines carrying mutations in genes coding for SWI/SNF subunits and BRCA1 protein. These lines were subjected for detailed phenotypic analyses including assessment of their response to the treatment with various hormones and abiotic stresses.

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You can't teach an old dog new tricks? Story about newly discovered lincRNA involved in seed dormancy

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Keywords: lncRNA; seed dormancy; polyadenylation; Arabidopsis

Choosing the best moment of germination is one of the most important decision in plants life. In nature the major regulator of the transition from a dormant seed to germination is *Delay of Germination 1* gene (*DOG1*).

DOG1 is regulated by a range of mechanisms including – alternative splice site selection, speed of Pol II elongation, alternative polyadenylation and antisense transcript (*1GOD*encoded within *DOG1* transcript unit).

Nevertheless, exciting things are taking place in *DOG1* genomic neighborhood. Firstly, expression of neighboring gene (*PKS18*) is regulated opposite way to *DOG1* during development. Secondly, we discovered a novel long intergenic non-protein coding RNA (lincRNA) - *3'1GOD* located between *DOG1* and *PKS18*.

3'1GOD region is needed for proper expression of both *DOG1* as well as *PKS18*. Detailed analysis show that *3'1GOD* inactivation leads to altered ratio between *DOG1* sense and antisense and affect choice of *DOG1* alternative polyadenylation site. Further, *3'1GOD* level is strongly induced in *cpl1* mutant which link levels of this lincRNAs with disturbance in Pol II elongation and recycling.

To explore the molecular mechanisms involved we mapped Pol II distribution at *3'1GOD* locus at a single-base resolution showing that Pol II is extensively pausing along this transcript. Our molecular dissection reveals that *3'1GOD* transcripts can be subjected to urydynalation and its expression is extensively upregulated in nuclear exosome mutant.

I will present how the discovery of this novel lincRNA originating from region next to *DOG1* locus opens the doors to exploring mechanistic of *DOG1* expression regulation and investigation of how upstream environmental signals control *DOG1* through *3'1GOD*.

Investigation of SWI/SNF chromatin remodeling complex composition using classical genetic and molecular biology approaches

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Keywords: epigenetics; chromatin remodeling; SWI/SNF; Arabidopsis

In all eukaryotic organisms nuclear DNA is packed into chromatin. Such packaging imposes serious restrictions on processes such as transcription, DNA repair or replication. This problem can be overcome by chromatin remodeling complexes, including SWI/SNF complex family. SNF5 is one of the core subunits of SWI/SNF complexes and it plays an essential role in complex formation and function in variety of organisms, from yeast to humans. Inactivation of the human SNF5 homolog results in malignant rhabdoid tumors formation. The only homolog of SNF5 in Arabidopsis is BSH. No mutant lines with complete *BSH* gene inactivation have been described so far. Here we present the preliminary results of functional BSH characterization, using classical genetics, protein complex immunoprecipitation and other approaches to show its unexpected roles in plant SWI/SNF complex function.

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RNA-seq analysis of differential gene expression during somatic embryogenesis induced by trichostatin A in Arabidopsis explants

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Keywords: Arabidopsis; histone acetylation; somatic embryogenesis; RNA-Seq; trichostatin A

Epigenetic modifications, including histone acetylation, play a significant role in the regulation of the genes involved in the embryogenic reprogramming induced in plant somatic cells. In line with this belief, we observed that treatment of the Arabidopsis explants with trichostatin A (TSA), an inhibitor of histone deacetylases (HDACi), results in somatic embryogenesis (SE) induction. Thus, TSA treatment seems to affect the explants similarly to 2,4-D of auxin-like activity, that is commonly applied to induce SE in different plants, including Arabidopsis.

We hypothesized that TSA-induced histone acetylation triggers the embryogenic pathway of development in the explants via modulation of the genes of regulatory role in SE induction. In order to verify this hypothesis and to identify the SE-regulatory genes that are controlled by histone acetylation, we conducted RNA-seq analysis in the TSA-treated explants of Arabidopsis. To this end, the explants (immature zygotic embryos) were cultured *in vitro* on E0-control and SE-induction medium (E0 +1 µM TSA). The explants for RNA-seq analysis were collected at 3 time points of *in vitro* culture, including 0, 5 and 10 day, and three biological repetitions were analysed. The sequencing libraries were prepared using Illumina ScriptSeq™ Complete Kit (Plant) following the manufacturer's instruction. A library quality was analyzed with Agilent Bioanalyzer and Agilent DNA 1000 Kit. Libraries for RNA-seq were prepared and sent to high-throughput sequencing in Illumina›s HiSeq sequencer.

Analysis of the RNA-seq data aimed at identificatio n of the genes that expression level and pattern was significantly different in response to TSA treatment. In total, 27,581 genes were analysed and expression of 12,9% (3558) of them was shown to be significantly modulated on TSA vs control medium. We indicated that significantly more of genes was de-regulated in the early (5 day) than in advanced (10 day) stage of the TSA-induced SE, accordingly, 2607 vs 2309. In addition, we found that among the genes that showed differential expression in response to TSA, over-represented (2.5 more frequent) were the down-regulated (2613) than up-regulated (945) genes. The RNA-seq data provide a valuable platform for identification of the histone acetylation-regulated genes of decisive function in the SE induction.

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Identification of evolutionary conserved functional interdependence between SWI/SNF-dependent chromatin remodeling and alternative splicing in Arabidopsis

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Keywords: SWI/SNF complex; alternative splicing; chromatin remodeling

SWI/SNF-type chromatin remodeling complexes (CRCs) are evolutionarily conserved multiprotein machineries controlling DNA accessibility by regulating chromatin structure. The core of Arabidopsis SWI/SNF complex is formed by association of SNF5 (BSH) with one of the four ATPases (BRM, SYD, CHR12, CHR23) and two of the four SWI3 type proteins (SWI3A, SWI3B, SWI3C, SWI3D). In eukaryotes, intron sequences from RNA polymerase II transcribed pre-mRNAs are removed by the spliceosome to produce mature mRNAs. Catalytic activation of the spliceosome is critically dependent on its association with the evolutionary conserved NineTeen Complex (NTC), which among others carries the core PRP19A/B, CDC5 and SPF27 subunits. The aim of our study was to identify a regulatory link between chromatin remodeling and control of pre-mRNA splicing by the NTC complex in Arabidopsis. RNAseq analysis revealed that inactivation of some subunits of SWI/SNF complex causes disturbed splicing on numerous SWI/SNF-target genes. In addition, we found that the SWI/SNF complex plays an important role in selection of alternative TSS (transcription start sites). Using combinations of existing T-DNA insertion mutations, we observed epistatic genetic interactions between SWI/SNF and NTC. Yeast two-hybrid (YTH) and BiFC interaction studies indicated direct interaction between particular subunits of NTC and SWI/SNF complexes. Subsequent ChIP analysis showed cooperative binding of SWI/SNF and NTC subunits to TSS regions of genes involved in i.e. hormonal signaling. Collectively, our results provide evidence for functional interdependence between chromatin remodeling and pre-mRNA splicing mechanisms in Arabidopsis.

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Session 4

PLANT INTERACTIONS WITH OTHER ORGANISMS

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Orchids eating fungi – when mycorrhizal symbiosis turns to exploitation

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Keywords: orchids; mycorrhiza; mixotrophy; mycoheterotrophy; stable isotopes

A particularity of orchids is their mycoheterotrophic germination, where reserveless seeds develop into a heterotrophic seedling, thanks to the colonization and carbon provided by a symbiotic fungus [1]. The seedling later forms green leaves in most case: the fungus, generally belonging to the polyphyletic 'rhizoctonia' aggregate [2], then turns into a purely mycorrhizal fungus, which colonizes roots only. At this adult stage, most green orchids are believed to become autotrophic and to reward the fungus with their own photosynthetic carbon, as in most other mycorrhizal associations.

However, some species rely on mycoheterotrophy at adulthood and lost photosynthesis. This evolution of non-green species occurred ca. 50 times independently in the orchid family. It was more recently realized that some green orchids, phylogenetically related to mycoheterotrophic species, although photosynthetic, are partially mycoheterotrophic, a strategy called mixotrophy [3, 4]. In the later species, difference in isotopic abundance (^{13}C) between fungal and photosynthetic carbon and the examination of albinos (rare achlorophyllous variants that survive *in natura*, thanks to full mycoheterotrophy) were instrumental in the elucidation of mixotrophy. Mycoheterotrophic and mixotrophic species rely on the symbiotic shifts from the usual rhizoctonia partners to taxonomically and ecologically different taxa, which are either saprotrophic (in the tropics mainly) or mycorrhizal on surrounding trees [5]. Moreover, mixotrophy is viewed as an evolutionary step toward mycoheterotrophy [6].

Recently, isotopic particularities found in most green orchids that are putatively considered autotrophic raised the possibility that they are mixotrophic as well [7]. Evidence exists that they receive carbon from their fungal partners, although a budget of the plant-fungal exchange remains elusive, questioning the exact definition to give to the concept of 'mixotrophy'. We discuss this issue in an evolutionary perspective, and also address the limits of isotopic approaches, in order to suggest next steps in research on mixotrophy in the orchid family.

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Ecological significance of the plant microbiome

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Keywords: root microbiome; rhizosphere; phosphorus; plant nutrition

The plants microbiome is a constant and important control of the plants growth and health. The composition and function of the plant microbiome were described in detail for a number of agricultural relevant crop species. Due to the current global need of changes in crop production, there is an urgent need and increasing interest to use the plant microbiome for an improved nutrient supply with decreased need of using fertilizers and pesticides. Different approaches for the improvement of the microbiome were tested and described in the last years by several authors. Microbial inoculums can be applied, but it seems most promising to use plant breeding strategies with consideration of the genotype-specific microbiome in the long-term. The root and rhizosphere microbiomes of arable crops offer promising strategies to improve the use of the soil phosphorus (P) pool. The global availability of rock phosphates for fertilizer production is limited and the quality is decreasing. For this reason international activities are running to find solutions for an increase P use efficiency of plants involving the microbiome. Farming practices like fertilization and crop rotation can affect the plant microbiome and its functioning significantly. Therefore, selection of suitable farming practices and plant breeding with consideration of the microbiome and its capacities are promising strategies. In the future the use of the plant microbiome is assumed to be a promising strategy to increase the yield security and quality with a decreased use of agrochemicals and under consideration of climate change.

Friend or foe: Crop plants between beneficial and pathogenic bacteria

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Keywords: Induced Resistance; priming; human pathogens

During the cultivation of crop plants, priming for enhanced resistance using biocontrol agents is an efficient disease management strategy. It results in robust resistance and higher yield. The beneficial effects of the bacterial QS molecules, e.g. *N*-acyl homoserine lactones (AHL), on resistance and plant growth have been shown in different plants. Presence of AHL influences the transcriptional of various defense and growth-related genes and modifies the physiology of primed plants. Here, we present the effects of the AHL oxo-C14-HSL and AHL-producing bacteria on the priming capacity of barley plants. Barley is one of the most important crop worldwide and an enhanced resistance against pathogens, such as the powdery mildew causing fungus *Blumeria graminis*, is of high importance to agriculture. We demonstrate here that barley, primed with the beneficial bacterium *Ensifer meliloti*, expresses enhanced resistance against *B. graminis*. We show also that the capacity to induce priming varies among different barley cultivars. This suggests that appropriate genetic equipmentis required in order to induce AHL-priming, at the same time it bears the potential to use this genetic feature for new breeding approaches. We further show that priming for enhanced resistance in barley involves stronger activation of the barley MAP kinases, regulation of defence-related *PR1* and *PR17b* genes and remodelling of the chemical composition of the cell wall. Noticeable was the stronger accumulation of lignin upon priming after chitin challenge. In the following, we focused on the impact of resistance induced in crop plants on the establishment of human pathogen in plant production environment and colonization of plants. In greenhouse experiments, crop plants were primed for induced resistance with *E. meliloti*. Our results show that priming has a negative effect on the persistence of *Salmonella*. Primed plants express the defence-related genes earlier than unprimed plants and are able to close their stomata for longer period. These results indicate the potential of priming for enhanced resistance against *Salmonella*. The use of biologicals or beneficial bacteria represents therefore a good strategy for sustainable plant protection measures and opens new opportunities for breeding approaches.

Microbes as drivers of plant success – new tools in agriculture, plant protection, phytoremediation and agromining

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Keywords: plant; fungi; bacteria; extreme conditions

A wide range of microbes can be used in cost-efficient and sustainable techniques in agriculture, phytoremediation, agromining and plant protection. Selection of the most efficient endophytic and mycorrhizal fungi assisted by symbiotic bacteria can be used to speed-up plant growth, to limit the use of fertilizers and pesticides and to defend plants from pathogens or harsh conditions. Above mentioned interactions can be additionally supported by animals that have been found to be an important factor serving as propagule vectors and sources of key nutrients.

Bacterial and fungal endophytes were isolated from plants colonizing post-flotation wastes rich in heavy metals, agricultural and agromining areas, and extremely harsh sites. Many of them were strongly mycorrhizal but this type of symbiosis can be hampered due to wrong use of agrotechnology or presence of soil toxicity and also has limits due to expensive inoculum production. The endophytic fungi in roots are generally more common than mycorrhizae. In addition, they also may colonize the shoots. On the basis of the recent research endophytic fungi appear to be important in association with AMF but they cannot substitute the AMF, especially in case of mycorrhizae responsive plants. Nonmycorrhizal plants depend only on endophytic microbes. These are very important eg. in optimization of Ni agromining in Europe while mycorrhizal plants used in agromining are known mostly from warmer climates. The presentation will show several data resulting from long – and short-term studies carried out under local and international cooperation.

Sucrose transporters are involved in abiotic stress response and biotic interactions

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Keywords: sucrose transporters; abiotic stress response; biotic interactions

The sucrose molecule is not only the most important long distance transport molecule of photoassimilates, but is also an important florigenic molecule involved in flower inducing signaling pathways, and plays a role under abiotic stresses and in biotic interactions such as symbiosis with arbuscular mycorrhizal fungi. We aimed at elucidation of the protein-protein interactome of all three sucrose transporters (SUTs) known from Solanaceae in order to elucidate their specific role in planta. Each of the three sucrose transporters has its own specific spectrum of interaction partners suggesting different functions for different sucrose transporters. Only few of the SUT-interacting proteins interact with all three of them. Several of these versatile interaction partners are involved in abiotic stresses such as cold stress, salt stress or drought stress. Sucrose accumulation in the leaves of stressed plants seems to be part of the strategy to cope with abiotic stresses. Plants with inhibited expression of these of stress-inducible SUT-interacting candidates do not recover from drought stress or salt stress, suggesting that sucrose plays an important role during recovery phase and resilience. Elucidation of the interactome of sucrose transporter SlSUT2 reveal interaction with several partners that are involved in either brassinosteroid biosynthesis or BR signaling. The membrane steroid binding protein MSBP1 is assumed to inhibit BR signaling and to affect mycorrhization efficiency in Medicago. MSBP1 interacts with SlSUT2 and with the BR co-receptor kinase BAK1. We asked whether MSBP1 impacts root colonization with arbuscular mycorrhizal (AM) fungi in a similar way as shown previously for SlSUT2. In addition, we asked whether brassinosteroids *per se* affect efficiency of root colonization by AM fungi. Phenotype and behavior of the transgenic tobacco plants with increased or reduced *MSBP1* expression is consistent with an inhibitory role of MSBP1 in BR signaling. *MSBP1* overexpression could be mimicked by brassinazole treatment. We observed that brassinosteroids indeed have a direct impact on the nutrient exchange in AM symbiosis and on the biomass production of colonized host plants. Furthermore, arbuscular morphology is affected by changes in *MSBP1* expression and brassinolide or brassinazole treatments. We conclude that host plant growth responses and nutrient exchange within the symbiosis with AM fungi is controlled by brassinosteroids and might be impeded by the MSBP1 protein. The role of SlSUT2 might be in sucrose retrieval from the periarbuscular space thereby inhibiting fungal growth. Sucrose retrieval might represent part of a defense strategy in biotic interactions.

Analysis of *Prune dwarf virus* intercellular transport and pathogenesis

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Keywords: viral intercellular transport; immunolocalization; plasmodesmata; 3D protein structure

Prune dwarf virus (PDV) is an important viral pathogen of plum, sweet cherry, peach, and many herbaceous test plants. Although PDV has been intensively investigated, mainly in the context of phylogenetic relationship of its genes and proteins, many gaps exist in our knowledge about the mechanism of intercellular transport of this virus. The aim of this study was to investigate intercellular transport of PDV and alterations induced during pathogenesis in PDV inoculated susceptible plant host: *Cucumis sativus* cv. Polan at ultrastructural level. To analyze the role of viral proteins in local transport, double-immunogold assays were applied to localize PDV coat protein (CP) and movement protein (MP). We observed structural changes in chloroplasts, mitochondria, and cellular membranes. We prove that PDV is transported as viral particles via MP-generated tubular structures through plasmodesmata. Moreover, the computer-run 3D modeling reveals structural resemblances between MPs of PDV and of *Alfalfa mosaic virus* (AMV), implying similarities of transport mechanisms for both viruses. All this data enables to assemble model of cell-to-cell transport of PDV.

Coat protein (CP) of conserved structure from multiple Potyviruses triggers Rysto-mediated immunity

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Keywords: Rysto; potato virus Y; PVY coat protein (CP)

Ry$_{sto}$ from the wild relative of potato, *Solanum stoloniferum*, confers extreme resistance to potato virus Y (PVY) virus that causes severe losses in potato production. Using SMRT RenSeq, we cloned *Ry*$_{sto}$ and showed that it encodes a TNL-class nucleotide-binding, leucine rich repeat immune receptor. To identify the elicitor of the *Ry*$_{sto}$-mediated immune response, the ORFs encoding putative viral proteins were cloned and transiently expressed in transgenic *Ry*$_{sto}$ tobacco plants. Among all tested proteins, only expression of PVY coat protein (CP) resulted in strong HR, showing that viral CP is an elicitor of *Ry*$_{sto}$-triggered immunity. To further understand the mechanism of *Ry*$_{sto}$-mediated resistance, we showed that *Ry*$_{sto}$ targets a conserved element of the CP structure, rather than a linear amino acid motif. Consistent with this hypothesis, we showed that Ry$_{sto}$-mediated resistance can be triggered by CPs of similar structure from multiple Potyviruses, but not by the ones lacking it. This allows to predict the range of viral pathogens against which Ry$_{sto}$ could confer resistance.

Fungal endophytes in non-host plant species: beneficial or detrimental association in salinity stress?

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Keywords: fungi; endophyte; halophyte; salinity; ryegrass; salt tolerance; Epichloë

Lolium perenne (perennial ryegrass) is a popular cool-season grass, one of the major turf and forage species in the world. Changes in climatic conditions and improper irrigation practices have led to soil salinization which drastically affects ryegrass yields since this species is moderately salt tolerant. Moreover, many studies refer to beneficial microbes inhabiting inner parts of the plant called "endophytes" that are known to benefit their host and play crucial roles in plant development, growth and diversification. But the question arises if they are able to confer these traits in other non-host plant species? This depends on a set of abiotic and biotic factors, including the genotypes of plants and microbes, environmental conditions and the dynamic network of interactions within the plant biome. Hence, our study hypothesizes that the fungal endophytes of the halophyte *Salicornia europaea* when inoculated in glycophyte *L. perenne* can improve the growth and salt tolerance of their new host.

This study employed selected fungal endophytes to combat salinity stress in *L. perenne*. Two grass varieties: one Epichloë infected and the other Epichloë free were chosen for *S. europaea* endophyte inoculation. Plants were subjected to three salinity stress levels ranging from no stress to high stress. Plant physiological parameters, biochemical analysis and transcriptome profiling of this plant endophyte association were recorded. Endophytic strains provided *L. perenne* with decipherable resilience under salinity stress. This provides a promising basis for the study of *S. europaea* endophytes as a solution for saline entrenched areas and in improving future farming systems.

■ ■ ■

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A comparison of selected physiological and molecular characteristics of strawberries and their susceptibility to diseases caused by pathogenic fungi

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Keywords: fungal pathogens; metabolites; phenylalanine ammonia lyase; *Fragaria* sp

Strawberries are one of the most commonly consumed fruits worldwide, mostly due to the health and nutrient importance and economical benefit. In recent years, their production has increased in a lot of countries, including both Poland and Turkey. However, strawberries cultivation shows many difficulties associated with their high susceptibility to diseases caused by pathogenic fungi which limit both strawberry fruit quality and yield. The decrease of strawberry (*Fragaria × ananassa* Duch.) yield is mainly due to fungal pathogens such as *Colletotrichum* and *Botrytis*. *Colletotrichum acutatum* is a principal pathogen known to be responsible for anthracnose. Strawberry cultivars exhibit wide phenotypic diversity in terms of their susceptibility to fungal pathogens. The metabolic and proteomic status of strawberries plays an important role in triggering defence strategies. In response to attacks of fungal pathogens, plants activate multiple defence reactions directed against the attackers both at the site of infestation and systemically. Besides, the changes in the level of metabolites in plant cells affect metabolic mechanisms of the pathogen involved in the pathogenicity process during the infection spread. Our experiments were conducted on resistant and susceptible varieties of *Fragaria × ananassa* Duch. organs, non-infected and infected with fungal pathogens. Plant materials were cultured at Poznań University of Life Sciences and Cukurova University. The concentration of selected metabolites, the activity of phenylalanine ammonialyase (PAL) and expression levels of PAL genes were determined. The obtained results provide new insights into the regulatory mechanisms during the plant-fungal pathogen interactions at metabolic and molecular level and contribute new insights to our understanding of the plant resistance mechanisms.

Changes in xylan-1/xyloglucan and xyloglucosyl transferase (XTH-Xet5) as a step-in of ultrastructural cell wall remodelling in potato–PVYNTN resistance and susceptible reaction

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Keywords: plant-virus interactions; hypersensitive response; cell wall; xyloglucan; xyloglucosyl transferase

One type of monitoring system in a plant cell is the cell wall, which intensively changes its structure during interaction with pathogen-stress factors. The wall plays a role as a dynamic and controlled structure, although it is not fully understood how relevant these modifications are to the molecular mechanisms during plant–virus interactions. In this work we localised the non-cellulosic polysaccharides such as xyloglucan, xylan (xylan-1) and xyloglucosyl transferase (XTH-Xet5), the enzyme that participates in the metabolism of xyloglucan. This provided us with information about the *in situ* distribution of the components of the hemicellulotic cell wall matrix in hypersensitive and susceptible potato–PVY^{NTN} interactions. The loosening of the cell wall was accompanied by an increase in xylan depositions during susceptible interactions, whereas, during the hypersensitive response, when the cell wall was reinforced, the xylan content decreased. Moreover, the PVY inoculation significantly redirected XTH-Xet5 depositions, regardless of types of interactions, compared to mock-inoculated tissues. Furthermore, the immunogold localisation clearly revealed the domination of Xet5 in the cell wall and in vesicles in the susceptible host. In contrast, in the resistant host increased levels of Xet5 were observed in cytoplasm, in the cell wall and in the trans-Golgi network. These findings show that the hypersensitive reaction activated XTH-Xet5 in the areas of xyloglucan endo-transglycosylase (XET) synthesis, which was then actively transported to cytoplasm, cell wall and to vacuoles. Our results provide novel insight into cell wall reorganisation during PVY^{NTN} infection as a response to biotic stress factors. These novel findings help us to understand the mechanisms of defense responses that are incorporated into the cell wall signalling network.

■■■

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Primary metabolism in *Brassica napus* cultivars resistant and susceptible to *Alternaria brassicicola* infection

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Keywords: black spot disease; carbohydrates; oilseed rape; photosynthesis

Oilseed rape (*Brassica napus*, OSR) is one of the most important crop cultivated all over the world and its seeds are used as a source of edible oils and biodiesel and leaves as feed for farm animals. However, one of the most frequent disease of *B. napus* is black spot disease caused by a necrotrophic fungus *Alternaria brassicicola*. In this study, four oilseed rape cultivars with a different level of susceptibility to *A. brassicicola* infection were selected from 160 OSR cultivars using WinDIAS_3 system. The area of the necrotic lesions was measured at 72, 120 and 240 hpi (hours post-inoculation). Two cultivars Askari and Savannah showed a high level of resistance to *A. brassicicola* infection and Monty-028DH and Zairai Chousenshu were highly susceptible. In general, infection by a necrotrophic pathogen can affect the primary metabolism of a host plant by release of phytopathogenic toxins and host`s tissue damage. Therefore analysis of chlorophyll *a* fluorescence using FluorCam system and content of carbohydrates using GC-MS analysis was performed. Fluorescence of chlorophyll *a* expressed as the photosynthetic parameters such as maximum quantum yield (QYmax) in a dark-adapted state, quantum yield (Fv/Fm) in a steady-state and non-photochemical quenching (NPQ) in a steady-state were measured in control leaves and in two areas of infected leaves: treated and untreated. In resistant cultivars Askari and Savannah, values of photosynthetic parameters in control and infected leaves did not differ much although at a significant level. However, values of photosynthetic parameters in infected leaves at both treated and untreated areas of susceptible Monty-028DH and Zairai Chousenshu cultivars significantly decreased compared with the control leaves. Analysis of carbohydrates revealed over 60 sugars with a various content dependent on a cultivar as well as a plant susceptibility to *A. brassicicola*infection. Primary metabolism analysis allowed estimating influence of *A. brassicicola* infection on selected OSR cultivars and in the future can be a valuable tool for selection of resistant cultivars in agricultural areas exposed to black spot disease.

■■■

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Early stages of symbiotic interactions between *Gunnera* sp. and *Nostoc* sp.

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Keywords: *Gunnera*; symbiosis; *Nostoc*; hormogonia; heterocyst

Gunnera is the only angiosperm plant known to have intracellular symbiotic association with *Nostoc* sp. cyanobacteria capable to fix molecular nitrogen. *Gunnera* forms special glands in the stem above the base of each petiole where the symbiosis takes place. *Nostoc*symbionts are not transferred between *Gunnera* generations, but seedlings have to be infected *de novo* by the soil-born *Nostoc* colonies. *Nostoc* hormogonia, short fragments of filamentous colonies consisting of several vegetative cells, surrounded by their own cell wall and cyanobacterial envelope, were found on receptory surface of glands. Hormogonia are claimed to be invasive form, although the way they enter *Gunnera* cells has never been described. Our examinations by using transmission electron microscope, conducted on *G. manicata* and *G. magellanica* glands, revealed that hormogonia became separated into the single cells being unicellular "invasive forms". During transition into the "invasive forms" cyanobacterial cells lost their cell wall and thylakoid membranes. They become surrounded by electrondense mucus produced apparently by host gland cells and entered intercellular spaces of receptory tissue. The mucus protects "invasive forms" and attaches them to *Gunnera* cell walls. "Invasive forms" induce host cell wall invagination that engulfs them and translocates across plant cell wall inside *Gunnera* protoplasts in structures surrounded by host's cell walls. Inside plant protoplast, "invasive forms" divide and re-transformed into regular vegetative cells that form filamentous hormogonia with heterocysts. Heterocysts have pronouncedly thicker cell walls than vegetative *Nostoc*cells. *Nostoc* hormogonia are ensheathed in a plant wall material tightly covered by host plasmalemma during entire time of symbiosis.

Activity of vacuolar processing enzymes in the response of spring barley to infection with cereal cyst nematodes

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Keywords: cyst nematode; *Hordeum vulgare*; legumain

Parasitic nematode infestation severely affects crop plants important for food and biofuels production. Cyst nematodes parasitize sugar beet, potato, corn, tomato, soybean as well as canola. Feeding of adult nematodes and their juveniles on host roots causes deep local metabolic changes in infected roots as well as systemic physiological effects in other plant organs (e.g. leaves). It should be noted that the most of international research teams focuses on studies on local plant responses at the site of infection (roots) and still there is little known about holistic plant responses induced by nematode parasitism. In our research this issue was treated with a special emphasis and performed analyses showed changes in gene expression and enzymatic activity of vacuolar processing enzymes (VPEs) in leaves and roots of spring barley infected with the cyst nematode *Heterodera filipjevi*. VPEs are cysteine peptidases produced in cytosol in the form of inactive zymogens and activated in vacuoles. In recent years, special attention has been put on involvement of VPEs in seed developmental processes, but there are also several references available showing their involvement in plant defence response to pathogen infection. However, their physiological role in cereal plants responses to cyst nematodes infection is unknown and never been examined before.

Interactions in the *Brassica* sp. – *Plasmodiophora brassicae* pathosystem. Reaction to various *P. brassicae* pathotypes in different *Brassica* genotypes and selection of candidate genes for molecular markers to clubroot

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Keywords: *Brassica* sp.; *Plasmodiophora brassicae*; clubroot

Clubroot disease caused by *Plasmodiophora brassicae* inflicts heavy losses in *Brassica* crops. The disease can be controlled chemically and by agronomic measures, but with limited effectiveness. One of the priorities of *Brassica* breeding programmes is resistance to clubroot. The aim of the study is to elucidate of mechanism of resistance to the disease.

Eight *Brassica* genotypes differing in the level and type of Clubroot resistance was used as the plant material for research. The reaction of Brassica plants to infection with three pathogens of *Plasmodiophora brassicae* (Pb2, Pb9 and Pb3) was observed.

Upon infection, the germs of the pathogen have been observed in the root hairs of all the genotypes tested. The presence of the pathogen both in the roots and in the soil has been confirmed by molecular tests. The results of the observation may indicate that resistance to clubroot was broken in majority of the tested *Brassica* plants. These results allow concluding that some of the following genotypes shall be considered as tolerant, but not resistant to the disease.

During the molecular analysis using cDNA-AFLP methods, 150 transcripts which expression were modulated during infection with Plasmodiophora brassicae pathotype 2 were identified. Analysis of their homology has shown that protein products encoded by these genes are involved in the defense reactions, regulation of gene expression, immune responses, cell transport, cytoskeletal structure, signal transduction, cell cycle regulation and post-translational protein modifications.

45 of the identified genes may be indicated as potential molecular markers to clubroot. Among them there are 19 genes encoding immune proteins. Genes which are probably involved in immune reactions based on hypersensitivity reactions and programmed cell death, like N protein, Pid3, TAO1, a protein with the MA3 domain and PDCD1 were analyzed using real-time PCR.

■■■

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Activation of defense responses by flg22 elicitor is dependent on the daytime and ethylene in intact tomato leaves

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Keywords: ethylene; flagellin; nitric oxide; reactive oxygen species; stomata

The first line of defence in plants against pathogens is induced by the recognition of microbe-associated molecular patterns (MAMP). The best-characterized MAMP is the flagellin (flg22). Flg22 induces stomatal closure which can be dependent on various external and internal factors. Here we studied the effects of the daytime-dependent flg22 treatments on salicylic acid (SA) or ethylene (ET) signalling in leaves of intact tomato plants. Flg22 was applied in the afternoon (late light phase) and at night (early dark phase) and defence responses of the plants were measured at several times after each treatment. Flg22 not only inhibited the light-induced stomatal opening at dawn, but it also induced the stomatal closure in the morning and afternoon, but the degree of stomatal closure was dependent on the daytime of the elicitor application. Flg22 perception in the first hours can be crucial to induce several defence responses in plants, thus signalling events after flg22 treatments were analysed following the different application times. Accumulation of reactive oxygen species (ROS) and nitric oxide (NO) was different after flg22 treatments in the late dark phase and early night. In addition, ET emission was not significant in the dark phase-treated leaves. Investigation of hormone response genes, *Pathogenesis-related 1* (*PR1*), *Ethylene response factor 1* (*ERF1*) and *Defensin* (*Def*) also showed significant differences in several daytimes upon flg22 treatments. Role of ET in flg22-induced defence responses was investigated in ethylene receptor mutant *Never-ripe* (*Nr*) tomato plants. Interestingly, significant stomatal closure was not induced after flg22 treatment in *Nr* plants suggesting the potential signalling role of ET in flg22-induced defence reaction. Moreover, flg22 caused ROS and NO levels were also different. These data demonstrate that early biotic signalling in intact leaves is an ET-dependent process, which has great importance on the guard cell-mediated plant defence responses.

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Organ-specific and daytime-dependent effects of exogenous flg22 elicitor treatments on the photosynthetic activity of tomato leaves

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Keywords: flagellin; nitric oxide; photosynthetic activity; reactive oxygen species; guard cells

Light is a fundamental factor in the control of many biological processes like plant development and defence responses. Chloroplasts, thereby photosynthetic activity may also be involved in the control of light-dependent immune responses. In this study, the organ-specific and daytime dependent effects of exogenous flg22 – a peptide derived from bacterial flagellin – treatments were evaluated in intact tomato leaves. Flg22 not only inhibited the light-induced stomatal opening at dawn, but it also induced stomatal closure in the morning and afternoon, which were independent on the daytime of the elicitor application. Flg22 caused significant accumulation of reactive oxygen species (ROS) and nitric oxide (NO) in stomata independently of the daytime of the flg22 treatments. Elicitors, like flg22, not only induce high ROS and NO production especially in chloroplasts but also have essential effects on photosynthetic activity. Therefore, the daytime-dependency of the PSII activity was investigated after flg22 treatments in order to ascertain the possible effects of flg22. Changes in the photosynthetic activity of guard- and mesophyll cells were also compared in flg22-treated leaves of intact tomato plants. The maximal quantum yield of PSII photochemistry (F_v/F_m) did not change significantly upon flg22 treatment in the first light phase of the day in none of them. In contrast, the actual quantum yield of PSII (F_{PSII}) and the photochemical quenching coefficient (qP) decreased slightly upon flg22 treatments in the guard cells, treated in the light phase of the day. At the same time, flg22 influenced significantly the non-photochemical quenching (NPQ) parameter in guard cells at 9:00 a.m. These physiological changes in guard cells can contribute to a faster and stronger stomatal closure at the early phase of the light cycle.

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Cyanogenesis in white clover (*Trifolium repens* L.) as an anti-herbivore defence mechanism

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Keywords: cyanogenic glycosides; cyanogenic polymorphism; HCN; insect feeding preferences

Cyanogenesis (an ability to release a highly toxic compound, hydrogen cyanide – HCN) occurs in almost three thousand plant species, functioning as an effective defence mechanism against herbivorous animals. In intact plant tissues, cyanogenic compounds, formed from amino acids with the participation of cytochrome P450 monooxygenases and *O*-glycosyltransferase, are constitutively stored as glycosides. Simultaneously, substrate-specific cyanogenic glucosidases are spatially separated from substrates in cells. The contact of substrates and enzyme, caused by tissue disruption (by chewing insect, animals, etc.), results in release of HCN, which is a respiratory poison and may cause severe acute or chronic poisoning. The cyanogenic glucosides themselves may act as phytotoxins and, when eaten by animals, may be hydrolysed in the digestive system causing HCN release and poisoning. Cyanogenesis is an ancestral plant two-component defence system, from which the other plant binary defence system has evolved. The 'mustard oil bomb' system occurring in Brassicaceae plants is based on glucosinolate synthesis pathway and β-glucosidase – myrosinase, stored either in specialized myrosin cells or ER-derived structures named ER bodies.

Due to high protein content in biomass, the ability to fix nitrogen from air and improve the soil structure, some leguminous species (e.g. white clover, alfalfa, etc.) are widely used as forage plants and are of a great economic importance. Amongst legume species, white clover (*Trifolium repens* L.) is one of the most widely grown species in pastures. White clover is not only characterized by possessing cyanogenic defence system, but also shows cyanogenic polymorphism (presence of cyanogenic and non-cyanogenic populations), which makes it a valuable model for ecological and evolutional investigations.

Here we show the feeding preferences of detritivorous woodlice (*Armadillidium vulgare*), model insect (*Drosophila melanogaster*), snail (*Cepea* spp.) and slug (*Deroceras* spp.) towards cyanogenic and non-cyanogenic genotypes of white clover in context of its potential repelling properties.

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Chitosan-induced plant defence responses are influenced by light and daytime in tomato

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Keywords: chitosan; chlorophyll a fluorescence; nitric oxide; reactive oxygen species; stomata

Fungal pathogen attack can be mimicked by elicitor molecules such as chitosan (β-1,4-linked glucosamine; CHT), which is a deacylated derivative of the fungal cell wall component chitin. Guard cells can sense the presence of these elicitors and then respond by closing the stomatal apertures. At the same time, plant defence responses are dependent on external factors, such as presence or absence of light and daytime, but their role is not known upon CHT treatments. Intact leaves of tomato plants were treated with CHT at various time points during the day and defence responses of the plants were measured at several times after each treatment. To examine whether light regulation plays role in CHT-induced defence reactions, artificial darkening experiments were also set in the morning. CHT not only inhibited the light-induced stomatal opening at dawn, but also induced stomatal closure in the morning, which were independent of the daytime of the elicitor application. CHT induced generation of reactive oxygen species in guard cells in the first part of the light phase independently of the daytime of CHT treatments, but significant CHT-promoted nitric oxide production was observable only in the afternoon. Moreover, the actual quantum yield of PSII (f_{PSII}) decreased upon CHT treatments at dawn both in guard and mesophyll cells. Expression of defence marker genes was also examined. Transcript level of *Pathogenesis-related 1* (*PR1*) was already increased at dawn in the CHT-treated leaves, which raised the maxima in the early light phase of the day. Interestingly, CHT-induced *PR1* expression was inhibited in the dark, suggesting the potential light-dependent regulation of CHT-induced defence responses and the importance of environmental factors on the intact plant responses after elicitor treatments.

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Mechanisms of resistance to nitrosative stress: implications for *Phytophthora infestans* survival in plant host

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Keywords: *Phytophthora infestans*; nitrosative stress; anti-nitrosative system

Reactive nitrogen species (RNS) are a family of molecules derived from nitric oxide (NO) and characterized by high reactivity. Among RNS, NO and peroxynitrite ($ONOO^-$) have a very well documented biological activity. Reactive nitrogen species that are overproduced or not efficiently eliminated may provoke nitrosative stress. Although RNS formation may be regulated by various balancing systems in living cells, the elements fulfilling an anti-nitrosative function are poorly recognized mainly in plant pathogens.

Phytophthora infestans (Mont.) de Bary is a fungus-like organism, belonging to oomycetes and as a causative agent of potato late blight it is one of the most dangerous potato pathogens worldwide. In order to verify whether and to what extent RNS are balanced in *P. infestans* structures, analyses using RNS modulators were undertaken. Briefly, the experiments were conducted on two pathogen isolates, i.e. avirulent (*avr*) and virulent (*vr*) to the ‚Bzura' potato genotype. Moreover, experiments were conducted under *in vitro*and *in planta* growth phases. To gain insight into metabolic changes associated with the potential anti-nitrosative response of the pathogen, studies involved determination of nitric oxide dioxygenase (NOD), peroxiredoxin (PRX) and S-nitrosoglutathione reductase (GSNOR).

In general, applications of NO donors resulted in a *ca.* 2-fold increase of *NOD* gene expression, NOD protein accumulation and higher GSNOR activity in both isolates. Moreover, GSNOR activity under the influence of exogenous NO correlated with an enhanced S-nitrosothiol pool (SNOs) and the pool of free thiol groups in *P. infestans* structures. In turn, the $ONOO^-$ donor treatment provoked 5-fold and 2-fold higher expression of the gene coding PRX2 in *avr* and *vr P. infestans*, respectively. The PRX activity differed between the tested isolates, with a higher activity noted in the *avr* isolate in both growth phases. The use of specific RNS scavengers produced an opposite effect.

The results indicated that the oomycete pathogen exposed to exogenous sources of RNS is able to tightly regulate their amounts using the anti-nitrosative system comprising NOD, PRX and GSNOR. Thus, the anti-nitrosative system may be a part of an adaptation mechanism to the presence of huge amounts of RNS derived from external sources, including plant host cells.

Precise phenotyping of traits related to plant susceptibility to two-spotted spider mite uncovers mite performance variation depending on the infested plant genotype and leaf age

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Keywords: *Tetranychus urticae*; *Arabidopsis thaliana* accessions; plant-herbivore interaction; leafdamage assessment; oviposition rate

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, represents one of the most destructive generalist mite herbivores. TSSM feeds on hundreds of plant species belonging to various botanical families. The ability of TSSM to flexibly adapt to multiple host plants resulted from innate tolerance to different plant xenobiotics. At the same time plants didn't evolve effective resistance loci which could give a wide and durable immunity to TSSM. Therefore novel approaches are required to identify quantitative phenotypes and to explain the genetic basis of traits related to TSSM susceptibility.

After the application of high-resolution imaging of infested leaves, we were able to define several parameters related to pest performance and plant susceptibility. Eight *Arabidopsis thaliana* accessions deriving from remote world locations were infested with TSSM and showed remarkable differences in the damaged leaf area, chlorotic area, and lay eggs number. The proposed method demonstrated also the accessions variation in the mite preference to the age of leaf and egg distribution on ab/adaxial leaf surface. Different methods for automation the image analysis were tested and their usefulness was discussed. We expect that proposed sensitive analytical pipeline will facilitate the screening of larger segregating populations of different plant species leading to the identification of loci for efficient breeding of TSSM tolerant plants.

The variation in glucosinolate biosynthesis and ABA signaling correlates with two-spotted spider mite susceptibility of selected *Arabidopsis* accessions

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Keywords: *Tetranychus urticae*; biotic stress; reproductive performance; molecular response; natural genetic variation

The two-spotted spider mite, TSSM (*Tetranychus urticae* Koch) is economically important herbivorous pest attacking a wide range of host plants and inflicting serious damage and crop yield losses. Mites are piercing-sucking herbivores, feeding mostly from the cells of mesophyll tissues. TSSM, as a generalist, is able to feed on a wide range of host-plants. It probably manipulates plant defense (e.g., by suppression, induction or counteraction) using effector-like proteins occurring in its saliva and digestive proteases in the midgut. In response to mite saliva and chelicerae wounding, the mite-infested host-plant defends itself by activating the JA-, ET-, SA- and ABA-dependent signal-transduction pathways although in the case of multiple stresses the main biotic stress hormones – SA and JA seem to lose their importance. Another mechanism which could play a role in the inhibition of the attacker relies on the toxicity and deterrence of the indole- and aliphatic-glucosinolates or their metabolites. In the presented research, we analyzed the correlation of ABA and glucosinolate related regulatory pathways with pest performance in selected infested and uninfested A. thaliana accessions. Based on the results we hypothesize that for several studied genes the insensitivity to down-regulate them upon infestation could be more important for building resistance than induction of other genes. Equally interesting was the correlation of some studied genes expression in consecutive leaves of the same plant and mite feeding preference. These results are discussed in the context of natural variation among *Arabidopsis* accessions and the possible use of studied genes in marker-assisted breeding.

Colonisation of *Raphus sativus* L. by human pathogenic microorgansisms (HPMOs)

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Keywords: human pathogenic microorganisms; endophytes; *Raphus sativus*

Most cases of foodborne illness are caused by consumption of raw or minimally processed vegetables and fruits. The most frequently identified human pathogenic microorganisms (HPMOs) in crops are: *E. coli* 0157:H7, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus*. The occurrence of HPMOs in crops are primarily associated with polluted soils enriched with organic fertilisers, crops irrigated with contaminated waters, or the faeces of free-living animals.

The aim of our research was to determine the interactions between selected species of HPMOs (e.g. *Salmonella enterica* subsp. *enterica* PCM 2565, *Listeria monocytogenes* PCM 2191) and vegetable crop radish (*Raphanus sativus* L.). We assume that colonization of HPMO can be specific to plant organs and the colonization of plant tissues by HPMO can affect growth parameters.

The results of our study revealed that colonization of radish was observed in the case of all tested HPMOs but the density of pathogens in plant organs (leaves, stems, roots) dependent on the HPMO species. The total number of bacteria depends on plant organs, pathogen species and plant age. Tested HPMOs had a negative effect on plants growth parameters including e.g. number of leaves, length of roots, as well as fresh and dry weight.

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Disorganization of tomato root apical meristem as a result of canavanine phytotoxicity

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Keywords: canvanine; flavonoids; meristem organization; root growth; starch

Canavanine (CAN) is non-protein amino acid produced by some Fabaceae species, found mainly in seeds and sprouts. In seeds of plant producers, it acts as a defense against herbivores. In insect cells CAN is incorporated into proteins instead of arginine, thus leads to the formation of abnormal and non-functional proteins. In mammalian CAN is best characterized as inhibitor of inducible nitric oxide synthase. Due to this action and cytotoxic and anti-proliferation activity CAN is implemented in anti-cancer therapy (Staszek et al. 2017).

In tomato, CAN inhibits root growth and disturbs ROS and RNS metabolisms (Krasuska et al. 2016a,b). Growth of roots of tomato (*Solanum lycopersicum* L.) seedlings was inhibited (in 50 or 100%) by supplementation with CAN (10 and 50 μM) for 24 or 72 h. Root tip organization and root tip cells ultrastructure were analyzed. Starch granules localization was performed to estimate root metabolic activity and flavonoids accumulation was investigated due to CAN dependent induction of nitro-oxidative stress.

After 24 h of CAN supplementation root tips structure resembled the control but after longer treatment it was highly affected. Meristematic region was strongly modified and all the cells (epidermis, cortex, vascular tissue and their histogens) underwent premature vacuolization, connected probably to the rapid differentiation. CAN led also to flavonoids accumulation, which pattern differed depending on CAN concentration and culture duration. In root tips of control seedlings, starch granules were limited to cells in root cap and in epidermis and cortex of elongation zone, while in CAN-treated tissue starch granules were widely distributed. After 72 h of 50 μM CAN treatment starch was present in almost all cells of the roots. We can conclude that microscopic observations confirmed sensitivity of the organization of primary root meristem to CAN.

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The effect of cadmium and *Colletotrichum acutatum* on nitrogen metabolism and the level of phytohormones in mung bean (*Vigna radiata*)

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Keywords: *Vigna radiata*; *Colletotrichum acutatum*; cadmium; nitrogen metabolism

The present study demonstrates the impact of cadmium (Cd) of different concentrations on nitrogen metabolism and the level of phytohormones in mung bean (*Vigna radiata*) seedlings. The objective of the research was to determine how the exposure of *V. radiata* seedlings to cadmium alters defense responses to pathogenic fungus *Colletotrichum acutatum* and whether these responses could affect the pathogen indirectly**.** The results of the study showed that cadmium in a chosen concentration range and *C. acutatum* triggered infection inhibited the growth of *V. radiate*. Additionally, transferring *V. radiate* seedlings cultured in cadmium medium to the cadmium-free one stimulated a slight increase in the length of seedling shoots. Furthermore, it was shown that cadmium alone in the chosen concentration range and the combined effect of cadmium and *C. acutatum* interaction caused stimulation of nitrogen metabolism due to a significant increase in the activity of nitrate reductase and changes in the level of nitrates. It is well known that nitrate reductase is a key enzyme in nitrogen assimilation, catalyzing the nitrate-to-nitrite reduction process in plants. A variety of factors, including nitrate, metabolites and phytohormones modulate the expression of both nitrate reductase genes and its activity. Additionally, the interaction of cadmium and *C. acutatum* caused changes in the level of phytohormones. Moreover, under *in vitro* conditions, cadmium inhibited the growth of mycelium of *C. acutatum*. Therefore, the following study sheds light on the current understanding of the role of nitrogen metabolism and phytohormones in plant defense strategy during heavy metal-pathogenic fungus interaction.

Brassinosteroid and sucrose interaction regulate sugar transporters and defense responses in pea seedlings (*Pisum sativum* L. cv. Cysterski) infected with *Fusarium oxysporum* f.sp. *pisi*

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Keywords: sucrose transporters; brassinosteroids; sucrose; *Pisum sativum-Fusarium oxysporum* pathosystem; phenolic compounds; defense enzymes

The sucrose transporter expression and activity is tightly controlled at the transcriptional, post-transcriptional, translational as well as post-translational level. Our first objective of the study was to determine the effect of epi-brassinolide and sucrose on the level of expression of genes encoding sucrose transporters in isolated embryo axes of pea seedlings (*P. sativum* L.cv. Cysterski) inoculated with *F. oxysporum pisi* and cultured *in vitro*under different trophic conditions. The second objective was to investigate crosstalk interactions of epi-brassinolide, sucrose and *F. oxysporum* on the total content of phenolic compounds, the relative level and localization of flavonoids in isolated embryo axes infected with the pathogenic fungus and cultured *in vitro* on medium with sucrose. In addition, the activity of β-glucosidase and peroxidase, enzymes involved in plant defense reactions against fungal pathogen was investigated. Real-Time PCR analyzes showed that sucrose alone, *F. oxysporum* infection and epi-brassinolide caused increased transcript accumulation for sucrose transporter SUT1 and sucrose facilitators SUF1 and SUF4 relative to the control. Moreover, the obtained results showed that sucrose and infection with *F. oxysporum* caused an increase in the total content of phenolic compounds and evidently stimulated the accumulation of flavonoids in the epidermal layer. The highest level of phenolic compounds was found in the pea embryo axes pre-treated or not with epi-brassinolide and infected with *F. oxysporum* and cultured on medium with sucrose. We also found high post-infection stimulation of peroxidase activity towards syringaldazine in infected tissues, pre-treated with epi-brassinolide in the presence of sucrose. Additionally, the highest β-glucosidase activity was recorded at epi-brassinolide pre-treated embryo axes and infected with *F. oxysporum* under sugar starvation. Additionally, one of the defense strategies of embryo axes pre-treated or not with epi-brassinolide and infected with *F. oxysporum* on medium with sucrose was the high generation of superoxide anion radical.

Metabolomic approaches reveal the impact of growth promoting fungal endophytes on Arabidopsis metabolom

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Keywords: secondary metabolism; metabolomic; root colonization; phosphor supplementation

In this study we investigated the impact of colonization of *Arabidopsis thaliana* roots with eight taxonomically distant endophytic fungi that have either positive or negative impact on plant growth under limited phosphate availability. To this end we applied metabolomics analysis based on combination of liquid chromatography with mass spectrometry (LC-MS), which is comprehensive and sensitive platform for studying changes in secondary metabolite profiles. Approaches based on unbiased LC-MS analysis enable comparison of a broad range of metabolites within large sample sets, as in our multifactorial experiment. Bioinformatic tools dedicated to processing (MZmine2, MarVis) and statistical analysis of metabolomic data (own R scripts and Genstat), enabled to (i) compare impact of different fungi on Arabidopsis metabolome, and (ii) select metabolites related to morphological effect of co-cultivation under different phosphate supply. Selected during these analyses signals were further subjected to the identification step by manual inspection of MS/MS spectra and annotation to metabolomics data bases.

Obtained PCA plots showed that colonization of roots with fungal strains had a higher impact on Arabidopsis metabolome than phosphate supplementation. Two-factor analysis of variance enabled to distinguish hundreds of LC-MS signals differentiating particular fungal strains at phosphate deficient conditions, which indicated strong involvement of plant secondary metabolism in the response to environmental stimuli. Most of the significantly affected signals had increased intensity in samples obtained from colonized roots as compared with respective controls. The most striking metabolome reprograming was observed during interaction of Arabidopsis with two fungal strains,which induced the strongest growth promotion under phosphate limitation. What is more, hierarchical clustering revealed that both strains grouped together and were distant from the remaining six strains used in the experiments.

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Session 5

ABIOTIC STRESS RESPONSES

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Is Arabidopsis LARP1 an effector of the TOR kinase in the control of Ribosomal Protein translation?

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Keywords: LARP1; TOR kinase; Ribosomal Protein

The ribosome is a macromolecular complex and a universal fundamental unit of life. The number of ribosomes is positively correlated with the cellular activity and strongly decreased in adverse environmental conditions. This contributes to the downregulation of the cellular activity when organisms need to shift from a growth mode to a survival mode. Eukaryotic cells exert a precise control of the production of ribosomes by tightly regulating in a coordinated and stoechiometric manner the production of ribosomal proteins (RPs). In mammals this control takes places at the level of translation and is dependent upon sensing of environmental conditions by the mTORC1 kinase complex. An effector of mTORC1 is the universally conserved RNA Binding Protein (RBP), LARP1. LARP1 directly binds mRNAs coding for RPs and when phosphorylated by mTORC1 in nutrient replete conditions, acts to stimulate their translation. Conversely, when mTORC1 is inactive, LARP1 binds to RP mRNAs to a different locus and downregulates their translation. In such conditions the highly conserved DM15 domain of LARP1 binds the cap structure and the following TOP sequence shared by mRNAs of RPs. LARP1 then likely blocks translation by displacing the EiF4F complex. How phospho-LARP1 acts as a positive regulator of translational and if and how mTORC1-induced phosphorylation prevents the DM15 domain to bind the cap remain unknown so far. In plants, *Arabidopsis thaliana* and *Chlamydomonas reinardhtii*LARP1 are phosphorylated by mTORC1 at two conserved serines located few amino acids upstream to the DM15 region. Is and how plant LARP1 acting downstream to mTORC1 to regulate RP production is unknown. Moreover plant mRNAs coding for RPs do not share TOP sequences leaving open the question of how these transcripts are co-regulated. We found that, under growth stimulating conditions, LARP1 binds to most RP mRNAs and observed that most of them carry a C as transcription start site. Using *in vitro* approaches we found that the DM15 domain of Arabidopsis LARP1 is also a cap-binding domain unable to accomodate TOP sequences but showing a higher preference for capped RNA probes with a C at +1. We are currently exploring the hypothesis that LARP1 cap binding activity could be regulated by the conserved phospho-serines located upstream to the DM15 domain.

Physio-genetic dissection of stress-induced leaf senescence and timing its reversal in barley

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Keywords: autophagy; chlorophyll fluorescence induction kinetics; gene expression profiling; stress induced senescence

The genomic resources available for *Arabidopsis* have made it a very attractive model for the identification and functional analysis of senescence-regulated genes. In many plants, such as barley, removal of the developing flowers and pods significantly extends the life of their leaves while in *Arabidopsis* male sterile mutants or plants from which the developing bolts are removed do not extend the lifespan of the leaves. Because of these differences, cereal leaves have been used over the years as a model for studying leaf development and senescence. Distinct differences in the senescence program of *Arabidopsis* as compared to that in the monocot plants have been revealed. Senescence in cereals is generally regulated at the level of an individual leaf. Nutrients from the older leaves are remobilized for the younger leaves and eventually for the flag leaf, contributing thereby to the nutrients required for grain development. Cereal leaves have a basal meristem, the leaf tip consists of older cells and the younger cells are concentrated at the leaf base. Such a cellular organization enables studies on senescence progression easier to differentiate. Nonetheless, the lack of coordinated development of the cells within an individual leaf introduces complexity in studying leaf senescence. Therefore, induced senescence that directs a synchronous process, such as the dark-induced senescence (DILS), has become more in vogue.

DILS is an extreme example of shading which induces senescence in leaves similar to that observed during normal plant development. The DILS model fits well with other important monocot crop plants, e.g., maize and rice, eliminating the confounding factors that overlap with developmental senescence such as bolting or flowering. Early and late events of leaf senescence in the DILS crop model were deciphered to reveal the time limit for dark to light transition in reversing the senescence process. Differences in gene medleys including the hormone-activated signaling pathways, lipid catabolic processes, glutamine catabolic processes, DNA and RNA methylation and carbohydrate metabolic processes between DILS and developmental senescence processes in barley leaves have been revealed. These studies also demonstrated that the DILS program is reversible by re-exposure of the barley plants to light prior to day-7 of dark exposure. The senescence

reversal involved regaining of photosynthesis, increase in chlorophyll and reversal of chlorophyll fluorescence vitality index (Rfd), inspite of the activation of macro-autophagy-related genes. Rfd was found to be an earliest parameter that correlated well with the cessation of photosynthesis prior to micro-autophagy symptoms, chromatin condensation and initiation of DNA degradation.

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The role of GLR channels in plant developmental and movement responses

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Keywords: Arabidopsis; chloroplast movement; hypocotyl; phototropism; root growth

GLRs (glutamate receptor-like channels) are plant counterparts of mammalian iGLR receptors involved in signal transduction in the central nervous system. The aim of this work was to test a working hypothesis that GLR channels are involved in blue light-controlled growth and movement processes in *Arabidopsis thaliana*. We investigated photomorphogenesis and skotomorphogenesis of seedlings, phototropism and chloroplast movement responses. Parameters of all studied processes were analyzed in the presence of inhibitors MK-801 and CNQX, known for blocking two subfamilies of iGLR channels, NMDA and AMPA respectively. Root growth was inhibited by CNQX and, to some extent, promoted by MK-801 in both, light and dark conditions. While in blue light hypocotyl growth was inhibited by CNQX only, in the dark MK-801 started to work as in roots. This shows that both types of channels, AMPA-like and NMDA-like, play different roles in modulating the seedling growth. Neither inhibitor affected phototropism. Thus calcium channels other than GLRs must be involved in the light signaling pathway that leads to hypocotyl bending. Both inhibitors were required to reduce chloroplast movement parameters. Currently chloroplast responses to blue light are investigated in GLR mutants to identify the channels involved.

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Plant Desiccation Tolerance: Still a mystery

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Keywords: resurrection plants; genome sequences

Desiccation tolerance in angiosperms is presented in seeds and a small group of resurrection plants that can be dehydrated to equilibrium with dry air and then on rehydration rapidly recover full metabolic activity. The mechanism of desiccation tolerance is multifunctional process comprising sugar accumulation, specific protein synthesis, cell structure changes and increased anti-oxidative reactions. Resurrection plants can be assumed as a gift of evolution – a suitable model for studying the molecular and genetic basis of plant desiccation tolerance. In last few years the first genome sequence of angiosperm plants has been presented and this was a great achievement of scientific and practical aspects in understanding the genetic basis and mechanisms of desiccation tolerance.

Comparative genomic and transcriptomic analyses revealed common and species-specific desiccation tolerance strategies comprising gene duplication, enhanced expression of LEA proteins, early light-inducing proteins, up regulation of unique genes for ROS generation and scavenging as well as distinct regulation of ABA biosynthesis pathway. Accumulation of sugars and LEA proteins in drying leaves of desiccation tolerant plants mirrors accumulation pattern in maturing seeds. Analysis of genomes and transcriptomes of resurrection plants strongly support the hypothesis that vegetative desiccation tolerance In angiosperms arose more recently, and mounting evidences suggest it evolved by redirection of genetic information from desiccation tolerant seeds. The connection of vegetative desiccation tolerance with existing seed desiccation and drought response pathways provide some candidate genes for engineering improved drought tolerance in crop plants. Genome analysis revealed a lack of desiccation tolerance-specific genome organizational feature, which suggests that desiccation tolerance has not been conferred by the acquisition of genes unique to resurrection plants, but rather by alteration in the regulatory control of genes that are present in genomes of most plants.

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Plastid membrane network in higher plants modified by environmental factors

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Keywords: thylakoid network; chloroplast; etioplast; light stress; chilling stress

Photosynthesis is a fundamental process performed in higher plants by specialized organelles – chloroplasts. The chloroplast thylakoid network is a site of photochemical reactions and it is one of the most complicated membrane systems in nature. Such a complex internal plastid membrane network is formed in developing chloroplasts during plant ontogenesis. Simple membrane system in proplastids develops without light to the paracrystalline prolamellar body (PLB) connected with porous prothylakoids. On light, this tubular spatial structure transforms, in a multistage process, to the linear one of parallel lamellae forming eventually a system of grana and stroma thylakoids.

Both the structure of developing plastids as well as one of the fully developed chloroplasts can be modified by different environmental factors. Among others, in a temperate climate, the most prominent are low temperature affecting young plants during spring and high light influencing the fitness of fully developed plants during summer.

In this study, we show how long-term and short-term stress can modify the structure of thylakoid network, arrangement/composition of pigment-protein complexes leading eventually to changes in photosynthetic efficiency. Experiments were perform using two plant species with different tolerance to chilling stress – pea (*Pisum sativum* L.) and bean (*Phaseolus coccineus* L.) and a model plant – *Arabidopsis thaliana*. We showed how formation and transformation of PLB into a linear system of grana and stroma thylakoids i.e. the process of chloroplast biogenesis is modified at different levels of plastid organization by long term night chilling. Moreover, we showed thylakoid network structural rearrangements (2D and 3D) present in fully developed chloroplasts exposed to short term high-light conditions. In conclusion, highly organized and flexible thylakoid membrane system enables maintaining the optimal photosynthetic capacity in young and fully developed plants in changing environmental conditions.

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Participation of the RNA-Binding proteins (RBPs) in heat and cold stress response in *Brassica oleracea var. botrytis*

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Keywords: abiotic stress; GRP; transcriptional response; proteomic response; RRM

Plants as sessile organisms with no ability to move are constantly exposed to the detrimental environmental conditions such as high or low temperature. During evolution,plants have developed a number of adaptive strategies to adverse conditions both at the morphological and physiological as well as on biochemical and molecular level. Organellar RNA-binding proteins (RBPs) have many regulatory functions and are involved in stress response in plants. Using qPCR we checked the level of different RBPs and chose two of them which are the most variable after stress treatment. Confocal microscopy analysis showed a speckles like pattern throughout protoplast suggesting mitochondrial localization. To further extend our study we performed transcriptomics and proteomics analysis. Results from these experiments proved the crucial role of RNA-binding proteins in temperature stress response.

Arabidopsis thaliana cation exchanger 4 (CAX4) is important for plant fitness and growth under Mn, Zn and Fe deficiencies

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Keywords: cation ion exchanger 4; abiotic stress; micronutrients; *Arabidopsis thaliana*; flowering time

The great importance of micronutrients for the proper functioning of all organisms has been understood a long time ago. However, until now, not all of the essential proteins associated with elements' homeostasis and their transportation within plants have been fully characterized. Here, we would like to present the study of significance of one of the ion exchangers of *Arabidopsis thaliana* – CAX4, for growth and general fitness of plants. For this purpose, we have analysed and compared the loss of function mutant in *CAX4* gene with Col-0 genetic background. We have performed a detailed phenotypic characterization of both lines grown in various conditions by conducting quantitative PCR, recording flowering time, measuring fresh weight of both roots and shoots, and determining mineral and chlorophyll content. Mutant line and Col-0 wild type plants were cultivated in soil as well as in hydroponic cultures. According to *in silico* analysis, plants were subjected to chosen environmental abiotic stresses which causes alterations in *CAX4* expression level. In the case of soil culture, plants were cultivated in optimal soil and soil with disturbed micronutrients ratio. Plants grown in hydroponic cultures were subjected to manganese (Mn), zinc (Zn) and iron (Fe) depletion. The phenotypic analysis revealed interesting growth-related changes of *cax4* mutant line depending on the culture conditions. We observed a significant correlation between tested traits like plant growth, fresh weight, rosette leave number, flowering time and micronutrients (Mn, Zn and Fe) availability. These results lead us to the conclusion that alterations in growth of *cax4* in comparison to wild type plants are linked to disturbed micronutrients content in the environment.

Root-type ferredoxin-NADP+ oxidoreductase isoforms in *Arabidopsis thaliana*

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Keywords: ferredoxin-NADP+ oxidoreductase; *Arabidopsis thaliana*; non-photosynthetic plastids; electron transport; stress responses

In the plastids of higher plants, ferredoxin-NADP$^+$oxidoreductase (FNR) mediates electron transfer between ferredoxin (Fd) and NADP$^+$. Leaf-type FNRs (LFNRs) function in the last step of linear photosynthetic electron flow by mediating electron transfer from Fd for the reduction of NADP$^+$ to NADPH, while root-type FNRs (RFNRs) have been shown to catalyze reduction of Fd in non-photosynthetic plastids. Reduced Fd in turn provides reducing power for the function of enzymes involved in anabolic processes like assimilation of nitrogen and sulfur, desaturation of fatty acids as well as redox regulation and amino acids biosynthesis. Both LFNRs and RFNRs exist as multiple isoforms in *Arabidopsis thaliana*. While the LFNR isoforms are extensively studied, only limited information is available on RFNR isoforms.

Here, we have used several distinct approaches to study in details gene expression of *RFNR1* and *RFNR2* as well as accumulation of RFNR proteins in different parts of *Arabidopsis thaliana*. *RFNR1* and *RFNR2* genes show unique expression patterns in roots and shoots suggesting different functions of the isoforms. Physiological characteristic of *rfnr1* and *rfnr2* mutants followed by studies on accumulation of the RFNR isoforms in response to different environmental conditions support above observation. Proteomic analysis of Arabidopsis root plastids show that RFNR proteins provide reducing power for several anabolic processes occurring within non- photosynthetic plastids, mainly amino acid biosynthesis. Moreover, involvement of RFNRs in dark-light transition as well as response to cold and ozone stresses are discussed.

Starvation-induced microautophagy in plants

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Keywords: autophagy; microautophagy; E-64d. FM4-64; starvation

Autophagy is an essential system to degrade and recycle cellular components to survive under the starved condition. Autophagy is classified as macro-, micro-, and chaperone-mediated autophagy and autophagy is refers macroautophagy in many cases. While most of the study about molecular mechanisms of the plant autophagy focus on macroautophagy, the knowledge about microautophagy in plant is little. Previously we reported that the application of a papain protease inhibitors E-64d and a fluorescent membrane marker FM4-64 to plant seedlings or tobacco BY-2 cells enabled to visualize the accumulation of vesicles and its aggregates at the border of tonoplast, which was induced under the sucrose-starved condition. This vesicle accumulation was stopped with several autophagy-related *atg* mutants. To understand the link between the vesicles formation and autophagy, we monitored the process after the E-64d treatment. At the early phase of the induction, we observed the invagination of the tonoplast and cytosolic acid granules are trapped into the tubule. FM4-64 signal is detected on the invagination site and colocalizes with tonoplast marker. Besides, In the later phase, FM4-64 was detected as a number of vesicles in the vacuole, and most of the vesicles indicate higher acidicity than that of the vacuole. In the *atg* mutants, vesicle formation was stopped on the tonoplast and formed ubnormal rod-shaped structures. We concluded that the starvation induces microautophagy in plant cells, which requires *ATG* genes to complete the process.

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Suboptimal conditions of growth trigger meristem arrest in the *ftsh4-1* mutant plants

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Keywords: Arabidopsis; *AtFTSH4* gene; SAM; RAM; oxidative stress

Shoot and root apical meristems (SAM and RAM, respectively) are vital structures for plant development, which ensure a constant delivery of cells for proper growth and organogenesis. We analyzed the mechanisms governing an interesting phenotype of precocious cessation of growth in plants lacking the mitochondrial protease AtFTSH4, during growth in the suboptimal temperature of 30˚C. We linked the phenotype of SAM arrest with the on-going accumulation of the internal oxidative stress and mitochondria impairments, both visualized *in vivo*. Concomitantly, we analyzed directly in the SAM and RAM of the wild type and loss-of-function *ftsh4-1* mutant plants, the expression of selected meristem and cell cycle related genes, the cell cycle progression, and the responsiveness of the two structures to hormones. We concluded that the growth arrest in the *ftsh4-1* mutant, triggered by the suboptimal temperature of 30˚C, is an outcome of the loss of stem cells identity and cell cycle dysregulation due to accumulating internal oxidative stress and mitochondria dysfunction. The results of our research will be discussed in the context of the role of the *AtFTSH4* gene for the apical meristems maintenance during growth in the phenotype-inducing conditions, at the cellular and molecular levels.

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Tetrapyrrole biosynthesis is hardwired to photosynthetic electron transfer

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Keywords: *Chlamydomonas reinhardtii*; chlorophyll biosynthesis; protoporphyrinogen oxidase; plastoquinone pool

In the last common enzymatic step of tetrapyrrole biosynthesis, prior to the branching point leading to the biosynthesis of heme and chlorophyll, protoporphyrinogen IX (Protogen) is oxidised to protoporphyrin IX (Proto) by PROTOPORPHYRINOGEN IX OXIDASE (PPX). The absence of thylakoid-localised PLASTID TERMINAL OXIDASE 2 (PTOX2) and cytochrome b_6f complex in the *ptox2 petB* mutant, results in almost complete reduction of the plastoquinone pool (PQ pool) in light. Here we show that the lack of oxidised PQ impairs PPX function, leading to accumulation and subsequently uncontrolled oxidation of Protogen to non-metabolised Proto. Addition of 3(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) prevents the over-reduction of the PQ pool in *ptox2 petB* and decreases Proto accumulation. This observation strongly indicates the need of oxidised PQ as the electron acceptor for the PPX reaction in *Chlamydomonas reinhardtii*. The PPX-PQ pool interaction is proposed to function as a feedback loop between photosynthetic electron transport and chlorophyll biosynthesis.

The recovery of soybean plants after short-term cadmium stress

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Keywords: cadmium; soybean; recovery; methylation; mineral homeostasis

Contamination of the environment with cadmium is a serious problem in various parts of the world. This non-essential metal is readily absorbed by plants leading to over-production of reactive oxygen species, membranes damage, hampered photosynthesis, increased mutation rate, enhanced cell mortality and hampered growth. Although the mechanisms of Cd toxicity are relatively well documented, little is known about plants recovery from Cd stress.

In present study soybean seedlings were treated for short time (48 h) with Cd at two concentrations: 10 and 25 mgl^{-1}. Thereafter they were transferred to optimal growing conditions for 7-days long recovery periods. The obtained results show that even relatively short-term Cd stress leads to hampered roots growth, loss of membranes integrity and increased cells mortality. After the recovery period previously Cd-treated plants exhibited the same growth parameters as the control plants with an exception of roots shortening in the case of seedlings exposed to higher metal concentration. No differences between Cd-stressed and control plants were noted in terms of cell viability, lipid peroxidation, chlorophyll content or photosynthesis efficiency. For the first time it was observed that plants recovered from metal stress showed alterations in mineral content. They contained higher levels of manganese, magnesium and sodium in relation to the non-treated control. To examine if past metal treatment led to epigenetic changes the level of DNA methylation marker, 5'-methyl-2'-deoxycytidine (5MedCyd), has been measured. No significant differences in global DNA methylation level have been noted.

The results indicate that soybean plants are capable of efficient recovery even after relatively severe Cd stress. The recovered plants contained higher amounts of manganese, magnesium and sodium indicating that past metal treatment led to changes in mineral uptake.

The anatomical structure, pigments content and antioxidant system efficiency in leaves of *Anthyllis vulneraria* calamine ecotype

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Keywords: calamine wastes; *Anthyllis vulneraria*; heavy metal stres

In the presented work, we investigated the structural and physiological changes in leaves of *Anthyllis vulneraria* that spontaneously appears on waste heaps created after Zn-Pb ores exploitation and processing.The anatomical structure of leaves, the content of metals, photosynthetic pigments, phenolic profile as well as activity of antioxidant enzymes were compared between specimens representing calamine ecotype (CAL) and plants from non-contaminated (NM) site.

The present study showed that absorbed heavy metals were mainly immobilized in the CAL root system. Microscopic analysis showed differences in leaf blade structure between analyzed ecotypes. Photosynthetic pigments content in CAL leaves was higher than in NM one, although the calculated ratio of chlorophyll a to b was similar in both ecotypes (about 3.2). The CAL specimens accumulated significantly higher amounts of total secondary metabolites, phenylpropanoids, flavonols, and anthocyanins in comparison with NM specimens. Nevertheless, the concentration of polyphenols did not differ between ecotypes and reached 9.4–10.4 mg per 100 g of fresh weight. Moreover, the measurements of enzymes activity demonstrated a significant diversification of tested specimens. The detailed results will be presented during the poster session.

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Comparative analysis of metabolic responses to salt excess in halophytic and glycophytic species from *Asteraceae* family

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Keywords: *A. alpinus*; *A. tripolium*; carbohydrate profiling; phytohormones; salinity

Comparative analyzes of plant responses to salt stress in genetically related taxa, but differentiated in ecological features can significantly extend the current state of knowledge of plant tolerance mechanisms to important abiotic stress factors. This experimental approach was applied in the current study, aiming at comparing metabolic responses to increased salinity between two species from Asteraceae: tolerant halophyte *Aster tripolium* (syn. *Tripolium pannonicum*) and sensitive glycophyte *Aster alpinus*. Plants were treated with 200, 400 and 600 mM NaCl solutions for four weeks and then data were collected on the plant growth performance and the content of endogenous phytohormones, carbohydrate profile and stress-related amino acids in leaf samples. Metabolomic profiling was conducted using HPLC and UHPLC methods.

As expected, under salinity halophytic species performed better than glycophytic one, exhibiting higher growth tolerance index and leaf water content. Progressive salt concentrations reduced the stem height in both species, however in a halophyte the reduction was less pronounced. Phytohormonal profiling revealed that *A. tripolium* was able to maintain higher content of active cytokinins, and lower level of deactivated growth promoting phytohormones, mainly auxins and cytokinins. Interestingly, the species has high initial concentration of ABA in untreated control plants, which declined under low and moderate salinity. The content of active jasmonates declined, while jasmonate precursor oxo-phytodienoic acid (OPDA) increased in salt-treated plants of both species. These reactions were more intensive in glycophyte than in halophyte.

Considering carbohydrate profile, massive accumulation of uronic acids occurred in halophyte in comparison with glycophyte in response to salinity. The initial content of sugar alcohols was high in *A. tripolium* and declined substantially under salt treatment. In turn, their content in *A. alpinus* was low and stable, irrespectively of salinity exposure. Opposite pattern was observed in the content of oligosaccharides. The accumulation of low molecular weight carbohydrates, namely mono- and disaccharides was more advanced in glycophytic than in halophytic species. Osmotic adjustment in *A. alpinus* involved also enhanced synthesis of betaines, while *A. tripolium*accumulated mainly proline.

The results of our study shed a light on comprehensive metabolic reactions involved in defense responses to salt excess in halophytic and glycophytic Asterace species, and may be used to decipher potential mechanisms conditioning tolerance to salinity stress in this plant family.

Geranium robertianum L. plants on railway tracks – microevolution aspect

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Keywords: *Geranium robertianum* L.; railroad tracks; pollutions; phenotypic and genetic diversity; microevolution

Geranium robertianum L. is a herb that prefers shaded, fertile and moist forest habitats. It can also be found outside the forests, in railway areas with different conditions than those preferred by *G. robertianum*plants. The conditions prevailing there include: insolation, water shortage, pollutions (herbicides, heavy metals, petroleum substances, PAHs, PCBs). Such difficult conditions for plant growth can lead to selection, which favors microevolutionary processes. Because the first observations showed that *G. robertianum* plants found on railway tracks differ significantly from plants found in forests, wider research into this species has been undertaken. *G. robertianum* plants were tested, which occur on railway tracks ("track populations") and in forests ("forest populations"), of north-eastern Poland. The research was carried out both on plants found in the field (F0 generation) and on plants grown in a greenhouse from collected seeds (F1 generation). The aim of the study was to check the phenotypic and genetic diversity of plants from these two types of population. Among the tested track and forest populations, the track population from Waliły-Station was the most distinguished, which showed adaptations to unfavorable conditions on railway tracks. The plants from this population in comparison to other studied populations were: smaller, had much smaller and darker leaves with an increased level of anthocyanins. The studies also showed an increased tolerance of *G. robertianum* plants from the track population to the Roundup herbicide, as compared to plants from forest populations. These features were preserved in the next generation (F1). However, tested track and forest populations of *G. robertianum* were not genetically separate. The observed differences in the phenotype of these two types of plants populations could have arisen as a result of the occurrence of epigenetic processes. The obtained results are an example of the initial stage of the process of microevolution in plants in anthropogenically transformed areas, such as railway tracks.

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Nitrogen metabolism of tomato seedlings roots under hypoxia stress

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Keywords: nitrate reductase; amino acids; nitrate; nitrite; low oxygen stress

Nitrogen affects plants growth, development and stress reaction. Plants can uptake nitrogen as organic or inorganic form. Nitrate and ammonium ions are used as inorganic substances from the soil or nutrient solution. Nitrate ions require reductions before incorporation into organic compounds in plant cell. The process of nitrate reduction is conducted by nitrate reductase, highly regulated plant enzyme. Due to climate change, the flooding frequency is increasing in the world. Many important crop plants are characterized by low tolerance for flooding stress, and their growth and yield are poor in such conditions. Also non-aerated medium (stagnant solution) causes low oxygen content stress for plant roots. The aim of the present study was to evaluate the effect of hypoxia stress on nitrogen metabolism of tomato roots, furthermore the hypothesis that calcium ions addition in nutrient solution might alleviate this stress was also tested. Tomato ‚Kmicic' was cultivated in hydroponics with aeration. Nutrient solution contained all macro and microelements. After 21 days of cultivation plants were divided into 3 groups: control (aerated, 1.75 mM Ca^{2+}), hypoxia (without aeration of nutrient solution, 1.75 mM Ca^{2+}) and hypoxia + calcium (without aeration but with higher content of calcium in nutrient solution, 3.5 mM Ca^{2+}). Plants material (roots) were collected after 24, 48, 72 hours after treatments started. Additionally plants were harvested also after 7 days of stress. Nitrate and nitrite reductase activities as well as content of free amino acids, total proteins, nitrate and nitrite were evaluated. Hypoxia stress affected enzymes activities and organic nitrogen compounds content in tomato roots. Increased activity of nitrate reductase was observed under stress alone or with calcium addition. Mechanism of calcium action on nitrogen metabolism under stress condition needs further investigation.

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Differential expression analysis of genes involved in tolerance to deacclimation in winter barley

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Keywords: RNAseq; differential expression; barley; deacclimation

Mechanisms involved in deacclimation of the plants caused by warm spells during winter aren't sufficiently recognized. It is unclear what makes some plants de-harden after only a few warmer days and why other plants need much more time for that. Also the mechanisms responsible for high survival rate of some plants after experiencing freezing temperatures following a warm spell are still unknown.

The aim of this study was to identify genes involved in tolerance of deacclimation in winter barley.

Four winter barley lines tolerant to deacclimation and 4 susceptible ones were subjected to cold acclimation followed by deacclimation. Leaves from each genotype were sampled before hardening, after hardening and after de-hardening. Total RNA was isolated from the leaves and subjected to RNAseq analysis. Differential expression analysis was performed to compare transcriptomes of control (pre-hardening), cold-acclimated and deacclimated plants as well as tolerant genotypes with susceptible ones.

■ ■ ■

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The role of calcium transporters in the formation of calcium homeostasis disorders under ammonium nutrition

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Keywords: ammonium syndrome; calcium transporters; calcium homeostasis; apoplast; cell wall

Plants are capable of utilizing different inorganic forms of nitrogen (N), nitrate (NO_3^-) and ammonium (NH_4^+). Although NH_4^+ should be the preferred form of N, because its assimilation is associated with a lower energy demand on the cell, surprisingly cultivation using NH_4^+ as the only source of N for most crop plants leads to severe growth suppression and development disorders, which are together commonly referred as the "ammonium syndrome". However, despite many years of research, the mechanism of plant sensitivity to ammonium is still unclear. In our research we showed that in NH_4^+-grown plants there are ionic imbalances. The greatest changes are in calcium (Ca^{2+}) content – approximately 3-fold drop in rosettes of *Arabidopsis thaliana*, while in the roots there was a 5-fold increase of Ca^{2+} content. We also observed changes in calcium distribution in tissues and altered expression profile of many calcium transporters (e.g. autoinhibitory Ca^{2+}-ATPases, cation cation-exchanged proteins, cyclic nucleotide-gated channel 2), which suggest disturbances of Ca^{2+} transport into cells and perhaps its accumulation in the extracellular space (apoplast) during ammonium nutrition. Accumulation of calcium in the apoplast may lead to the stiffening of cell wall structure due to cross-linking of pectins, in which process Ca^{2+} are involved. In our research, we have observed that in plants under the ammonium nutrition, the cell walls are stiffer (Podgórska *et al*. 2017). Therefore, the inhibition of plant growth in response to NH_4^+ feeding may result from calcium homeostasis disorders mediated by changed calcium transporters activity and changes in the cell wall structure.

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In search for annexin 1 scavenging activity in Arabidopsis: the case of *vtc* mutant.

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Keywords: *vtc*; ascorbate acid; annexin; glutathione

Annexins are a family of calcium and membrane binding proteins. It has been demonstrated, partially thanks to our research, that certain annexins are involved in the signal transduction and, in yet unknown way, improves the plant tolerance and its adaptation to sub-optimal external conditions. Preliminary data obtainded in our lab suggest that annexin 1 from *Arabidopsis thaliana* (ANXD1) can adjust the redox poise of the most abundant antioxidant – ascorbate. We are exploring the potential mechanism of this process. To confirm our hypothesis we examined if ANXD1 have an impact on the balance between oxidized and reduced ascorbate. For these experiments we have used the single knock-out mutant of ANXD1 and the plants overexpressing ANXD1. The level of ANXD1 correlated positively with the level of ascorbate and glutathione, but the redox state of these molecules were unaffected. Observed effect was confirmed in vivo by studying the double mutants with reduced level of both ascorbate and annexin as well as mutant with reduced level of ascorbate and elevted level of ANXD1. We have examined the level and the redox state of low-molecular-weight antioxidants and the expression level of genes encoding for scavenging enzymes. Our data suggests that ANXD1 plays an important role in the reactive oxygen species metabolism.

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This work was supported by grant: 2015/19/B/NZ3/01476 Project Opus „Search for molecular mechanism of annexin-mediated chloroplast protection against high light stress in *Arabidopsis thaliana*".

In search for annexin 1 scavenging activity in Arabidopsis: the case of *flu* mutant

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Keywords: annexins; redox poise; *flu* mutants; non-photochemical quenching; deep RNA sequencing

Genomes of almost all plant species encode several annexins and the expression levels of certain of them are rather high. The contribution of annexins to plant cell adaptation to adverse environmental conditions is well documented. It was shown that annexins participate in membrane-related events, such as cellular transport, membrane-cytoskeleton interactions, or endo-/exocytosis. But their cellular functions go far beyond and include regulation of redox poise and protection against chloroplast-derived oxidative stress. Ectopic over-expression of annexins alleviated oxidative stress in prokaryotic (bacterial) and animal cells, which strongly suggests that they cross-talk with a very basic mechanism/s, common to different forms of the living kingdom. In transgenic Arabidopsis plants, over-expression of annexin 1 (ANN1) resulted in retard degradation of photosynthetic pigments and longer maintenance of photosynthetic activity during stresses[1,2]. However, the precise molecular mechanism of this process stays unknown. From our previous experiments, it is clear that during photooxidative stress ANN1 over-expression can modulate both H_2O_2- and singlet oxygen-mediated signaling pathways. To show precisely which one is affected by ANN1 we employ conditional flu mutant which is the best model to study biological functions of singlet oxygen. Due to a point mutation in FLU gene (At3g14110) flu1-1 plants lack negative feedback control in tetrapyrrole biosynthesis and in dark they over-accumulate direct precursor of chlorophyll, Pchlide. As a result, they can grow without any visible phenotype in constant light but while exposed to light after staying in the dark they massively produce singlet oxygen and the length of the dark period is correlated with the intensity of singlet oxygen production. In mature plants induction results in the development of necrotic lesions and growth inhibition. flu1-1 plants respond to photooxidative stress triggered by singlet oxygen by reprogramming their transcription and within the first 30 min, approximately 5% of the total Arabidopsis mRNAs are modified. We modified flu1-1 toward the over-expression of ANN1 and subjected them to photooxidative stress. Next, we analyzed the plants' transcriptome, and the functioning of scavenging systems, especially of low-molecular-weight antioxidants. We assessed also the chlorophyll turnover and analyzed parameters of photosynthesis in these plants. This approach enables us to study the ANN1 effect on chloroplast maintenance during stress.

■ ■ ■

This work was supported by an Opus grant from the National Science Centre 2015/19/B/NZ3/01476.

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CRISPR/Cas9-editied *Nicotiana tabacum* CPK11 gene decreases tolerance of seedlings to salt and osmotic stresses

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Keywords: tobacco; CPK11; salt stress; osmotic stress; CRISPR/Cas9

Calcium-dependent kinases (CPK) are a large family of proteins found mainly in plant organisms. These proteins contain a protein kinase domain at the N-terminus and a calcium-dependent regulatory domain at the C-terminus. The presence of both of these domains (effector and regulatory respectively) causes that CPK proteins are one of the most important regulators of stress response in plants, because they can simultaneously detect and respond quickly to changes in intracellular Ca^{2+} ions appearing in response to stress. In the course of evolution, the plants have created many orthologs of CPK kinases. And so far, 34 orthologs have been detected in *Arabidopsis*, and 41 in maize. Despite such a variety of kinases, their functions are often superimposed, because the paths of stress signaling in plant cells may overlap, and local abiotic stress may cause a systemic response of the whole organism. This implies that often the activation of a given signal pathway occurs due to many stress factors. Studies using *Arabidopsis* have demonstrated that the *AtCPK11* kinase is involved *in vivo* in the signaling of abscisic acid (ABA) in the plant, which is one of the major plant hormones responsible for stress response. *CPK11* disruption results in a phenotype of increased resistance to the presence of ABA in the medium, while overexpression of this gene has the opposite effect. The presence of mutations also increases the sensitivity of *Arabidopsis* seedlings to high concentrations of sodium chloride. It was also found that overexpression of the *CPK11* gene from maize in *Arabidopsis* causes an increase in tolerance to salinity. This allows us to suppose that the product of this gene may be involved in the response to salt stress also in other plants. Due to the ease of genetic transformation, tobacco (*Nicotiana tabacum*) is a very good research model. In addition, this plant grows relatively large, which facilitates physiological studies (eg leaf size measurements, root lengths, etc.). In addition, ease of breeding, lack of sensitivity to the photoperiod (laboratory varieties used) and high fertility suggest using this plant as a model for studying abiotic stresses.

In this study we have constructed tobacco lines lacking functional *NtCPK11* gene by CRISPR/Cas9 system. Mutations introduced by gene-editing decreased tolerance of seedlings to salt and osmotic stresses. This suggests that *NtCPK11* and *AtCPK11* may be a functional orthologs as well as sequence orthologs and play a role in a similar stress signal-transducing pathways.

Silicon ions as a factor regulating the accumulation of selected aquaporins in the leaves of *Brassica napus* var. *napus* L. in the response to a periodic shortage of water in the soil

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Keywords: oilseed rape; water channel; silicon; abiotic stress

The correct course of the main metabolic processes in plants depends significantly on the proper water relations balance. The flow of water in plants is controlled among others by the regulation of aquaporins activity. They create water channels that belong to the conservative family of membrane proteins. Most of the abiotic environmental factors have a direct impact on the water homeostasis and stimulate complex responses that lead to the induction of water saving strategies in the plant cell. Among all types of aquaporins, the most significant changes caused by drought stress concern: *Plasma membrane Intrinsic Protein* (PIP) and *Tonoplast membrane Intrinsic Protein* (TIP). Silicon is one of plant's microelement however, its role in plants physiological processes has been underestimated so far. Silicon is accumulated in cell walls where it interacts with its various components. It was found out that plants supplementation with silicon improves their functioning under the conditions of various environmental stresses. It was also stated that in some of plants, enriching the soil with silicon ions during drought stress increases the water absorption and maintains its high level in the leaves.

The aim of our studies was to investigate the influence of silicon ions supplemented with solution of orthosilicic acid or a commercial product *Optysil* in optimal conditions and under drought stress applied during growth of *Brassica napus* var. *napus* L. Total silicon concentration in shoots and biochemical analyzes of some aquaporins content were determined.

The analysis of silicon content in the aboveground part of investigated plants showed significantly higher content of this ions in the samples collected from plants watered with *Optysil* and orthosilicic acid in the relation to the control plants. The same tendency was observed in the conditions of drought stress. In addition, plants growing under drought stress showed significantly higher silicon content when compare with plants growing under optimal conditions. Biochemical analyzes showed the accumulation of PIPs aquaporins – BnPIP1 and BnPIP2-1-7 in the leaves of investigated plants. It was observed that supplementation of plants with silicon ions from *Optysil* significantly increases the accumulation of BnPIP1 and BnPIP2-1-7 in the leaves, when compare with samples collected from plants supplemented with orthosilicic acid. In addition, the accumulation of BnTIP1;1 aquaporin was higher in plants grown under optimal conditions than in plants kept under conditions of periodic water deficiency. Moreover, supplementation of plants with *Optysil* significantly lowered BnTIP1;1 aquaporin accumulation in both above-mentioned growth conditions.

Our studies showed that silicon ions significantly regulate accumulation of BnPIP1, BnPIP2-1-7 and BnTIP1;1, that might participate in the amelioration of drought stress.

Comparison of fluorescent lamps and light emitting diodes in terms of influence on growth and secondary metabolites production in plants from *Droseraceae* family

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Keywords: Droseraceae; secondary metabolites; LED; fluorescent lamps

Light is an essential component for the process of photosynthesis. Photosynthesising organisms utilise radiation in the wavelength range of 400–700 nm. Chlorophyll, the main plants' dye, absorbs blue and red light most efficiently. It is therefore assumed, that photosynthetically active radiation (PAR) covers the range of 400–550 nm and 620–700 nm. Light also affects plant metabolism. The illumination with narrow spectral bands in the range of blue light (430–500 nm) increases the production of various types of secondary metabolites (Stutte et al., 2009; Choi et al., 2013).

As standard, fluorescent lamps are used as a light source in specialised plant breeding rooms. The fluorophores emit light consisting of several narrow bands of various wavelengths. Their biggest disadvantage is the harmful effect on the environment through the content of mercury and high energy consumption. These problems do not occur when using light emitting diodes (LED). Low energy consumption is also associated with the cost reduction of the process of cultivating plants under controlled conditions. LEDs can emit light similar to sunlight, containing waves of all lengths in the range of visible light. What is more, LEDs emit much less heat than fluorescent lamps during operation, so they can be placed almost directly over bioreactors with plants, thus allowing for more efficient space management by growing plants on shelves of a lesser height.

At HerBioPharm, we measured light spectra from fluorescent lamps and LEDs. The measurement also provided information on colour temperature and PAR and PPFD values of light from both sources. Then, two species of plants from the *Droseraceae* family were grown on two variants of Murashige & Skoog medium (Murashige & Skoog, 1962) and after six weeks the increase in plant biomass was weighed, and the secondary metabolites were extracted and measured by HPLC. The results indicate that LEDs can replace fluorescent lamps in specialised plant breeding rooms.

■■■

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Verification of the engagement of cyclic electron transport (CET) in the salinity resistance

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Keywords: electron transport; halophyte; salinity stress; photosynthesis

In this work we verified whether an acclimation of photosynthetic electron transport to high salinity in the halophytic plant (*Mesembryanthemum crystallinum* L.) is associated with the induction of cyclic electron transport (CET). Salinity treatment led to the raise of PSI/PSII ratio and of the maximal redox change at PSI. These two cues suggest an increased importance of PSI for salinity resistance. However, this was associated by an intensified whole-chain electron transport, as shown by measurements with isolated thylakoid membranes. The engagement of chloroplast NAD(P)H dehydrogenase-like complex (NDH) in CET was monitored as the chlorophyll fluorescence rise in darkness following the adaptation to actinic irradiation. Salinity-evoked changes in the contribution of CET were also monitored by changes of NPQ during the induction of photosynthesis and by redox changes of P700 evoked by far red light. The activity and amount of plastid terminal oxidase (PTOX) was evaluated by western blotting and by the effects of PTOX inhibitor 2-octyl gallate. Altogether, our data suggests that acclimation of the photosynthetic electron transport to this extreme environmental conditions in ice plant is rather dependent on the improvement of the linear electron flux than of CET.

Reduction of *NtZIP5* expression level in tobacco (*Nicotiana tabacum*)

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Keywords: NtZIP5-RNAi; tobacco; ZIP; zinc; cadmium; metals translocation

Zinc (Zn) is an essential micronutrient. Cadmium (Cd) is a highly toxic heavy metal. Efficiency of Zn and Cd uptake and their translocation to shoots depend on mutual concentrations of both metals in the growth medium. Recent study suggested that NtZIP5 might be involved in the regulation of this phenomenon at the molecular level in tobacco.

The aim of this research was to generate tobacco lines with reduced expression of *NtZIP5* as a tool for examination of the role of *NtZIP5* in the regulation of Zn/Cd concentration-dependent root-to-shoot translocation.

To generate the tobacco lines with reduced levels of *NtZIP5* expression the RNAi technique was used. The 200bp (from ATG) of the ORF of *NtZIP5* was cloned into binary expression vector pK7GWIWG2 generating an intron spliced hairpin construct with antisense *NtZIP5*fragment – intron – sense *NtZIP5* fragment configuration downstream of the CaMV 35S promoter. Created construct *NtZIP5-RNAi* was used for stable tobacco transformation.

To initially characterize generated transgenic plants the *NtZIP5* expression level was determined in *NtZIP5-RNAi*tobacco lines. 5 week-old plants were grown in the control ¼ Knop's medium and under Zn-deficiency (no Zn was added to the control medium; *NtZIP5* is Zn-deficiency inducible) for 4 days.

It was shown that *NtZIP5* transcript abundance in tested RNAi lines decreased between 80% up to 10% of wild-type levels. Next, selected lines will be used for determination of Zn/Cd root/shoot distribution.

■■■

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The regulation of PSI and PSII enzyme kinetics in maize mesophyll chloroplasts by light intensity and quality

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Keywords: maize; state transitions; Michaelis-Menten's kinetics; PSI; PSII; light intensity and quality

State transitions is considered as short-time response of photosynthetic apparatus to variable intensity and quality of light which supports the excitation balance between PSI and PSII by the migration of LHCII antenna. In our work we used the photosystems as the enzymatic complexes (PSII is water-plastoquinone oxidoreductase, PSI is plastocyanin/cytochrome c6 to ferredoxin oxidoreductase) which carry out oxidation and reduction reactions using the solar energy, thus can be regarded as a Michaelis-Menten's type enzymatic reaction. The main interest was to understand how the light conditions affected PSI and PSII activity, and values of Michaelis-Menten's kinetic parameters: Km, Vmax and k2, also composition of the antenna proteins of LHCII in PSI-PSII- LHCI-LHCII supercomplexes were determined.

Plants were grown at irradiance of 80 (LL) and 800 (HL) µmol photons m^{-2} s^{-1}. Maize mesophyll chloroplasts were isolated from the leaves of plants from the light or after the dark period, and also after treatment one hour far red light (which is preferentially absorbed by PSI). Results indicated that all light conditions did not significantly affect the value of Vmax for PSI and PSII. Darkness induced strong decrease of Km values for both photosystems compared to the light. This indicate better capacity of the transfer absorbed excitation to the reaction centers of PSI and PSII. The PSI turnover time was 84 ms, independently to light conditions.

Besides, we identified three PSI-PSII-LHCI-LHCII supercomplexes, all containing Lhcb proteins (trimers and monomers), whereas PSI-LHCI-LHCII complex bound only the Lhcb1, 2 and 4 proteins.

We conclude that the state transitions do not change of photons absorption by the photosystems. But, must exist mechanism(s) which allow to increase the such ability for dark adapted chloroplasts, as it is explained by the decrease of Km value.

Chromatin-level differences suggest efficient metabolism as element of maize cold-tolerance

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Keywords: maize; cold; chromatin; tolerance

Stress limits the productivity of plants, so there is significant effort to bred tolerant crop plant varieties. Maize is a subtropical species and although it is now cultivated in the temperate climate its full potential is not realized there, despite intensive research in this field. It is known that maize lines differ in cold-tolerance and highly-tolerant lines exist. Uncovering genetic basis of cold-tolerance could lead to lines displaying both high-productivity and tolerance traits. Maize is an extraordinarily variable species at both phenotype and genome level, inbred lines differ in genome content, in terms of Presence Absence Variation and Copy Number Variation at the gene level. Eventually produced proteins have the greatest effect on the phenotype, mRNA and corresponding protein content correlation is low to medium. However, production of mRNA determines transcriptional potential, and its first prerequisite is physical accessibility of DNA for regulating proteins. Importantly, the level of DNA sequence can be regulated by a human.

The aim of our study was the determination of key differences in chromatin accessibility between two maize lines of contrasting cold-tolerance level grown in the cold. We studied tolerant Polish S68911 and susceptible B73 reference maize lines, first-leaf was sampled at three early growth stages: coleoptile (VE), the appearance of the second leaf, and mature first leaf (V1). Nucleosome Depleted DNA Regions – so accessible for transcription factors – was extracted according to Formaldehyde Assisted Isolation of Regulatory Elements method and sequenced (FAIRE-seq).

The tolerant line response to cold was twofold, developmental processes were boosted, as shown by potential expression of MADS and homeobox genes as well as activation of carbon metabolism and photosynthesis, what is surprising but confirm previous research on cold-tolerant lines. Also, activation of auxin reception and signaling and gibberellin signaling was observed. At the other side, in the tolerant line stress-response processes were activated, i.e., abscisic acid and jasmonate biosynthesis as well as expression of several AP2/EREBP transcription factors. Also, Reactive Oxygen Species scavenging was activated. The susceptible line showed mainly activation of expression of developmental genes, with only a few AP2/EREBP genes potentially expressed at the V1 stage. Also in this line auxin signaling seems to be repressed.

Taken together, it seems that several components are responsible for high cold-tolerance of S68911 maize line. The key to withstanding the cold-stress could be a balance of developmental and defense processes. Some transcription factors identified in protein-protein interaction networks were upregulated at the transcript level in tolerant-lines in our previous experiments, and those are good targets for further studies.

Is it possible to detect macronutrient deficiency before its appearance?

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Keywords: chlorophyll a fluorescence; photosynthesis; nutrient deprivation; transpiration; maize

The aim of the research was to compare the effect of short-term deprivation of selected macronutrients (Ca, K, Mg and P) on the yield of the photosynthetic apparatus, transpiration and pigment content in maize (*Zea mays*). Seedlings were cultivated in a hydroponic culture on a full-strength Hoagland medium for two weeks after which they were transferred to a medium without the selected macronutrients for another week (nutrient deprivation stress). The strongest inhibition of both the light and dark phases of photosynthesis was caused by a deprivation of Mg, which was visible as a decrease in the photosynthetic and transpiration rates (ca. 75 and 85% of the control, respectively), stomatal conductance (80% of the control), photosystem II (PSII) performance, chlorophyll and flavonol content with a simultaneously increased content of anthocyanins (300% of the control). In the K-deprived plants, a decrease in the photosynthetic rate was observed. However, the transpiration rate and stomatal conductance did not differ significantly compared to the control. The PSII performance under K starvation was similar to that for the Mg-deprived plants, which was observed as a decrease in, e.g., the electron transport flux (85% of the control), the percentage of active reaction centers (82% of the control) and the plastoquinone pool. In the K-deprived plants, a decrease in chlorophyll (75% of control) and an increase in the anthocyanin content (340% of the control) were also found. We showed that Ca starvation resulted in a decrease in the photosynthetic and transpiration rates, stomatal conductance and PSII performance, while the pigment content was not significantly affected. In the case of P-deprived plants, we observed a decrease in the photosynthetic and transpiration rates. Interestingly, the inhibition of stomatal conductance was the strongest in the P-deprived plants compared to all of the investigated elements (75% of the control). However, the performance of PSII was not significantly affected by P starvation compared to the control. The measurements of Ca, K, Mg and P concentration in shoots showed that 7 days deprivation of these macronutrients did not caused their deficiencies in plants. Thus, presented results suggest that described above parameters may be used as the physiological markers of the beginning of selected macronutrient deficiencies. A simultaneous comparison of the effect of Ca, K, Mg and P deprivation on the chlorophyll *a* fluorescence, photosynthetic and transpiration rates, stomatal conductance and chlorophyll, anthocyanin and flavonol content was performed on maize plants for the first time.

Germin – like protein from *Mesembryanthemum crystallinum* (McGLP) exhibits superoxide dismutase activity and enhances resistance to dehydration

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Keywords: germins; germin-like proteins; dismutase; stress responses; dehydration

Germins and germin – like proteins (GLPs) constitute large and diverse family of plant proteins involved in numerous physiological processes like germination, growth and development and stress responses. Different functions of germins and GLPs are linked to specific enzymatic activity, e.g. oxalate oxidase (OxO) or superoxide dismutase (SOD). Being involved in diverse physiological events germins and GLPs are considered promising subjects of research to increase, by genetic manipulations, the yield of crops and other useful plants. *Mesembryanthemum crystallinum* L. (ice plant) is a model for studying stress-tolerant mechanisms in higher plants. Among stress-responsive ice plant genes, the expression of GLP gene (*mc_glp*) has been proved in roots, but the enzymatic activity of encoded protein and its biological function have not been studied so far. The aim of our study was to purify McGLP, determine its enzymatic nature, subcellular localization and effect on selected aspects of plant physiology. We amplified *mc_glp* using cDNA as a template and successfully incorporated it in pMCSG19 plasmid using Ligation Independent Cloning (LIC). Sequencing of PCR product revealed additional cytosine at 590 position in comparison with previously published report. In a result of altered reading frame a shorter McGLP protein was expressed *in vivo*. We obtained an effective heterologous *Escherichia coli* based system for McGLP expression as a fusion with maltose-binding protein (MBP) after several rounds of testing different expression conditions. Downstream processing enabled us to obtain single fraction of pure protein that was further submitted to biochemical assays. Spectrophotometric measurements and activity staining of purified McGLP excluded OxO activity, but confirmed its MnSOD nature. In order to determine the effect of McGLP on plant physiology, the *mc_glp* was overexpressed in *Arabidopsis thaliana*. The transgene was highly expressed in *Arabidopsis* plants and McGLP-GFP fluorescence showed punctate distribution throughout the cytoplasm with preferential localization in cell wall and intercellular spaces. When grown in well-watered soil the transformants showed similar growth rate to WT plants but produced more branched shoots and roots with longer and more abundant root hairs. No differences in physiological parameters like chlorophyll content or photosynthetic efficiency were observed between WT and transformants

growing under non-stress conditions. However after exposure to drought the transformants grew faster and produced higher shoots with bigger leaves with more chlorophyll than WT plants. Transgenic plants also displayed markedly higher photosynthetic efficiency as well higher total antioxidant capacity under stress conditions. Overall, our findings indicate that overexpression of *mc_glp*provides protection against dehydration. The question as to whether its beneficial effect relay on SOD activity of encoded protein needs to be elucidated in future.

Dark chilling induced changes of phosphorylation of LHCII proteins of thylakoid membranes in chilling sensitive and chilling tolerant plant species

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Keywords: LHCII; phosphorylation; chilling stress; pea; runner bean

Low temperature is one of the abiotic stresses that affect plant growth and productivity. Plants are divided into chilling-sensitive (CS) plants, susceptible to temperatures below 12°C, and chilling-tolerant (CT) plants, resistant to low but non-freezing temperatures. A third group, freezing-tolerant plants, acquired frost tolerance after a period of exposure to low, non-freezing temperatures. The chilling impact causes a decrease in the photochemistry of lowering Calvin cycle enzyme activity and decrease in PSII activity, as well as an increase of ROS. Dark chilling of CS plants decreases the stability of LHCI-PSI supercomplexes as well as trimeric LHCII and increases free fatty acids level. The inhibition of photosynthesis by chilling is quite well described, however little is known about the state transitions mechanism under chilling stress. Aim of this study was to determine the role of LHCII antenna complex phosphorylation in dark chilling response. The study included an experimental model based on 3-day long dark chilling at 4°C of detached CS runner bean (*Phaseolus coccineus* L.) and CT garden pea (*Pisum sativum* L.) leaves. This model is well described in the literature as used for the analysis of chilling impact without any additional effects caused by light. Analysis of LHCII phosphorylation level changes was performed by modified Phos-tag™ SDS-PAGE electrophoresis followed by Western blot. Interactions between phosphorylated LHCII and photosynthetic complexes were determined by low-temperature (77K) chlorophyll fluorescence and two dimensional Blue Native-SDS PAGE electrophoresis, followed by ProQ Diamond and SYPRO Ruby staining. The PSII photochemical efficiency was monitored by measuring in vivo chlorophyll a fluorescence parameters. Our results indicate that different responses of the LHCII phosphorylation to chilling stress take place in CT and CS plants, and that kinetics of LHCII phosphorylation and interactions of phosphorylated LHCII with photosynthetic complexes may be crucial to chilling stress response.

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Overexpression of triticale proline aminopeptidase gene (*TsPAP1*) enhances the tolerance of Arabidopsis transgenic plants to abiotic stress factors

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Keywords: proline; prolyl aminopepidase; stress tolerance; triticale

Prolyl aminopeptidase (PAP; EC 3.4.11.5) is an enzyme that specifically removes proline or hydroxyproline from the N-terminus of short peptides, however, knowledge about physiological functions of PAP in plants is very limited. Due to the osmoprotective properties of proline, and its contribution in the stabilization of proteins and sub-cellular structures, as well as the regulation of intracellular redox potential, the participation of PAP in the response of plants to stress factors was suggested. In our previous studies, an increase of the prolyl aminopeptidase activity as well as the expression of an prolyl aminopeptidase gene, *TsPAP1*, was observed in triticale seedlings in response to drought, salinity, and the presence of metal ions in the nutrient medium.

To further examine the role of *TsPAP1* under stress conditions, we developed transgenic Arabidopsis plants overexpressing *TsPAP1*. The hygromycin resistant Arabidopsis seedlings (12-day old seedlings of T_4 progeny) were plated on a ½ Murashige and Skoog (MS) medium or on a ½ MS medium containing 125 mM NaCl, 75 mM mannitol, 150 μM $CdCl_2$, 500 μM $ZnSO_4$ or 20 mM $LiCl_2$, and the adequate analyses were performed after 6 days of the transfer. Under control conditions, the wild type plants and the transgenic lines exhibited similar biomass production, chlorophyll content and abscisic acid content. Under stress conditions, however, the transgenic lines revealed higher biomass production, higher chlorophyll content and lower abscisic acid content. The transgenic lines demonstrated also a several times higher prolyl aminopeptidase activity and twice higher proline content, both under control as well as under stress conditions. The results indicate that *TsPAP1* is involved in the regulation of proline content not only under physiological conditions but also in response to various abiotic stress factors, contributing to enhanced tolerance of plants under unfavorable growth conditions.

May stem photosynthetic apparatus provide an effective alternative to leaves in acclimatization strategy to salinity stress (NaCl) in non-halophytes plants? – the case of grasspea

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Keywords: *Lathyrus sativus*; photosynthetic apparatus; RC PSII; ROS; salt stress

The rapid climate changes observed in recent decades lead to the expansion of areas with disturbed water management. It results in an increase of areas with high NaCl concentration, what leads to salt stress in plants. Salt stress (40 mM NaCl according to FAO, 2015) in sensitive plants (glycophytes) first disturbs the water management of the plant leading to imbalance of the plant osmotic homeostasis (Fast Phase). Subsequently, salinity causes ionic stress (Ion Phase) leading to disruption of nutrition balance, damage of enzymes and biological membranes leading to a rapid metabolism reduction. The most sensitive metabolic processes to high salt concentration are mitochondrial respiration and photosynthesis. Disorders of photosynthesis are associated with: 1) the closure of the stomata (Fast Phase) which leads to a decrease of CO_2 concentration what causes photorespiration; 2) a direct toxic effect on the photosynthetic apparatus and a thylakoids membranes (Ion Phase) – substitution of divalent cations (Ca^{2+}, Mg^{2+}, Mn^{2+}) by Na^{+} which results in a disorder of the linear electron transport leading to ROS formation and photooxidation. The consequence is a photosynthesis rate decrease, which in conditions of prolonged stress leads to the plant death. In contrast, resistant plants (halophytes), due to adaptation mechanisms, are tolerant to high NaCl concentration. Grasspea (*Lathyrus sativus*) is a moderate salinity tolerant crop. Previous studies showed that a low concentration of NaCl has a stimulating effect on this species. While, NaCl concentrations above 50 mM led to a progressive chlorosis of leaves resulting in the photosynthetic apparatus degradation. In contrast, plant stems remained green.

Therefore, the aim of presented study was to compare the content of the photosynthetic apparatus key elements in the leaves and stems in the context of grass pea acclimatization to salinity stress.

Our study indicated that the stem photosynthetic apparatus of plants treated with 100 mM NaCl was characterized by reduced size of the photosynthetic antenna, maintained amount of Photosystem II Reaction Center elements and simultaneously high levels of electron membrane transporters. Such structure limited the risk of ROS generation, while providing conditions for effective carboxylation of CO_2 in these organs.

Contribution of GLR channels in the regulation of Arabidopsis seedlings growth

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Keywords: GLR channels; photomorphogenesis; seedling growth; skotomorphogenesis

GLRs are plant homologs of animal ionotropic, glutamate receptors, which are ligand-gated amino acid-activated ion channels. The *Arabidopsis thaliana* genome contains 20 sequences of GLR channels. Upon binding to the channels, ligand amino acids activate influx of ions, in particular Ca^{2+}, into the cytosol. Animal iGluRs are divided into three families: NMDA, AMPA and kainate receptors. In plant studies, inhibitors of animal AMPA channels were mainly used. Early studies suggested that inhibition of hypocotyl growth by light is partially blocked by GLR antagonists. The aim of this work was to assess the role of two types of GLR receptors in photomorphogenic and skotomorphogenic growth of seedlings. We tested hypocotyl and root growth in the presence of inhibitors of NMDA and AMPA channels, MK-801 and CNQX respectively. Root growth was inhibited by CNQX and, to some extent, promoted by MK-801 in both, light and dark conditions. In light, the hypocotyl growth was inhibited only by CNQX, whereas in in the dark it was stimulated by MK-801 similar as in roots. Our research indicates that different GLR channels take part in photomorphogenic and skotomorphogenic growth of Arabidopsis seedlings.

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Treatment with chemical inhibitors and transcriptional response of HpCDPK1 kinase during mechanical injury of various *Hippeastrum* x hybr. tissues leading to phytoalexine synthesis

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Keywords: mechanical injury; CDPK; calcium; phytoalexine

Phytoalexin (PA) from *Hippeastrum* plant belongs to the group of isoflavone compounds as one of the secondary metabolites formed on the phenylpropanoid pathway. Synthesis of PA is strongly induced by mechanical injury of *Hippeastrum* tissues. The intracellular signal transduction pathway activated after wounding and leading to the production of PA is still unknown. Calcium-dependent kinases (CDPKs) are plant Ca^{2+}signal receptors, which participate in the regulation of various growth and development processes as well as different stress responses. Our previous research showed possible link between cytosolic Ca^{2+} signaling, including CDPK kinases, and the control of PA production in wounded *Hippeastrum* bulbs.

The aim of this report was to examine the participation of HpCDPK1 kinase gene and protein in the response to the wounding stress which results in PA formation. Therefore, we analyzed: (1) the effect of selected inhibitors of CDPK kinases on the rate of PA synthesis in damaged tissues; and (2) dynamics of *HpCDPK1*transcript level changes in wounded leaves, roots and flower shoots. Treatment of *Hippeastrum* cut bulbs with chemical inhibitors allowed for the indirect determination of the participation of active HpCDPK1 protein in controlling PA synthesis. This was necessary to do due to the inability to obtain transgenic *Hippeastrum* plants. In our study, four chemical compounds–trifluoperazine dihydrochloride (TFP), calmidazolium chloride (CMZ), N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7), and chlorpromazine hydrochloride (CPZ) – were used, with two concentrations of 100 μM and 200 μM for each. Measurements of the amount of PA showed that the individual inhibitors had different inhibitory effects on the amount of PA, often depending on the concentration used. Therefore, we showed that the HpCDPK1 kinase may be a regulator of phytoalexin synthesis in the bulbs of *Hippeastrum* at a given time point after injury. Moreover, the analysis of *HpCDPK1*transcript level in wounded roots, leaves and flower shoots of *Hippeastrum* revealed that the profile of the examined mRNA changes was dependent on tissue type and time point after wounding stress, proving that HpCDPK1 kinase is also active in those organs where phytoalexin synthesis occurs at a lower level than in bulbs.

Based on the results of these studies, it seems that both HpCDPK1 kinase protein and gene can be important elements of intracellular signaling pathway during mechanical injury of *Hippeastrum* tissues leading to phytoalexin synthesis.

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The effect of decreased expression of different RBOH isoforms on the metabolism in *Arabidopsis thaliana* under ammonium nutrition

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Keywords: ammonium syndrome; NADPH oxidase; reactive oxygen species

Plants cultured on ammonium ions (NH_4^+) as the only nitrogen source in the soil solution show stress symptoms like growth inhibition and developmental disorders known as "ammonium syndrome". Changes in the metabolism of reactive oxygen species (ROS) in the apoplastic space can have an impact on the cell wall structure and a variety of processes connected with plant growth and cell cycle. Respiratory burst oxidase homologues (RBOHs) are the main enzymes which participate in increased ROS production in the apoplastic space under stress conditions. Plasma membrane NADPH oxidases in *Arabidopsis thaliana* are encoded by 10 genes (*RbohA-RbohJ*). The main RBOH isoforms (RBOHD and RBOHF), which are known to be the most expressed isoforms in *Arabidopsis* leaves, were analysed in the studies. In addition, also RBOHC, RBOHG, RBOHE, RBOHI isoforms were selected for further examination.

The aim of this study was to analyse the effect of decreased expression of various RBOH isoforms (RBOHD, RBOHF, RBOHC, RBOHG, RBOHE, RBOHI) on ROS metabolism and the growth of *Arabidopsis thaliana*under ammonium nutrition.

The growth abilities, ROS content and low molecular mass antioxidant concentration were examined in all tested mutants grown on NH_4^+ as the sole nitrogen form. During the ammonium treatment, a difference in the length and a number of lateral roots in tested mutants were discovered. Our studies showed that the decreased expression of RBOH isozymes affects the apoplastic ROS metabolism under ammonium treatment. Obtained results indicate that the expression of RBOHD and RBOHF isoforms is an important factor of ROS production in the apoplastic space under ammonium nutrition.

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Phytohormones synthetized in tomato (*Solanum lycopersicum* L.) under oxygen deprivation in the root zone

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Keywords: waterlogging; hypoxia; tomato; phytohormones

Phytohormones participate not only in the regulation of plant development, but also mediate between the perception of environmental stimuli and the plant's responses to these stimuli. They are transported from the place of their synthesis to place where they reveal their activity. Under hypoxia stress, phytohormones are involved in i.e. aerynchyma and adventitious root formation, shoot elongation and stomatal closure (Bashar et al., 2019).

The aim of the study was to determine the changes in levels of phytohormones involved in response to excess water in the root zone. The content of auxins, cytokinin, abscisic acid and 1-aminocyclopropanecarboxylic acid (ACC) were estimated in roots, hypocotyls and leaves of two tomato genotypes: POL 7/15 and PZ 215, described as sensitive and tolerant to excess water conditions, respectively.

Tomato plants were exposed to the hypoxia stress by flooding for 7 days, then plants recovered for 14 days and another 7 days of the hypoxia treatment was applied. Root, hypocotyl and leaf samples were collected at 0, 1, 2, 3 and 7 day of first and second hypoxia treatment. Auxins (IAA, IBA), ACC and ABA were determined according to the procedure of Dziurka et al. (2016). Results showed differences in phytohormones concentrations in response to hypoxia in different parts of plant. The increased level of ACC in the leaves was observed after 24 hours of the first hypoxia treatment in tolerant genotype in contrast to roots, whereas the ACC content in sensitive genotype was at the same level in control and treated plants in roots and leaves. The highest level of auxin (IAA) was estimated in roots of both genotypes under second hypoxia treatment at 72 h time point, whereas the highest level in leaves was only observed in sensitive genotype at 48 h time point. No changes in level of IAA were observed in leaves of tolerant genotype. The content of ABA was higher in leaves of treated plants in comparison to roots in both genotypes.

Many processes occurring in plants depend on the ratio of concentrations of diverse growth and development regulators, so complex analysis of biosynthesis and transport of phytohormones from roots to leaves is needed to understand the regulatory function of phytohormones in response to hypoxia stress.

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Various exogenous purine and pyrimidine nucleoside 5'-phosphoramidates (NH_2-pNs) induce phenylpropanoid pathway leading to biosynthesis of stilbenes and lignin in *Vitis vinifera* cells suspension culture

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Keywords: nucleoside 5'-phosphoramidates; phenylpropanoid pathway; *Vitis vinifera*; stilbenes

Adenosine 5′-phosphoramidate (NH_2-pA), one of the nucleoside 5′-phosphoramidates (NH_2-pNs), was first discovered in *Chlorella pyranoidosa* [1]. Its biochemistry and function are poorly understood. In high plants NH_2-pA can be synthetized by Fhit/adenylylsulfate:ammonia adenylyl transferase [2, 3]. The supposition that NH_2-pA occurs in other organisms and that its concentration can be enzymatically controlled is supported by the existence of nucleoside-5′-phosphoramidate hydrolase activities. NH_2-pA can be hydrolyzed by at least two enzymes: dinucleoside triphosphatase/Fhit protein and nucleoside 5'-phosphoramidate hydrolase/Hint1 protein [4–6]. Our studies showed that exogenous NH_2-pAadded to *Arabidopsis* seedlings induced expression of several genes of phenylpropanoid pathway and caused accumulation of lignin, 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. We wondered if induction of phenylpropanoid pathway by NH_2-pA is a common phenomenon in plants and if NH_2-pN other than NH_2-pA can also enhance the biosynthesis of phenylpropanoids such as stilbenes (*t*-R, *trans*-resveratrol and its glucoside, *t*-P, *trans*-piceid), lignin as well as phenolamides, conjugates of the phenolic acids with amines, which can also lead to lignin synthesis and are involved in response to pathogen infection [8]. We studied the effect of 5 microM NH_2-pA, NH_2-pG, NH_2-pU or NH_2-pC on the expression of the following genes of phenylpropanoid pathway in *Vitis vinifera* cv. Monastrell cells: *phenylalanine ammonia-lyase* (*PAL1*), *cinnamate-4-hydroxylase* (*C4H1*), *4-Coumarate:CoA ligase* (*4CL1*), *stilbene synthase* (*STS1*), *caffeic acid 3-O-methyltransferase* (*CCR*) and *cinnamyl alcohol dehydrogenase* (*CAD*). In addition, we have checked if the applied compounds induce gene expression of *ATP binding cassette (ABC) transporter* (*VvABCG44*) that is involved in the transport of *t*-R. The results obtained in this work show that NH_2-pNs evoke in *V. vinifera* cells the induction of *t*-R synthesis which is a result of the induction of relevant

genes of phenylpropanoid pathway. In particular, NH_2-pC affects the 12-times higher than in controls expression of the *STS1* gene, encoding the enzyme catalazing *t*-R synthesis. NH_2-pNs enhanced also expression of *VvABCG44* transporter gene and that results in accumulation of *t*-R in spent medium.

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The expression of *RPB1* the largest subunit of RNA polymerase II in mesophyll cells of *Arabidopsis thaliana* during submergence and after recovery of aerobic conditions

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Keywords: hypoxia; RNA polymerase II; transcription

Modulation of transcription is crucial in acclimatization of plants to abiotic stress. The core gene involved in the stress response usually undergoes increased the transcriptional activity, while some of the others may be silent. Here, the RPB1 the largest RNA polymerase II subunit gene expression as a key of transcription factor was studied.

qPCR results indicate that the amount of *RPB1* mRNA is dependent on the availability of oxygen. After one and five days of hypoxia, the amount of transcripts drops sharply compared to the control of conditions and the reoxygenation stimulation of the increase level of mRNA *RPB1*. Next analysis of retained introns in *RPB1* mRNA was performed using PCR. The results suggest the retention only of 12 intron. Additional the possibility of alternative polyadenylation sites in 3 and 11 introns was analysed.

To study the transcriptional activity we measured quantity of the phosphorylated ser2 in CTD of Pol II RNA (the elongated form of RNA Pol II) in the nuclei of the mesophyll cells. During 1 and 6h of hypoxia, there was increase the process of the transcription. However in the stages with a reduced amount of *RPB1* mRNA also decreased the amount of the active RNA Pol II. The transcription level returned to the level that was observed in normoxia conditions after removal of the stress. Subsequently the efficiency of RNA polymerase II was study by detecting and measuring the amount of poly(A) RNA. In the most stages level of poly(A) RNA correlates with the transcription. However, despite the increase in the amount of RNA pol II after 6h reoxygenation, the increase of poly(A) RNA was observed in the next stage. The results obtained reveal that the expression of the *RPB1* gene as well as the transcription i s strongly regulated during hypoxia and after removal of the stress.

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Genome-wide identification of calcium-dependent protein kinases (CDPKs) in *Solanum tuberosum*, their expression profiles in organs and in response to abiotic stresses

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Keywords: *Solanum tuberosum*; calcium-dependent protein kinases; abiotic stresses

One of the earliest plant reaction to various environmental stresses is transient increase in the concentration of calcium ions (Ca^{2+}) in the cytoplasm. Calcium-dependent protein kinases (CDPKs) are both sensors as well as effectors of these changes in Ca^{2+}concentration. They are involved in growth and developmental processes as well as in defence strategy against different environmental stresses. CDPKs, unique for plants, are encoded by multi-gene families. Despite extensive studies of *CDPK*s in many species, still little is known about this gene family in the important crop – potato (*Solanum tuberosum*).

We have performed bioinformatics analysis of the potato whole genome sequence and identified 23 potential *CDPK* genes. The phylogenetic relationships and expression profiles of the *CDPK* genes were estimated.

Identified *CDPKs* were divided into four subfamilies based on a phylogenetic tree and gene structures. QPCR expression analysis was carried out for the *CDPK* genes in nine different organs of potato such as young and mature leaves, stems, young shoots, roots, stolons, swollen stolons, flowers and tubers. Transcripts of all 23 *CDPKs* were present in all the samples analysed, although their level varied greatly. The organs differed from one another not only in the overall level but also in the spectrum of the expressed *CDPKs*. Moreover, we performed gene expression profiles of *CDPKs* in potato plants in response to abiotic stressses: mechanical wounding, salinity and drought. Expression of *CDPK*increased in response to abiotic stress although both the level and kinetics of their expression differed between leaves and roots as well as between individual stresses. In roots subjected to the salt stress or drought, we observed an increase in the expression level of the same *CDPKs*: *StCDPK2/4/5/11/12/15/17* and *23*. Such a similarity was also noted in leaves subjected to salt stress and wounding. In this case transcript of *StCDPK2/10/16* was mostly pronounced.

The results presented here would be helpful for the better understanding of the evolutionary history and general biological roles of the potato CDPK family.

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Antioxidative mechanisms involved in carrot response to soil salinity

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Keywords: carrot; salinity; ascorbic acid; glutathione; proline

Salinity is a major problem in crop production faced by farmers in modern agriculture. Due to climate changes and necessity for land irrigation the situation may deteriorate further. Carrot is important vegetable not only due to economic reasons – Poland is one of the leading producers in the world; but also from a consumer point of view – it is the main source of provitamin A in human diet. Unfortunately carrot is susceptible to soil salinity. Reactive oxygen species (ROS) are created continuously as metabolic products involved in cellular signalling and can be neutralized by antioxidative compounds such as phenolics, glutathione or ascorbic acid. Under stress condition the balance between ROS formation and scavenging can be disturbed which leads to impairment of cell function and in severe cases may cause plant death.

Two carrot varieties, a salt-sensitive doubled haploid line (DH1) and a salt-tolerant variety DLBA were grown in control (0.2 $dS \cdot m^{-1}$) and saline soil (3 $dS \cdot m^{-1}$). After harvest plants were subjected to biometric measurements and biochemical analyses. The content and profile of phenolic compounds was determined, as well as proline, glutathione and ascorbic acid contents.

The differences in antioxidant response to salt stress were observed between varieties and their organs. The leaves of both varieties had a higher concentration of phenolic compounds than the roots, although in the green organs of plants growing under salt stress the concentration of phenolics decreased. Also the total content of phenolics in a salt-sensitive DH1 was much higher than in a salt-tolerant DLBA indicating that these compounds do not play essential role in carrot tolerance to salinity. In both varieties glutathione content increased in roots exposed to salinity, yet the quantity of glutathione was the highest in a salt-tolerant DLBA. The most notable changes were observed in the leaves of salt-sensitive DH1 plants where glutathione content decreased almost 10-fold in comparison to the control plants. In both varieties and organs proline content increased under salt stress – in DLBA the proline levels were 4 and 7-fold higher than in control in leaves and roots, respectively while in DH1 the proline levels were 3 and 2-fold higher in these organs. In roots of DLBA and DH1 ascorbic acid content was higher in plants exposed to salinity, whereas in leaves it decreased, especially in DH1 plants where the observed value was 5-fold lower under salt stress.

The obtained results suggest different antioxidative response to salinity in tolerant and sensitive carrot varieties. Potentially, in carrot tolerance to salt stress the key role may play proline as well as efficient operation of the ascorbate-glutathione cycle.

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Genome-wide characterization of polymorphism and gene expression of barley near-isogenic lines

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Keywords: barley; gibberelin regulation; gene expression; termal stress; semi-dwarfism

The reported research concerns molecular mechanisms responsible for production and regulation of gibberellins in barley at tillering stage. We are interested in explanation of pleiotropic action of the semi-dwarf *sdw1* gene encoding oxidase GA-20, determining the juvenile growth habit (erect or prostrate), and influencing many other morphological, anatomical and physiological traits. The study is based on the variety Bowman (wild type) and its two near-isogenic lines BW827 (*sdw1.a*) and BW828 (*sdw1.d*). Any functional disorder in essential enzymes of GA biosynthesis can affect the plant morphology. Hence, effects of both genetic background and environmental conditions on plant stature were investigated. All three genotypes were characterized with respect to the genomic sequence of the *sdw1* region and the genome-wide polymorphism obtained by 50k Infinium iSelect SNP genotyping. Their gene expression profiles, phenotypic properties and physiological parameters were studied at early developmental stage under normal conditions and under increased temperature. In addition to the major yield components observation, the in-depth characterisation of peduncle and internodes of primary and secondary stem was performed. All this provided a novel information on characterization of the studied lines. Integration of obtained data provided a solid background for further studies on regulation of expression of potentially involved genes.

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The different composition of thylakoid lipids and proteins in pea and bean determines the type of structural rearrangements induced by the dark-chilling treatment

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Keywords: thylakoid membranes; chilling stress; pea; runner bean

The chloroplast thylakoid network, a site of photochemical reactions, forms an intricate spatial structure assembly of proteins, polar lipids, and pigments. This system evolved multiple regulatory mechanisms which can trigger dynamic rearrangement of the chlorophyll-protein (CP) complexes in response to diverse environmental factors. One of the important factors affecting the efficiency of photosynthesis in a temperate climate is low temperature. We examined two plant species: chilling sensitive runner bean (*Phaseolus coccineus* L.) and chilling tolerant pea (*Pisum sativum* L.) in order to determine changes in protein and lipid composition and their impact on thylakoid network structural dynamics under dark-chilling conditions. We found out that the dark-chilling treatment of bean leaves induced substantial changes in lipid and protein composition of thylakoid membranes including the association of soluble proteins with thylakoid membranes and the increase of LHCII proteins phosphorylation. These changes were accompanied by partial dispersion of grana structure and the increase of unstacked thylakoid area. On the contrary, in pea, no significant changes in protein and lipid composition were observed and the grana membranes maintain their native structure. Analysis of membrane dynamics by means of FTIR and fluorescence spectroscopy showed a decrease of protein aggregation under dark-chilling conditions in pea. In bean, the opposite effect was observed. In conclusion, in pea dark-chilling induced changes of protein and lipid composition, and of CP complexes interactions eventually leading to the stabilization of spatial thylakoid structure, while in bean the reverse effect was noticed.

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Unravelling novel intracellular trafficking regulators involved in plant stress response

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Keywords: vesicular trafficking; intracellular localization; stress response and adaptation

The sessile life style of plants creates the need to utilize a diverse set of mechanisms to maintain homeostasis in changing environment. Endosomal trafficking is vital for plant development both in optimal and stress conditions. Short-term extracellular osmotic treatments are followed by a shift in the balance between endocytosis and exocytosis in root meristem cells acting through clathrin machinery. Moreover, stress conditions result in elevation of reactive oxygen species (ROS) production. The oxidative stress has an impact on subcellular trafficking and localisation of plasma membrane proteins vital for plant development, among them PIN auxin efflux carriers by affecting endocytic recycling. We have shown that regulated vesicle trafficking is necessary for membrane integrity preservation and therefore for plant resistance to acute osmotic stress. We also reported that intracellular trafficking of PIN2 protein during adaptation to oxidative stress requires the function of the ADP-ribosylation factor (ARF)-guanine-nucleotide exchange factor (GEF) BEN1. Characterisation of the role of BEN1 and other intracellular trafficking regulators in cellular stress response is a main focus of our further research.

Anti-ageing action of NO in warm stratified apple seeds

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Keywords: *Malus domestica* Borkh; nitric oxide; seed ageing; seed dormancy; seed viability

Seed dormancy allows seed to overcome unfavorable conditions for establishment of young seedlings, therefore this feature is important in plant ecology and agriculture. Apple (*Malus domestica* Borkh.) seeds are characterized by a deep embryonic dormancy, which may be overcome by 3 months long cold (5°C) stratification (Lewak 2011). Seed ageing and viability are affected by a number of factors during seed maturation and storage. It was demonstrated that warm (25°C) stratification of dormant apple seeds resulted in ageing leading finally to their death (Dębska et al. 2013). Nitric oxide (NO) is regarded as a regulatory agent in various physiological processes in plants, and particularly acts as a dormancy removal factor, stimulating seeds transition from dormant to non-dormant stage. NO is known also as a protective compound in plants exposed to different, harmful environmental conditions. Apple seeds were stratified at warmth for 7, 14, 21 or 40 days. Such treatment led to artificial ageing of the seeds, while short term (3 h) fumigation with NO was used for restoration of seeds vigor. We determined seeds germination after various period of warm stratification followed by NO treatment. Seeds quality was assessed as the loss of viability by TTC tests and NBT staining. The additional aim of our work was to examine in the embryonic axes the level of proteins considered as longevity markers, to verify the hypothesis of beneficial effect of NO in preservation of artificial seeds aging.

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The nature and the scale of plant root alterations in response to trace elements toxicity

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Keywords: arsenic; heavy metal; cell wall; clearing technique; vesicular transport

We analyzed root alterations in two tree species, *Tilia cordata* and *Acer platanoides* growing 3 months on mining sludge containing extremely high concentrations of TE, i.e. As 18022, Cd 1030, Cu 4511, Pb 3865, Tl 669, Zn 1565, Cr 1260 [mg kg^{-1}] dry weight (DW) as well as in *Populus tremula x tremuloides* and *Arabidopsis thaliana* exposed to Pb (1mM,4h aqueous solution) in laboratory conditions.

Combining clearing and confocal laser microscopy analysis allowed us to demonstrate, the nature and the scale of the root apex alterations in *T. cordata*. We observed the absence of the root cap, a reduction in the size of the root apex zones and an irregular arrangement of the cells building the root apex tissues, the occurrence of vascular tissues abnormally close to the root apex, and the collapse of the internal tissues. Interestingly, clearing of *A. platanoides* roots was not successful. We suppose that the markedly higher accumulation of TE in roots of this species (As – 541, Cu- 1300; Pb -55 mg kg^{-1} DW) than in *T. cordata* (e.g. As 541, Cu, 1300; Pb 55mg kg^{-1}DW) hampered clearing of *A. platanoides* roots The high TE content in CWs could make the roots of *A. platanoides* impermeable for reagents. Opposite to this, the roots of plants exposed to Pb in laboratory conditions did not show any visible alterations in their architecture.

At the cellular level we focused predominantly on the alterations in root cell structure which can be considered as symptoms of defence strategies to TE, such as the increase of CW thickness and the formation of local CW thickenings that are particularly abundant in low-methyesterified pectins (up to 40%), the CW compound predominantly binding and immobilizing toxic TE. It is worth noting that similar CW thickenings were detected in all tested plant species both growing on mining sludge and in laboratory conditions. Moreover, in the protoplasts of all examined plant species we observed highly active vesicular transport. Numerous transporting vesicles containing Pb deposits, evidenced by X-ray microanalysis, were detected near CWs and CW thickenings.

The obtained results show that root alterations in plants exposed to mining sludge containing a mixture of TE, over long time exposure, are much more severe than in response to Pb in laboratory conditions. In both cases, however, plants cope with TE using a similar defence response which leads to an increase of plant CW capacity for TE sequestration. The defence strategy is also involved in the intensification of vesicular transport. It suggests that TE, already present within the endomembrane system, can be removed from the protoplast by the secretion pathway and sequestered in CWs and their thickenings

■■■

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Response of common tree species to organic arsenic – a dendroremediation trial

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Keywords: arsenic; dimethyloarsinic acid; organic acids; phenolics; tolerance

Arsenic (As) is a redox-active metalloid existing in the environment on four oxidation states and in organic and inorganic forms, and As(V) (arsenate), As(III) (arsenite), dimethylarsinic acid (DMA(V)), and monomethylarsonic acid (MMA(V) are the most abundant. The interest on arsenic is related to its high toxicity, and mainly inorganic forms of the metabolite are considered to be extremely toxic. Recently, organic As has gained a considerable attention due to its probably higher toxicity to plants and human beings. Formation of organic As in the environment occurs via methylation of its inorganic forms by soil microorganisms and some plants. Additionally, organic As(V) was a component of herbicides used in the 20th century and is therefore accumulated in soil.

The aim of the study was to evaluate the effect of cocadilic acid (DMA(V)) on growth and selected stress-related metabolites of three woody species, i.e. *Acer pseudoplatanus*, *Betula pendula* and *Quercus robur*. Speciation analysis of As was also performed to asses As conversion to other forms showing diffrent toxicity thresholds and mechanisms.

Two-year-old plantlets of comparable size were used in hydroponic experiment. Plants were obtained from the forest nursery in April 2018. Leafless plants were weighted and transfered to hydroponical pots filled with quartz sand. The following concentrations of DMA(V) ($(CH_3)_2As(O)OH$) (Merck, Germany) in modified Knop's solution were employed: 0 (control), 0.06, 0.1, 0.3 and 0.6 mM. During the experiment plants were constantly watered with deionized water. The 14-week long experiment was performed in a greenhouse equipped with mechanical ventilation system and automatic data loggers recording ambient parameters.

Determinations of total As and PEAs were performed with the inductively coupled plasma optical emission spectrometer Agilent 5110 ICP-OES (Agilent, USA). The arsenic speciation was performed with high performance liquid chromatography coupled with optical emission spectrometry detection and hydride generation (HPLC-HG-OES) using Shimadzu chromatograph equipped with anion-exchange column (Supelco, USA) LC-SAX1 (250 × 4.6 mm).

Studied species showed diverse tolerance to organic As. Increased concentration of DMA caused a progressive decrease of plant biomass compared to control, the lowest for *A. pseudoplatanus* and *Q. robur* and the highest for *B. pendula*. *Acer pseudoplatanus* and *B. pendula* were characterized by BCF<1, indicating the As exclusion mechanism. For *Q. robur* BCF>1 was recorded with the exception of 0.06 mM pointing at the effective uptake of As by this species. Additionally, effective accumulation of As in stems and leaves of *Q. robur*translated into TF>1 for all addition levels. Species-specific tolerance to organic As was reflected by changes in metabolic profile including phenolic compounds and low-molecular-weight organic acids secreted into rhizosphere and accumulated in roots and leaves of investigated tree species.

The mechanism of selenium uptake and its influence on enrichment of the edible plants with this element

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Keywords: selenium; plants; foods

Selenium is an essential element for human health. The low content of this element in Polish soils results in insufficient amount of this element in daily diet. Some plant species are able to efficiently absorb selenium and convert it to organic compounds with anticancer properties. Such plants may be a way to supplement selenium deficiencies and be an important part of antitumor prevention. Bulb onion (*Allium cepa* L.) plants were used for the study of selenium uptake. The aim of the research was to find such modification of plant cultivation, that would enhance the process of selenium uptake, as well as its translocation and biotransformation to selenoaminoacids with the most health-beneficial properties. The research showed the possibility to increase the selenium uptake by changing the concentration of phosphate and sulfate in the mineral feed. On the other hand, those changes caused lowering of the translocation factor and share of the organic selenium compounds in totally extracted selenium. The onions absorb Se (IV) more slowly than Se (VI), but with higher biotransformation factor to organic compounds. Use of the CCCP inhibitor enabled to indicate active nature of transport of Se (VI), whereas uptake of Se (IV) can be at least partially passive.

Light regulation of *GLR* gene expression in Arabidopsis leaves

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Keywords: *Arabidopsis thaliana*; GLR; light; gene expression

Glutamate receptor-like (GLR) channels are ubiquitous in plants. They are involved, among others, in light-regulated processes including hypocotyl growth, chlorophyll synthesis in seedlings and/or senescence. 20 *GLR* genes have been identified in the genome of *Arabidopsis thaliana*. The available microarray and RT-PCR data point to the dependence of *GLR* gene expression on light. However, the conditions of the experiments were not always precisely described.

We tried to identify potential light-regulated motifs in promoter sequences of *GLR* genes. We tested G-box, I-box and other motifs found in the promoters of 30 genes which are known to be regulated by light, e.g. *LRS3*, *PIF*, *CCA1*, *ATMYC2*, *CPRF*, *RBCS*, *phyA*, *FHL*, *COP1*, *HY5*, *CRY1* etc. The analysis did not show the presence of motifs related to light regulation in the promoter sequences of *GLR* genes.

In addition, we studied effects of blue and red light on *GLR* gene expression profiles. Arabidopsis plants grown in standard light conditions were illuminated for 3h with blue or red light of ca 35 µmol $m^{-2}s^{-1}$. The expression of most tested *GLR*s is light-dependent, and both spectral regions up-regulate the expression in most cases. Our results suggest a concerted action of phytochromes and cryptochromes on the expression of the studied genes.

■ ■ ■

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New insight into possible role of TsGSI from triticale

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Keywords: glutamine synthetase; triticale; nitrogen metabolism

Glutamine synthetase (GS, EC 6.3.1.2) is the enzyme that is mainly responsible for the assimilation of ammonium in plants. GS catalyzes the ATP-dependent condensation of ammonium with glutamate to produce glutamine. Three GS types, GSI, GSII and GSIII, have been described in living organisms. GSI and GSII are both present in eukaryotes and prokaryotes, with GSI being more abundant in prokaryotes and so far little is known about the function of *GSI* genes in plants.

Previous research based on the RT-PCR strategy has allowed to isolate the full-length gene encoding glutamine synthetase type I in triticale (*TsGSI*, GenBank JX 040632.1). The main goal of this project was to examine the function of the *TsGSI*. The transgenic *Arabidopsis thaliana* plants were obtained by introducing the coding region *TsGSI* linked to the 35S CaMV promotor, via *Agrobacterium tumefaciens* transformation. We also used a homozygous *Arabidopsis* line with an insertion in the coding region of GSI (At3G53180) and a wild-type ecotype as a control. All analyzed lines were cultured on standard medium with different concentrations of ammonium (0,2 mM, 5 mM and 50 mM). Under high and low concentrations of ammonium a greater shoot and root biomass, an increased GS activity and chlorophyll content was observed in transgenic line. In non-transgenic control plants and mutants we observed significant reduced the fresh weight and chlorophyll content which could be due to the toxic effects of the high concentration of ammonium. The obtained results suggest that *TsGSI* may modify the efficiency of ammonium assimilation.

Session 6

PLANT HORMONES AND HORMONAL REGULATIONS

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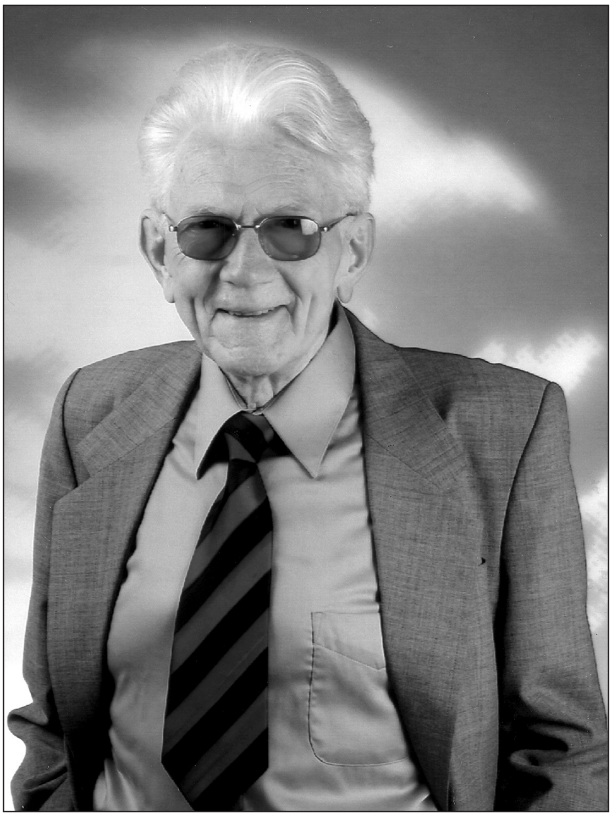
Session dedicated to the memory
of Professor Marian Michniewicz
(1922–2008)



Marian Michniewicz

(1922–2008)

Professor Marian Michniewicz was born on December 5, 1922 in Vilnius to a family of a railway clerk. He graduated from high school in May 1940. In the autumn of the same year, he began his biological studies at the University of Vilnius. After taking over Vilnius by the Nazis in 1942, he was removed from the university and worked physically for the railway company. In 1943 he joined the Home Army, and from January 1944, as partisan of the III Vilnius Home Brigade „Szczerbiec", fought against the German occupant. After the Bolsheviks captured Vilnius, in order to avoid being arrested by the NKVD in the autumn of 1944, he joined the Polish People's Army. Demobilized in the autumn of 1945, he continued his biological studies at the University of M. Curie Skłodowska (UMCS) in Lublin. In 1948 he obtained a master's degree in philosophy in the field of botany, and in 1951 a doctorate in mathematical and natural sciences. In 1964 he received the title of associate professor, and in 1971 he became full professor.

He began his scientific work in 1946 as a deputy assistant at the Department of Plant Physiology UMCS in Lublin. In 1953 he took over the leadership of the Chair of Plant Physiology UMK in Toruń, where he worked until his retirement in 1991. In the years 1956–1958 he was a deputy dean, and in the period 1958–1961 dean of the Faculty of Biology and Earth Sciences, from 1969 to 1978 the first director and organizer of the Institute of Biology. He was a member of numerous scientific societies, including an honorary member of the Polish Botanical Society and a member of the Scientific Society in Toruń. From 1971 he was a correspondent member of the Polish Academy of Sciences, and became a real member in 1986.

Scientific achievements of prof. Michniewicz encompass about 150 publications, including 115 original works. They mainly concern the physiology of growth and development of plants with particular emphasis on the role of the hormonal factor. The scientific achievements also include the co-authorship of a number of books: „Outline of Physiology of Scott Pine" – 1976, „Fusarium, Mycotoxins, Taxonomy and Pathogenicity" – 1989, „Biology of Scots Pine" – 1993.

Cooperation with US scientific institutions, established in 1961 and 1962 during an internship at the California Technical Institute in Pasadena, resulted in obtaining four five-year grants from the Ministry of Agriculture (PL-480 agreement). The research conducted within the framework of this cooperation (1963–1979) mainly concerned the role of the hormonal factor in the growth and development of forest trees, especially the pine tree.

Professor Marian Michniewicz made many foreign scientific trips, especially to the USA and Canada. He participated in many conventions and international symposia and he organized four international symposia devoted to research on plant growth regulators (1959, 1963, 1968 and 1974) in Toruń.

The outcome of prof. Michniewicz's didactic activity included supervision of 229 master's theses, 12 doctoral dissertations and co-authorship of the academic textbook ppt. „Plant Physiology", 1977 and 1984. For his scientific and didactic activity, he received a number of rewards, distinctions and orders, among others, Medal of the National Education Commission (1978), Order of Polonia Restituta, Officer's Cross (1985), and a number of war decorations.

In February 2005, prof. Michniewicz was awarded the „Convallaria Copernicana" distinction by Nicolaus Copernicus University for his exceptional contribution and merit.

Professor Marian Michniewicz died on April 30, 2008.

How jasmonate metabolism controls defense responses against biotic stress

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Keywords: jasmonate; catabolism; signaling; defense; biotic stress

Jasmonates (JAs) regulate major sectors of immune responses and mediate also developmental processes related to growth or fertility. Jasmonoyl-isoleucine (JA-Ile), the active hormone controlling most responses is embedded in a complex metabolic grid where its dynamic accumulation is shaped by multiple upstream and downstream enzymatic modifications.

We are interested in elucidating new metabolic steps in the JA pathway that govern JA-Ile accumulation and impact defense signaling. We have characterized two integrated JA-Ile catabolic pathways that are stress-induced in Arabidopsis leaves, concomitantly to JA biosynthesis and signaling. They consist in: a) two-step JA-Ile oxidation mediated by cytochromes P450 of the CYP94 family to form signaling-inactive derivatives [1, 3]; and b) the cleavage of JA-Ile and 12OH-JA-Ile conjugates by amidohydrolases [2]. Their genetic impairement has profound impacts on JA signatures, including JA-Ile overaccumulation, but surprisingly minor if any consequences on defense output. In addition, we have recently elucidated a direct oxidation route of JA to OH-JA by JA oxidases (JAO) of the 2-oxoglutarate-dependent oxidase family [4]. Inactivation of a particular JAO isoform revealed a new metabolic diversion mechanism, that is needed to repress JA-Ile responses in unstimulated wild-type leaves. Consequently, in unstressed leaves lacking this enzyme, expression of defense is constantly upregulated, resulting in enhanced tolerance to biotic stress, with minor plant growth penalty. The remarkably different impact of deficiency in pre- and post- JA-Ile metabolic steps on defense signaling will be discussed.

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Looking for a needle in haystack – major aspects of analysis of brassinosteroids and other plant signalling molecules

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Keywords: plant hormones; brassinosteroids; phytoecdysteroids; ultratrace analysis; signalling molecules

Chemical signalling is extremely ancient since the most organisms use chemical signals in cell–cell communication. Unlike animals, plants never developed a nervous system for intercellular communication, but they did evolve hormones as chemical messengers. Plant hormones play essential roles (individually and in concert) in the regulation of myriads of physiological processes involved in plants' growth, development and responses to environmental stimuli. Plant hormones (phytohormones) are usually present at extremely low concentrations in plant tissues, generally pg/mg fresh weight (FW), while substances that interfere with their analysis are present in far greater concentrations. This is the major problem associated with plant hormone analysis, which will be discussed focusing on steroid chemical messengers including the factors that complicate their extraction and isolation from the highly complex matrices.

A Non-Canonical Nuclear Function of ERECTA Family Proteins in Arabidopsis

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Keywords: ERECTA; SWI/SNF; gibberellin response and biosynthesis; chromatin remodeling

The ERECTA family (ERf) of leucine-rich repeat receptor-like kinases (LLR-RLKs) consists of ERECTA (ER), ERECTA-LIKE 1 (ERL1) and ERECTA-LIKE 2 (ERL2) proteins. In Arabidopsis, ERfs play important role in the control of epidermal patterning, stomata development, hormonal signaling, meristem size and inflorescence architecture. In this work we show that inactivation of Arabidopsis ERfs causes severe impairment of gibberellin (GA) response, down-regulation of gibberellin receptor *GID1* (*GIBBERELLIN INSENSITIVE DWARF 1*) genes and broad transcription changes affecting other important regulatory processes. Moreover, the triple *er/erl1/erl2* mutant exhibited reduced levels of bioactive GA_4 indicating impairment of gibberellin biosynthesis. We found that upon endocytosis, ERECTA migrates to the nucleus, where interacts with core SWI3B subunit of the SWI/SNF chromatin-remodeling complex (CRCs). Similarly, the ERL1 and ERL2 kinase domains bind SWI3B in the nucleus. Occupancy of promoter regions of *GID1* genes by ER and SWI3B proteins, abolishment of SWI3B binding to *GID1* promoters in *er/erl1/erl2* mutant together with genetic and comparative transcriptomic studies, suggest that ERfs and SWI/SNF CRC cooperate in transcriptional control of GA receptors.

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Transcriptional and post-transcriptional mediated regulation of expression of genes related to ABA/GA metabolism and signalling during light-dependent germination of *Arabidopsis thaliana* seeds

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Keywords: gibberellin; abscsic acid; germination; dormancy; ARGONAUTE

Induction and maintenance of seed dormancy by abscisic acid (ABA) and dormancy release by gibberellin (GA) and light occur in seeds of many dicotyledonous plant species. It is known that in plant cells light received by phytochromes (PHYs) induces a signal leading to changes in expression of genes involved in metabolism and/or signal transduction of phytohormones. Increasing number of evidences helped also to better understand how germinating seeds response to light stimulus. However, up to date the possible molecular mechanisms of interactions between hormonal (ABA, GA) and environmental (light) factors in the regulation of seed-related events remain unclear. Among few transcription factors responsible for the light-related modulation of genes expression in plant cells, the HFR1 (Long Hypocotyl in Far-Red) was identified. Taking into account that the role of *HFR1* gene in processes occurring in seeds is poorly understood, the aim of this particular study was to elucidate the role of *HFR1* gene in hormonally- and light-dependent germination of *Arabidopsis thaliana* seeds. Due to the fact that the gene expression may be regulated also at post-transcriptional level, the possible role of *ARGONAUTE1* (*AGO1*) gene coding the regulatory protein known as a significant element of the RNA-induced silencing complex (RISC), was also evoked. Looking for the answer for the scientific question stated above several experiments using wild type (WT) and *hfr1 A. thaliana* (Columbia-0 ecotype) mutant seeds (characterized by different levels of dormancy) were performed. The germination assays were conducted in the presence of water or treated by ABA, NOR (norflurazon, an inhibitor of ABA synthesis), GA, or PAC (paclobutrazol, an inhibitor of GA synthesis), at various light conditions, at 25°C. In addition, the expression profiles of *HFR1* and *AGO1* genes were examined in samples obtained from germinating WT seeds imbibed in conditions analogous as these during biological assays. To characterize the effect of light on the expression of genes related to ABA/GA metabolism and/or signaling (such as i.e.: *ABI*, *NCED*, *RGL*, *GID*) in germinating *A. thaliana* seeds, the qRT-PCR analysis was performed. The obtained novel results indicated that regulation of seed germinability and dormancy alleviation by light involves modulation of ABA/GA signaling and metabolism in *HFR1*- and *AGO1*-dependent manner (see also poster by Rosińska et al.).

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Regulation of seed germination and root morphology by abscisic acid transporters in model legume plant *Medicago truncatula*

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Keywords: ABC transporters; abscisic acid; legumes; germination; root organ formation

The phytohormone abscisic acid (ABA) plays a crucial role in different aspects associated with plant growth and development, including embryo and seed maturation, post germinative growth, and responses to environmental changes. It is growing evidence that ABA movement within plant can determine proper ABA perception and subsequently trigger adequate responses under unfavorable conditions. It was reported that in a model legume plant *Medicago truncatula*, ABA affects as a negative regulator the nodulation process to reduce costly establishment of nodules under water deficiency. Our previous studies have shown that in Medicago ABC transporter form the G subfamily (MtABCG20) is functioning as ABA exporter from its biosynthesis site in Medicago roots influencing lateral roots and nodules formation. Moreover, spatial expression pattern analysis with GUS reporter gene revealed that *MtABCG20* promoter is active in the hypocotyl-radicle transition zone of the seed embryonic axis. Phenotypic analysis and transport assay revealed that MtABCG20 promotes seed germination by ABA extrusion from embryo. Further investigation based on phylogenetic tree of the half-size ABCG transporters, allowed us to identify another Medicago ABC protein (MtABCG26), possibly involved in the ABA transport. We have shown that its expression is strongly upregulated upon drought stress, mimicked by PEG and ABA. The MtABCG20 and MtABCG26 share 50% identity, are located in the plasma membrane and have an ability to form homo- and heterodimers. A spatial expression pattern revealed that the *MtABCG26* promoter is active mostly within vascular bundles in roots, similarly to the *MtABCG20*. Interestingly, a spatial expression pattern of these two transporters is different in embryo. The *MtABCG26* is expressed peculiarly in cotyledons of Medicago what is in contrast to *MtABCG20* expressed only in radicle – hypocotyl transition zone. We postulate that MtABCG26 can also play a role in controlling/modulating seed germination of Medicago however distinct/supplementing that of MtABCG20.

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The looped strive towards unravelling PIN structure-function connections

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Keywords: PIN membrane topology; protein motifs; auxin transport; plant development

The signalling molecule auxin provides instructive cues guiding the entire plant ontogenesis. Its orchestrating role largely depends on the directional transport between cells facilitated by a plant-specific group of auxin transporters named PINs. While the physiological and developmental roles of PINs along with their intracellular dynamics have been extensively studied, the biochemical and structural underpinnings of PIN activity are still scarcely characterized.

Here we strive to provide further insights into PIN structure-function relationships encompassing the plasma membrane topology of Arabidopsis PINs and characterization of major protein domains of those carriers

The auxin orchestra. Transcriptome-wide identification of genes encoding elements of auxin signal transduction pathway in yellow lupine flowers

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Keywords: auxin; RNA-Seq; *Lupinus luteus*; flowers

Yellow lupine (*Lupinus luteus*), with its elevated high-quality protein content and low alcaloid biosynthesis level, has a great potential to become one of the leading legumes in Europe. It is especially vivid to increase pulse production in the face of a strong trend to reduce meat consumption and replace animal protein with high-value vegetable protein. To improve lupine grain yield and thus encourage farmers to grow this crop, first we have to learn the molecular mechanisms that orchestrate development of generative organs, so that it will be possible to use more sophisticated techniques in the near future. Studies on numerous model plants have shown, that one of the crucial players in flower development is auxin. Binding of this hormone to TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX1 auxin receptor (TAAR) family member triggers ubiquitination and degradation of Auxin/Indole-3-Acetic Acid (AUX/IAA) repressors. This event liberates Auxin Response Factors (ARF) from repression, so that they can fulfill their function as transcription factors. In this study, we aim to identify elements of auxin signal transduction pathway in yellow lupine flowers.

For this purpose we have constructed RNA-Seq libraries using RNA extracted from flowers of *L. luteus* (v. Taper) at four different stages of development. Flowers at the first stage are green buds with closed anthers and small, intensively growing gynoecium. Second stage comprises flowers where most anthers started to open, third one is a stage of full anthesis and flowers at the fourth stage are fertilized with slightly enlarged pistil. Among assembled transcripts we have identified mRNAs encoding full-length homologues of the following elements of auxin signal transduction pathway: TIR1, AFB2, AFB3, AUX/IAA8, AUX/IAA11, AUX/IAA14, AUX/IAA16, AUX/IAA17, AUX/IAA26, AUX/IAA27, ARF2, ARF3, ARF5, ARF6, ARF9, ARF17 and ARF18. Expression of these genes varied in flowers depending on the developmental stage.

These findings suggest that the above-listed genes play a role in the development of yellow lupine flowers and represent potential targets for crop improvement.

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Occurrence of brassinosteroids in the seedlings of *Hordeum vulgare* L. cv. Golden Promise

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Keywords: brassinazole; brassinolide; brassinosteroids; castasterone; occurrence; phytohormones

The occurrence of mainly castasterone, brassinolide and cathasterone and lower amounts of 24-epibrassinolide, 24-epicastasterone, 28-homobrassinolide, typhasterol, 6-deoxocastasterone and 6-deoxotyphasterol in 14-day-old de-etiolated barley (*Hordeum vulgare* L. cv. Golden Promise) was noted. The endogenous level of brassinosteroids (BRs) in barley seedlings treated with 24-epibrassinolide (EBL) and/or brassinazole (Brz; an inhibitor of BR biosynthetic reactions) was also investigated. To our knowledge, this is the first report related to the occurrence of BRs and application of EBL and Brz in terms of the endogenous content of BRs in barley. Brz decreased the level of BRs in the leaves. Application of EBL showed a weak promotive effect on the BR content in Brz-treated seedlings. Brz also inhibited growth of the seedlings; however, addition of EBL overcame the inhibition. The EBL applied alone at 0.01–1 μM increased the BR level in the leaves but at 10 μM lowered the BR content. In opposition to leaves, the Brz in the concentration range from 0.1 to 1 μM did not significantly affect the content of BRs in the roots. However, application of 10 μM Brz caused BRs to decrease, but treatment of EBL concentrations overcame the inhibitory effect of Brz.

Phytohormonal regulation of *Populus trichocarpa* fine root senescence

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Keywords: senescence

Plant senescence is the age-related developmental process leading to death. Despite its destructive nature, senescence is a well-regulated process with plenty of developmental and environmental signals controlling this process. The role of phytohormones in the senescence of leaves is well documented, but still, there is no information on their effect on senescence of another ephemeral organs such as fine, absorptive roots.

The aim of our study was to check whether phytohormones are engaged in the regulation of root senescence. For this purpose, we have conducted analyses of genes expression which are related to phytohormone signalling. Moreover, we performed quantitative analyses of selected phytohormone content, as well as histochemical analyses to check their localization.

Our research has shown that phytohormones play a crucial role also in fine root senescence. We have selected a large group of hormone-related genes which expression changed during senescence. In addition, quantitative analyses have shown that the content of abscisic acid (ABA) and jasmonic acid (JA) increased in senescent roots. These results suggest that ABA and JA are those phytohormones which may control and/or promote root senescence. The results are another common feature of senescence of fine roots and leaves, which may indicate that mechanisms of senescence might have a universal character.

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Enhancement of biosynthesis of jasmonates by lead and *Acyrthosyphon pisum* infestation in *Pisum sativum* L.cv. Cysterski seedlings

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Keywords: aphid *Acyrthosiphon pisum*; lead; crosstalk between heavy metal and aphid; hormetic and toxic doses of lead; signalling molecules

Jasmonates are important regulators in plant responses to abiotic and biotic stresses. The regulation of jasmonate biosynthesis is determined by a positive feedback loop, substrate availability and tissue specificity. The primary aim of the research was to determine the effect of an abiotic factor, i.e., lead (Pb) at various concentrations (low causing a hormesis effect and high causing toxicity effect), on the generation of jasmonates in pea (*Pisum sativum* L. cv. Cysterski) seedlings and then during infestation by the pea aphid (*Acyrthosiphon pisum* Harris). The second objective was to verify whether the presence of Pb in pea seedling organs and crosstalk of Pb and *A. pisum* modulate the activity lipoxygenase (LOX) and expression levels of selected genes encoding enzymes of the jasmonate biosynthesis pathway. Already at the beginning of the experiment, i.e., 4 days after the administration of Pb, before aphid transfer to pea seedlings, a significant accumulation was recorded for jasmonates, i.e., free (JA) and methyl jasmonates (MeJA) in the roots and leaves of pea seedlings growing on the Hoagland medium with 0.075 and 0.5 mM $Pb(NO_3)_2$. Results obtained within these studies provided evidences of the involvement of these molecules in the defense response of pea to the abovementioned stress factors. Therefore, a significant accumulation was recorded for JA in the roots of pea seedlings growing on lead-supplemented medium at the toxic dose. Additionally, crosstalk of Pb and pea aphid raised generation of JA, especially in the early stages of *A. pisum* feeding. In turn, in the case of the applied hormetic dose of Pb, a significant increase in level of MeJA was observed in leaves of pea seedlings in relation to other experimental variants. Moreover, aphid infestation alone caused an increase in the level of generation of jasmonates in organs of pea seedlings. Also, our research demonstrated that the increase in jasmonates in pea seedlings growing on lead-supplemented medium and during Pb and *A. pisum* interactions was accompanied by increase of LOX activity. This research provides insights into the cross-talk between the abiotic (Pb) and biotic factor (aphid infestation) on the level of the generation of jasmonates involved in the defense mechanism of *P. sativum* seedlings.

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A comparison of biochemical composition and yielding of fruits in apple cultivars (*Malus domestica* Borkh)

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Keywords: *Malus domestica*; metabolites; abscisic acid; semiquinone radicals; fruit yielding

The aim of the research was to determine biochemical composition of fruits in apple varieties (*Malus domestica* Borkh) such as Gala Schniga, Beni Shogun® (Fuji) and Ligol, grafted on M9 rootstock grown in the Greater Poland Region. In this study, metabolites such as sugars, organic acids, alcohols and amino acids in organs of apple trees were identified and relatively quantified using gas chromatography coupled with mass spectrometry (GC-MS). An important indicator, which allows for better definition of sweetness is the ratio between single sugar content, sorbitol and organic acids. Apart from the above-mentioned metabolites detected in organs of the tested apple varieties, we also determined the level of abscisic acid (ABA). Different levels of growth regulating hormones, including ABA in apples seems to be comprehensible on the specified phenotype. It is known that ABA regulates the organic substance translocation and metabolism in plants. Moreover, this is the first report revealing generation of semiquinone radicals in organs of apple trees. Semiquinone and phenoxyl radicals in plant cells are formed as a result of oxidation of hydroxyl groups in phenols and polyphenols and their generation was positively associated with markers of oxidation such as protein carbonyls and total peroxides. GC-MS analysis revealed differences both in the profile as well as the levels of sugars and other metabolites detected in the tested apple varieties. Moreover, EPR analysis showed the lowest contents of semiquinone radicals in apple fruits and the highest in leaves of apple trees. The results obtained indicate that fruits of the analyzed apple varieties differed both in terms of their biochemical composition and demonstrated a significant variability in yields.

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Investigation on crosstalk between different long distance signaling pathways in *Arabidopsis thaliana*

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Keywords: jasmonic acid; jas9:VENUS; stress

The plants exchange information about mechanical damages, pathogens, insects, or environmental stresses, between different organ through long distance signaling. There are different types of signaling like electrical signaling, calcium waves, ROS etc. In the response for these events frequently Jasmonic Acid (JA) is being produced. To study the long distance signaling we used the Jas9:VENUS *Arabidopsis thaliana*, a biosensor line. An increase in Jasmonic Acid level leads to Jas9:VENUS degradation and result in fluorescence intensity decrease. We are using different knockout lines of *Arabidopsis thaliana* to cross them with Jas9:VENUS and mJas9:VENUS lines to study effects of the different genetic backgrounds on the long distance signaling. To investigate the protein-protein interaction and localization the gene cloning of crucial for long-distance signaling pathways is being conducted.

Differential expression analysis of genes involved in tolerance to deacclimation in winter barley

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Keywords: cold; gibberellin; maize

Mechanisms involved in deacclimation of the plants caused by warm spells during winter aren't sufficiently recognized. It is unclear what makes some plants de-harden after only a few warmer days and why other plants need much more time for that. Also the mechanisms responsible for high survival rate of some plants after experiencing freezing temperatures following a warm spell are still unknown.

The aim of this study was to identify genes involved in tolerance of deacclimation in winter barley.

Four winter barley lines tolerant to deacclimation and 4 susceptible ones were subjected to cold acclimation followed by deacclimation. Leaves from each genotype were sampled before hardening, after hardening and after de-hardening. Total RNA was isolated from the leaves and subjected to RNAseq analysis. Differential expression analysis was performed to compare transcriptomes of control (pre-hardening), cold-acclimated and deacclimated plants as well as tolerant genotypes with susceptible ones.

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Formation of vascular system in Arabidopsis leaves

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Keywords: auxin; Arabidopsis; leaves; veins

Plant vascular system is a network of veins mostly composed of phloem and xylem tissues. The pattern of veins in a leaf is strictly related with leaf growth and its shape, that is manifested in branching pattern in dicot leaves and parallel pattern in monocot leaves. In *Arabidopsis* leaves vascular veins form a hierarchical branching network. The main vein (midvein) is formed first followed by emerging of secondary and next-order veins that ultimately generate closed loops. Vascular tissue differentiates from procambium. During a leaf formation at the shoot apex, procambial cells develop from inner tissues as narrow continuous strands which obtain their shape through cell elongation and oriented cell divisions. Mechanisms that control the formation of veins is not fully understood, but many studies indicated that the main factor for the induction and development of vascular tissues is plant hormone auxin. The pathways along which auxin is transported across cells form „canals" marking procambial cells. PIN1 protein is auxin efflux carrier that is crucial in polar auxin transport and play a major role in the formation of auxin canals. Establishment of secondary and next-ordered veins in *Arabidopsis* leaf is thought to be initiated by auxin „convergence points" in the epidermis and by random domains in subepidermal tissues, where auxin is subsequently transported into inner tissues and towards pre-existing veins.

The proposed models of the formation of vascular system do not explain how new veins are connected with existing ones or how auxin transport is directed towards existing veins. Thus, the main question of our study is: Are there any signals from existing veins that guide the formation of the next-order veins?

To analyse the formation of vascular system, first we present live imaging of growing leaf primordia soon after the initiation at the shoot apex by laser confocal microscopy. We use the different transgenic lines related with auxin signalling and auxin biosynthesis. Subsequently, we show results of mechanical disturbance (cell ablations) of potential signalling from pre-existing vasculature and the visualization of procambial vein patterns obtained by clearing method.

Transcriptome analysis of jasmonic acid-induced gene expression during late stamen development of yellow lupine (*Lupinus luteus* L.)

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Keywords: stamens; jasmonates; RNA-Seq; yellow lupine

Proper stamen formation and development is crucial for the productivity of plants, especially crop species. The key event in the functioning of stamens is anther dehiscence and consequently release of viable pollen grains. In addition to genetic and environmental control, the process of anther opening is regulated by plant hormones, including jasmonates (JAs). This is confirmed by the results of studies conducted mainly on *Arabidopsis thaliana*, which indicate that disturbances in JA biosynthesis or signaling pathway lead to delay in anther dehiscence, as well as disorders in the maturation and release of pollen grains. On the other hand, accumulation of JAs promotes these processes. Until now, little is known about the molecular network controlling the stamen development in legumes. Due to this fact, the main purpose of our study was to establish the differences in transcriptional networks in the JA-treated and untreated yellow lupine (*Lupinus luteus* L.) stamens during late developmental stage. Comparison of the cDNA libraries revealed the presence of 157 differentially expressed genes (DEGs). The most important DEGs were associated with metabolism and signaling of other phytohormones, circadian rhythm, and adaptation to changing environmental conditions. Furthermore, GO enrichment analysis revealed that DEGs are mainly connected with rhythmic process, regulation of photomorphogenesis, aging, as well as response to stress or phytohormones, including abscisic acid, ethylene, gibberellin and salicylic acid. A holistic approach in this type of research may allow a better understanding of interactions between different plant hormones or the influence of other factors on stamen development. Moreover, results of transcriptome analyses provide a valuable source of data for the future planning of experiments and explore the molecular mechanisms of stamen development.

Expression, purification and kinetic characterization of recombinant maize (*Zea mays*) IAA glucosyltransferase

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Keywords: auxin; IAA ester conjugate; IA-glucose; *Zea mays*

Indole-3-acetic acid (IAA) homeostasis is regulated by number of mechanisms, including its conjugation to various molecules. Predominant form of IAA in monocotyledonous plants, such as maize (*Zea mays*), are its ester conjugates, e.g. 1-*O*-indole-3-acetyl-β-D-glucose (1-*O*-IAGlc) synthesized from IAA and UDP-glucose by UDP-glucose dependent IAA glucosyltransferase (IAGlc synthase). We have optimized production of recombinant maize IAGlc synthase in *Escherichia coli* strain BL21-CodonPlus(DE3)-RIL (different induction conditions: temperature, time and IPTG concentration). Under all tested conditions most of the recombinant protein accumulated in the form of inclusion bodies, however some of the enzyme existed in soluble, catalytically active form. For further analysis we have used soluble protein fraction obtained from bacteria induced with 0.2 mM IPTG in 18˚C overnight. His-tagged recombinant IAGlc synthase was partially purified on HisTrap™ FF crude column with ÄKTA™ chromatography system. The enzyme was eluted with 170,5 mM imidazole. The recombinant IAGlc synthase, similarly to the native enzyme, is most active in pH 7.4. Besides IAA, it can also utilize indole-3-butyric acid as a glucose acceptor. The enzyme displays Michaelis-Menten kinetics and K_m for UDPG determined by Hanes-Woolf method is 1.23 mM, however the synthase is inhibited by high (above 6 mM) concentration of this substrate.

The effect of light conditions and polar auxin inhibitors NPA and TIBA on ABCB19-GFP localization and fluorescence intensity in *Arabidopsis thaliana* callus

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Keywords: auxin; callus; ABCB19; GFP; Arabidopsis

Indole-3-acetic acid (IAA) is the most common form of auxin – phytohormone, which controls and influences many morphogenetic processes, plant growth and callus formation. Only a small part of endogenous IAA can diffuse freely through the cell membrane, whilst the anionic form ($IAAH^-$) is mostly transported via the appropriate proteins. The AUX/LAX proteins family transport auxin inside the cells (influx transport), while the PIN proteins (along with PILS proteins), ABCB1 and ABCB19 transport IAA outside of the cell (efflux transport). ABC proteins are characterized by the presence of an ATP binding cassette (ABC) and the hydrolysis of ATP allows to transport the compounds against concentration gradient. ABCB transporters are essential for plants development, playing a significant role in such processes as: gametogenesis, development and seed maturation, organ formation, or secondary growth. The best known ABCB transport proteins are ABCB1 and ABCB19, found in *Arabidopsis thaliana*, which act as exporters of both endogenous and synthetic auxin.

The aim of the study was to determine the effect of light conditions and polar auxin transport inhibitors: NPA and TIBA on the localization and level of ABCB19-GFP fluorescence intensity in the 14-day *Arabidopsis thaliana* callus obtained from 7-day old tips of shoots with cotyledons.

Within the control variant grown in the constant light the ABCB19-GFP fluorescence signal occurred mostly in the cell membrane and rarely within cytoplasm in the callus external cell layers. Occasionally fluorescence has been observed in the deeper layers especially nearby the prevascular tissue. In contrast, the addition of 2.5 μM NPA resulted in change of localization of ABCB19-GFP to mostly cytoplasmatic. The signal was visible only in deeper callus cell layers. Addition of 2,5 μM TIBA to the growth medium resulted in decreased fluorescence level within the plasma membrane whilst NPA increased fluorescence intensity in cell cytoplasm. ABCB19-GFP signal in callus cultivated in the dark was observed mainly in cell cytoplasm within external as well as internal layers of callus cells. The fluorescence level within plasma membrane was much lower in callus cultivated in dark compared to control (cultivated in constant light). On the contrary the fluorescence intensity within cytoplasm was greater in callus grown in darknes than in constant light.

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Role of ARGONAUTE in ABA – and light-dependent germination of *Arabidopsis thaliana* seeds

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Keywords: dormancy; germination; light signalling; phytohormones; seeds

The numerous aspects of seed physiology are controlled by various endogenous (i.e, phytohormones) and environmental (i.e. light) factors. Among plant hormones, abscisic acid (ABA) is the major negative regulator of seed germination, responsible for induction and maintenance of dormancy. The ABA content, signalling and interactions with other stimuli play important roles in determination of the physiological state of the seed and in regulation of germination process. Seed dormancy can be alleviated during after-ripening (in dry seed) and/or by light (in imbibed seed). It is also well known that both transcription and translation are required for the completion of seed germination and seedling establishment. Regulation of gene expression in higher plants results from transcription but also from post-transcriptional processes, and the ARGONAUTE (AGO) proteins comprise the catalytic engine of the complex modulating these processes. In plants, the conserved AGO protein family coded by 10 genes in *Arabidopsis thaliana* (from *AGO1* to *AGO10*) have been implicated to play important and diverse roles in the regulation of various aspects of development. In spite of the fact that AGO are expected to participate in key cellular and organismal processes, the knowledge about their function in seed physiology is lacking. Taking into account all these facts, it was suggested to perform a functional analysis of gene coding AGO1 protein in ABA- and light-dependent germination of seeds characterized by different depth of dormancy. Characteristic of germination of freshly harvested (dormant) and after-ripened (non-dormant) wild type (WT) *A. thaliana* (Col-0 ecotype) seeds was made in the presence of exogenous solutions of ABA and NOR (norflurazon) – its biosynthesis inhibitor, at different light conditions. The possible role of *AGO1* in modulation of expression of genes involved in metabolism and signalling of ABA in light-dependent germination was verified by the qRT-PCR analysis of relative expression of *AGO1* gene and by Western blot of AGO1 protein, in dormant and non-dormant seeds imbibing on water and ABA, in various light conditions. Additionally, selected genes encoding elements of ABA biosynthesis and signal transduction pathway (e.g. *NCED*, *CYP707A2*, *ABI*, *HAI*) were also examined. The obtained results provide new valuable information concerning the possible role of *AGO1* in the ABA- and light-dependent seed germination.

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Strigolactones are involved in response to drought stress

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Keywords: strigolactones; drought; ABA; stomata; respiration

Strigolactones (SLs) are a recently described group of phytohormones. They are involved in many developmental processes. It was shown that SLs play a crucial role in plant adaptation to the nutrient stress conditions, mainly to nitrogen and phosphorus deficiency, via modification of root/shoot architecture and promotion the symbiosis with N-fixing rhizobial bacteria and arbuscular mycorrhizal fungi (AMF). The function of SLs in plant response to other biotic stresses such as drought or salt stress was also disclosed.

Recently, the barley *HvD14* gene encoding α/β hydrolase that is involved in strigolactone signalling was identified. The TILLING strategy was used to perform a functional analysis of the identified gene in order to obtain a series of *HvD14* alleles. The phenotype of the plants that carry one of the identified alleles, *hvd14.d*, corresponded to the phenotype of the SL mutants that have been described in other species. Plants with the *hvd14.d* allele were also insensitive to treatment with GR24, which is the synthetic analogue of SL.

In presented studies the response to water deficiency of barley SL signalling mutant was investigated. Obtained results indicated that *hvd14.d* mutant is hypersensitive to drought. In agreement with the drought-sensitive phenotype, mutant exhibited increased leaf stomatal number and density relative to the wild type variety Sebastian. Additionally the stomata in *hvd14.d* were open for the longer time during drought and mutant loses water faster than Sebastian. Finally the activity of photosystem II after drought stress was more affected in SL mutant, in comparison to wild type. All data together indicated the positive role of SLs in plant response to drought stress.

Subcellular localization of *Medicago truncatula* polar auxin transporter PIN9

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Keywords: PIN protein; auxin; Fabaceae; nodules

Gene encoding *Medicago truncatula* auxin transporter PIN9 was previously shown to be highly expressed particularly in root nodules [1, 2]. Furthermore, MtPIN9 is a direct ortholog of *Arabidopsis thaliana* transporter PIN5, which localizes predominantly in the membranes of endoplasmic reticulum (ER) and mediates auxin transport from cytosol to the ER's lumen [3]. Here we verified the MtPIN9 subcellular localization by fusion with fluorescent protein dsRED and transient transformation of living onion epidermis [4]. During visualization in confocal microscope, we applied fluorescent marker of ER's localization – ER-tracker. Signals from both fluorochromes overlapped, proving that MtPIN9 localizes in the ER. Moreover, we performed transient transformation of *M. truncatula* roots and detected fusion protein by Western blot analysis using anti-dsRED primary antibodies. Afterwards, same primary antibodies were used to visualize the subcellular localization of PIN9 in transformed *M. truncatula* hairy roots in electron microscope. This research confirmed that, similarly to its *A. thaliana* ortholog, MtPIN9 is ER-localized. Our finding supports the previous assumption [1] that ER-dependent intracellular homeostasis of auxin level could be crucial for development and functioning of indeterminate root nodules.

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■■■

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How changes in auxin metabolism under ammonium nutrition affect growth of *Arabidopsis thaliana*?

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Keywords: auxins; *Arabidopsis thaliana*; ammonium nutrition

Auxins are important plant hormones regulating growth and mediate responses to environmental signals. The aim of the project was to check alterations in plant growth caused by changes in auxin metabolism in ammonium nutrition. Arabidopsis thaliana was cultivated in hydroponic culture on nitrate ions (control) and ammonium ions as the only nitrogen source. Plants that were treated with ammonium showe d retarded growth and altered root phenotype. Level of auxins in leaves and roots was measured using ELISA method. Transcript level of genes encoding enzymes which take part in auxin synthesis was measured by RT-qPCR and levels of their proteins were determined by Western blotting. Changes in auxin localization were visualised using confocal microscopy. Ammonium nutrition caused modifications in expression of most genes encoding enzymes of auxin biosynthesis pathway: their transcript levels were highly decreased in leaf tissue. Besides, ammonium treatment resulted in changes in their protein levels. Moreover, alterations in auxin localization and lower concentration of auxin in roots of ammonium grown plants were observed. It has been concluded that ammonium nutrition affects auxin metabolism in plants which can be issue of weaker plant growth compared to nitrate nutrition.

■■■

This work was supported by grant 2018/29/B/NZ3/02687 from the National Science Centre (NCN, Poland) given to A. Podgórska.

The activity of fast vacuolar (FV) channels in red beet (*Beta vulgaris* L.) taproots vacuoles in response to auxin (IAA)

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Keywords: FV channels; auxin; patch-clamp; vacuole; *Beta vulgaris* L

Auxin (IAA) plays an essential role in plant cell growth and development driven i.e. by the osmotic uptake of water into the vacuoles. The turgor pressure of expanding cells is achieved by transport of osmotically active substances like ions into the vacuole to maintain its osmolarity.

In the tonoplast of higher plants two types of channels represent the major ion currents: non-selective slowly (SV) and fast (FV) activating cation channels.

Our earlier results on vacuoles isolated from red beet taproots suggested that the SV channels are modulated by auxin (IAA), however the role of FV channels in regulation of vacuole volume still remains unclear (Burdach et al., 2018).

Hence, the effect of IAA, in the range of 10^{-5} to 10^{-7} M, on the red beet FV channels was examined by the use of patch-clamp techniques. Their activity was measured in whole-vacuole and cytosolic side-out patch configurations.

It was found that activity of FV channels was modulated by auxin depending on its concentration in the medium.

■■■

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Exogenous amines as the enhancers of Cucumis sativus physiological state in in vitro culture

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Keywords: *Cucumis sativus*; *in vitro* culture; physiological state; polyamines

Plant polyamines (PAs) are low molecular compounds involved in regulating of many plant developmental and growth processes and response to environmental factors, however it is still not fully understood how, those biostimulants regulate plant growth.

The aim of this study was to evaluate the physiological response of *Cucumis sativus* cultured *in vitro* to different exogenously applied PAs putrescine (PUT), spermidine (SPD), spermine (SPM) and cadaverine (CAD) in concentrations 0.1 mM and 1.0 mM . Biomass, leaf area, flowering as well as chloroplasts' pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) concentrations were monitored. Additionally, parameters based on fluorescence of chlorophyll *a* – maximum quantum yield (Fv/Fm) and Performance Index (PI) was determined.

Supplying culture medium with PAs caused decrease in biomass production in *Cucumis* plants. The most severe depletion was observed under 1.0 mM SPD supplementation (approx. 30%). Exogenous PAs promoted female flower development. CAD, PUT and SPD at 1.0 mM concentration caused increase of about 20% in levels of Chl *a* and Chl *b* as well as Performance Index of the leaves. Plants treated with 0.1 mM solutions of PAs contained less pigments in comparison to the control plants.

Session 7

BIOTECHNOLOGY AND TISSUE CULTURE

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Can plant hormones be used to enhance the value of microalgae biomass for biotechnological application?

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Keywords: microalgae; phytohormones; growth; bioactivity

Due to a high lipid content and a diversity of other metabolites e.g. proteins, amino acids, omega-3-long chain polyunsaturated fatty acids and antioxidant compounds, microalgae are ideal candidates for a biorefinery approach. This encompasses extraction of lipids for biodiesel production, proteins for animal feed and secondary metabolites for nutraceutical and pharmaceutical applications. Before the commercial potential of microalgae can be fully realized, their production needs to be optimized to be more cost effective. This includes producing sufficient biomass with a high yield of the target compounds.

A hormonal network regulates growth processes and stress responses in vascular plants. Phytohormones are a valuable tool in land-based agricultural systems for enhancing plant growth and yields and to mitigate against environmental stress. There is increasing evidence for a similar hormonal network in microalgae. This includes i) identification of endogenous hormones including auxins, cytokinins, gibberellins and brassinosteroids, ii) changes in hormone content in response to diurnal cycles and stress e.g. increase in brassinosteroids content in response to salinity and temperature stress and iii) application of phytohormones eliciting physiological responses. The use of hormone-rich biostimulants such as the seaweed-derived Kelpak® can potentially improve growth and enhance the metabolite content of microalgae. Understanding the role of phytohormones in microalgae physiology provides a means to increase biomass accumulation, to overcome some of the environmental challenges encountered in mass culturing and to enhance biomass quality for a biorefinery approach, thereby increasing profitability of microalgae biotechnologies.

Cyclic nucleotide monophosphates & their cyclases in plant responses

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Keywords: plant stress signalling; cGMP; cAMP; cyclic nucleotide cyclases; *Arabidopsis thaliana*

Cyclic nucleotide monophosphates, in particular cGMP and cAMP, and the enzymes that can generate them are of increasing interest in the plant sciences since these molecules have central roles in many biological processes including responses to the environment. Arguably, the major recent advances came after the release of the complete *Arabidopsis thaliana* genome that has enabled the systematic search for adenylate and guanylate cyclases (ACs and GCs) and this has eventually led to the discovery of a growing number of these cyclases in higher plants. Firstly, we will review the role of cGMP and cAMP in plant responses, including responses at the systems level. Secondly, we will outline the computational and experimental approaches that have led to the identification and characterization of novel ACs and GCs. Interestingly, the functional cyclase domains have turned out to be part of complex dual functioning enzymes [1], e.g. receptor kinases. In some of these receptor molecules cGMP can tune kinase activity and hence downstream signalling and thereby profoundly affect plant responses.

■ ■ ■

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Susceptibility of *Chlamydomonas reinhardtii* developmental stages to atrazine – cell cycle studies

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Keywords: *Chlamydomonas*; cell cycle; atrazine; abiotic stress

Atrazine (AT) is one of the most toxic, photosynthesis-inhibiting herbicides. It is known that AT blocks photosynthetic electron transport chain and inhibits transfer of excitation energy from photosystem II (PSII) to photosystem I (PSI) reaction center. As a consequence, reactive oxygen species (ROS) are overgenerated, leading to oxidative damages of cell compounds. However, little is known about the sensitivity of different ontogenetic stages of the plant cells to AT. Thus, the aim of the study was to estimate AT influence on the particular developmental stages of the model green alga *Chlamydomonas reinhardtii*.

Synchronously growing population of *C. reinhardtii* (wild type CC-1690) was treated with 78 μg/L of AT. The herbicide was applied at specific time-points of the cell cycle to investigate the sensitivity of dark-adapted zoospores (progeny cells, 0h of the cell cycle), young light–adapted cells (3h of the cell cycle), highly photosynthetically efficient cells (6h of the cell cycle) and mature mother cells (9h of the cell cycle) to the toxicant. The cells growth (cell volume), photosynthetic parameters (photosynthetic pigments content, oxygen evolution, chlorophyll *a* fluorescence *in vivo*) and oxidative stress markers (H_2O_2 level, antioxidative enzymes activity) were analyzed.

Treatment of zoospores (0h) with AT resulted in the diminishment in photosynthetic pigments content (about 50% of control) in the mother cells that developed at the end of the growth phase of the cell cycle. AT-treatment of young light–adapted cells (3h), photosynthetically efficient cells (6h) and mother cells (9h) had no influence on pigments content. AT lowered photosynthesis efficiency in the cells in all experimental variants, however inhibition was the most pronounced for the AT-treated zoospores (0h). It was found that the main reason of photosynthesis inhibition was lowering of the efficiency of electron transport between PSII and PSI, with the enhanced energy dissipation *via* non-photochemical processes. Further, AT caused transient overproduction of ROS. H_2O_2 level increased within 1h after AT application in all experimental variants and after 4 to 5 hours decreased to the control level. The probable reason of such tendency was the fast increase in antioxidative enzymes activity.

A final consequence of photosynthesis disruption by AT was diminishment of size of mother cells that developed at the end of the growth phase of the cell cycle as well as decrease in the number of progeny zoospores released in the dark phase of the cycle. Mother cells that developed from AT-treated zoospores (0h), young light–adapted cells (3h) and photosynthetically efficient cells (6h) were about 30%–15% smaller than the control ones. It was visible that the susceptibility of the cells to AT decreased with the progress of the cell cycle and cells maturation.

■■■

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Comparison of the effectiveness of cleaning of municipal wastewater treated in experimental hydrophyte systems with *Hippuris vulgaris* and *Hydrocharitetum morsus-ranae* group

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Keywords: phytoremediation; wetlands; aquatic plants

Water is the most valuable natural resource that determines the proper functioning of the natural environment and human life. Water protection requires the use of effective technologies for the treatment of domestic, municipal or industrial wastewater. Since Poland's accession to the European Union and the introduction of modern and more efficient wastewater treatment systems in the coming years, significant progress has been made in 2017 in increasing the effectiveness of municipal wastewater treatment both in urban areas and in rural areas. Nevertheless, until 2015, it was not possible to achieve good status of all surface water assessed. Hence, in many wastewater treatment plants, constructed wetlands are introduced to improve the efficiency of conventional wastewater treatment technologies. The constructed wetlands use biological processes related to the transformation of biogenic compounds under the influence of microorganisms and the uptake of nitrogen and phosphorus compounds dissolved in wastewater by aquatic plants. The aim of the conducted research was to compare the effectiveness of cleaning of municipal wastewater treated in experimental constructed wetlands with *Hippuris vulgaris* and *Hydrocharitetum morsus-ranae*.

The experiment was carried out at the West Pomeranian University of Technology in Szczecin for 9 weeks (16.06 ÷ 29.09) in three cycles digesting 3 weeks each. Up to each 2 of four containers equipped with a drain valve were introduced 30 dm^3 of treated sewage obtained from the municipal treatment plant „Zdroje", in which 30 individuals of *Hippuris vulgaris* were grown in the gravelite layer. After a period of 2 weeks, the remaining two containers with 6 *Stratiotes aloides* and 20 rosettes of *Hydrocharis morsus-ranae* were filled 20 dm^3 of pre-treated sewage from *Hippuris vulgaris* containers and were kept for 1 week. Each time in the sewage samples spectrophotometric measurements of the concentration of nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$) and phosphorus (PO_4^{3-}) were made, the chemical oxygen demand was determined by permanganate method, electrolytic conductivity, general alkalinity and pH. On the basis of the results obtained, the reduction of the physicochemical parameters of treated wastewater was determined. It was found that a better cleaning effect was achieved in containers with *Hippuris vulgaris* than with the *Hydrocharitetum morsus-ranae*. Within one week, the content of orthophosphate(V) in sewage with a common mare's tail rooted in a layer of gravelite decreased on average by 15.9 mg, and nitrate nitrogen(V) by 32.8 mg. On the other hand, in the presence of the water-soldier-frogbit community, the content of ammonium nitrogen decreased by 83.6 mg. The chemical composition of the treated sewage influenced faster the decomposition of *Hydrocharis morsus-ranae* than on *Stratiotes aloides*.

ABCG46 (PDR10) from *Medicago truncatula* as a model for substrate specificity investigation of ABC transporters

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Keywords: ABC transporters; legumes; phenylpropanoids

The ABCG46 (PDR10) from a model legume plant *Medicago truncatula* was shown as transporter facilitating the unidirectional and selective transmembrane movement of two phenolic compounds namely *p*-coumaric acid and liquiritigenin (Biala *et al*., 2017). These compounds differ in structure and transport dynamics. Experimental results suggest that these ligands do not stimulate MtABCG46 ATP-ase activity – in the contrast to phenomenon observed in e.g. yeast homologues. Moreover, the selectivity of the MtABCG46 applies also to structurally similar compounds like liquiritigenin, isoliquiritigenin, 4'7-dihydroxyflavone (5-deoxyflavones) or naringenin (5-hydroxyflavone). This observation may support the assumption that plant ABCGs have higher substrate selectivity than their yeast homologues. Based on 3-D model structure of the MtABCG46, phylogenetic analyses as well as comparison of amino acid distribution/position conservatism across different taxa we are tackling a selectivity issue. By combining various transport assays, analyses of kinetics and the MtABCG46 mutants we intend to find molecular determinants responsible for selective transport.

■ ■ ■

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Contribution of NtZIPs in Zn and Cd uptake and root-to-shoot translocation in tobacco

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Keywords: zinc; cadmium; tobacco; NtZIP

Contrary to most agronomic plants, tobacco accumulates a greater proportion of cadmium (Cd) in its foliage than in any other part. Furthermore, in tobacco Cd stimulates Zn translocation to shoos in Zn/Cd-concentration dependent manner. The aim of the study was to understand mechanisms underlying Cd-dependent Zn translocation to shoots, and also Zn-dependent Cd translocation.

It is known that ZIP proteins (ZRT-IRT-like Proteins) transport Zn (and some of them also Cd) across membranes to the cytoplasm. The presence of Cd modifies Zn status in plants, and thus affects the expression of tobacco *ZIP* genes. To determine their contribution to the regulation of Zn/Cd-dependent translocation to shoots of both metals, we cloned and characterized four tobacco *ZIP* genes (*NtZIP1-like*, *NtZIP4*, *NtZIP5* and *NtZIP11*). Encoded proteins were localized in the plasma membrane. Each of them mediated uptake of Zn, but only NtZIP4 and NtZIP5 participate in Cd uptake.

To determine the role of *NtZIP4* and *NtZIP5* in the uptake and root-to-shoot Zn/Cd translocation, their expression in in apical, middle and basal parts of roots was investigated by qPCR. Obtained results indicated that Zn deficiency induced their expression in the root-part dependent manner. Expression of *NtZIP4* was comparable in the apical, middle and basal root parts. For *NtZIP5* the highest expression was noted in the apical part. Their tissue-specific expression was also examined in the transgenic tobacco expressing *GUS* under the control of their promoters. The results showed that NtZIP4 and NtZIP5 can participate in direct absorption of metals from the medium and from the apoplast by cells comprising internal tissues.

The transgenic RNAi plants with reduced level of *NtZIP4* mRNA were generated. The translocation of Cd to shoots was less efficient in these plants. This showed that NtZIP4 protein participates in the mechanisms controlling the root:shoot Cd distribution.

■ ■ ■

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Somatic embryogenesis in new lines purple coneflower

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Keywords: somatic embryos; breeding; growth regulators

The aim of the study was to determine the effect of different concentrations of auxin NAA (alfa-naphthaleneacetic acid) and IAA (3-indolylacetic acid) at a constant content of cytokinin BAP (6-benzylaminopurine) on the efficiency of somatic embryogenesis in new cultivar lines purple coneflower: L1, L4, L14, L47, L52 and L54. For the experiment, modified MS media was prepared with a constant addition of cytokinin BAP at a concentration of 1 mg·dm-3 and various concentrations of auxin: IAA and NAA. After sterilization, leaf explants were prepared, which were then inoculated abaxially into the prepared media. After 10 weeks of regeneration, it was found that the cultivar line L52 was distinguished by the best regeneration of embryos and shoots after conversion in most media. MS 1B0.5I medium proved to be the best for regeneration. On the control medium (MS 1B) and on MS 1B1N medium, the regeneration proceeded least efficiently in most cultivar lines. Differences in the effectiveness of regeneration of specific lines on individual media can be the basis for the development of a more optimized composition of nutrients for somatic embryogenesis of each of the new cultivar lines.

Characteristics of the oat (*Avena sativa* L.) plants obtained by crossing with maize (*Zea mays* L.)

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Keywords: doubled haploids; oat x maize hybrids; wide hybridization

Distant crosses from different genetic environments can be a useful tool for producing new cultivars of arable crops, especially in the context of climate change. In crosses between Pooideae and Panicoideae, e.g. oat × maize or oat × pearl millet, Panicoideae chromosomes are eliminated shortly after fertilization at the beginning of embryogenesis. Occasionally, however, incomplete removal of Panicoideae chromosomes may occur. When crossing cereals with maize, elimination of maize chromosomes results from their limited mobility during mitosis (during metaphase and anaphase) and failure to attach spindle fibres to centromeres. The aim of the research was the identification and molecular-cytogenetic characterization of oat × maize hybrids. Induction and regeneration of doubled haploid (DH) lines and hybrid lines were carried out by the method described by Noga et al. (2016). A total of 138 oat lines obtained by crossing over 2,000 oat plants from 80 genotypes with maize cv. Waza were tested for the presence of maize chromosomes (Skrzypek et al. 2018). The presence of maize chromatin was indicated in 66 lines due to the amplification of the *Grande-1* maize retrotransposon fragment (500 bp) by PCR. The maize chromosomes were detected in 14 lines using *in situ* genomic hybridization (GISH). All analysed plants had a full set of oat chromosomes. The number of maize chromosomes differed between the lines. Twelve lines had two maize chromosomes of similar size, one line – one maize chromosome and one line – four maize chromosomes. The presence of six 45S rDNA *loci* was detected on oat chromosomes, but none of the added chromosomes of maize in either line had a 45S rDNA *locus*. Four of the analysed lines did not have whole chromosomes of maize, and only fragments of maize chromatin embedded in oat chromosomes. Five out of 66 hybrids were characterized by a grassy type and did not produce panicles. Twenty-seven lines were fertile and produced grains in numbers from 1–102 per line (613 in total). Sixty-three fertile DH lines, out of 72, which did not contain chromosomes or maize chromatin, produced seeds in numbers from 1–343 per line (3760 in total).

■■■

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Regeneration of fertile *Avena sativa* L. plants in anther culture

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Keywords: oat; androgenesis; embryo-like structures; plant growth regulators

In vitro production of doubled haploid (DH) plants through androgenesis is a promising and convenient alternative to traditionally used techniques for rapid production of fully homozygous plants. In the present study, the effects of cultivar, induction medium and distance from the base of the flag leaf to the penultimate leaf of the panicle on the efficiency of oat (*Avena sativa* L.) androgenesis were tested. Tillers of oat 'Akt', 'Bingo', 'Bajka' and 'Chwat' were cut when the panicle was enclosed within the leaf sheath and the distance from the base of the flag leaf to the penultimate leaf of the panicle was designated as: (A) 0.0–4.0 cm, (B) 4.1–8.0 cm, (C) 8.1–12.0 cm and (D) 12.1–16.0 cm. For embryo-like structures (ELS) induction, anthers were isolated on C17 (Wang and Hu 1984) and W14 (Ouyang et al. 1989) media supplemented with auxins: 2.0 or 5.0 mg/dm^3 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg/dm^3 picloram, 0.5 mg/dm^3 dicamba and 2.0 mg/dm^3 naphthyl-1-acetic acid (NAA) and cytokinins: 0.5 mg/dm^3 kinetin, 0.5 mg/dm^3 6-benzylaminopurine (BAP).

All tested cultivars produced ELS and two of them, 'Akt' and 'Chwat', produced fertile DH plants. The highest number of ELS and haploid plants was obtained from *cv*. 'Chwat' (3.6% and 0.8%, respectively). ELS formation also depended on the distance from the base of the flag leaf to the penultimate leaf of the panicle. Most of them were observed on anthers harvested from panicles of which the distance from the base of the flag leaf to the penultimate leaf was less than 4 cm. The presence of the induction medium supplemented with different plant growth regulators was essential for the induction of ELS, but did not increase the production of haploid plants and DH lines. The highest number of ELS and plants was obtained on W14 medium with the addition of 2.0 mg/dm^3 2,4-D and 0.5 mg/dm^3 kinetin (2.7%). The low haploid plant regeneration rate (from 0.03 to 0.05%) still limits the practical application of oat anther culture for the DH lines production (Warchoł et al. 2019).

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Effect of glucose, sodium nitroprusside and nitric oxide elicytation on phytoalexin production by microbulbs of *Hippeastrum hybridum* (*Amaryllidaceae*)

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Keywords: elicitation; *Hippeastrum*; twin-scales; phytoalexin

Hippeastrum hybridum is a bulbous plant belonging to *Amaryllidaceae* family and it naturally occurs mainly in South and Central America. This species has great potential in horticulture as ornamental plant. Aditionally, it has the ability to produce phytoalexin, which can be used in agriculture as plant protecting agents or in medicine due to its antioxidant properties. Phytoalexins belong to the group of secondary metabolites that are released in response to a pathogen attack. These substances can be used as potential agents against fungal pathogens. However, the extraction of these compounds from plants grown in the traditional way is ineffective, because such plants synthesis them at a low level. With the use of cell and tissue cultures, by adding specific elicitors to media, stimulation of plants to produce secondary metabolites is possible.

The aim of the study was to increase the synthesis of phytoalexin by *Hippeastrum hybridum* in *in vitro* culture using the technique of elicitation. In the course of the experiment following elicitors were used: sodium nitroprusside (SNP), nitric oxide (NO) and glucose instead of sucrose, at a concentration comparable to sucrose in the sandard medium and 2-fold higher.

The microshoots were obtained from bulbs (twin-scales) and flowers (peduncles). The microbulbs were capable of producing phytoalexins without elicitation. However, the level of these secondary matabotates was higer in microbulbs that were cultivated on MS medium supplemented with glucose. Both glucose concentrations increased phytoalexin synthesis in microbulbs and this possitve effect was observed at all analised timepoints (0, 24, 48, 72 and 96 hours after wounding). In the case of SNP and NO elicitation no significant differences in the level of phytoalexins between the elicited and control plants were observe. Nevertheless, a slight increase in phytoalexins content was noted for NO elicitation, starting from the 48 hour after mechanical wounding.

In vitro and *ex vitro* cultured carnivorous plants as a source of biologically active compounds

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Keywords: *Dionaea muscipula* J. Ellis; *Drosera peltata* Smith; *Droseraceae*; phenolic compounds; acclimatization

Carnivorous plants of the Droseraceae family have been known for centuries as medical herbs. Their antibacterial, antifungal and antiinflamatory properties are related to the ability to synthesize various biologically active secondary metabolites, especially phenolic compounds. Nowadays, researchers postulate, that such plant-derived chemicals can be used against pathogenic microorganisms, since the use of antibiotics on a large scale has resulted in the emergence of drug-resistant strains.

The main goal of presented study was to evaluate whether acclimatization process to *ex vitro* conditions will intensify phenolic compounds synthesis in *Dionaea muscipula* J. Ellis (Venus Flytrap) and *Drosera peltata* Smith (shield sundew) plants.

Tissue cultures of plants were cultivated on agar-solidified ½ MS medium, with 3% of sucrose and pH=5.5. 50 plants of each genus were transferred to fresh medium (composition as above) and the same amount to pots with peat and sand (2:1). Plants were acclimatized 5 weeks to *ex vitro* conditions (initial humidity 80%, each week by 10% less). After 5 weeks of experiment biometric parameters were estimated. Plants were lyophilized, homogenized and of the following parameters were measured: total phenolic content, fenylopropanoids and flawonols concentration using spectrophotometric methods. Also, selected phenolic derivatives were analyzed using high pressure liquid chromatography (HPLC).

Results have shown that both genera from Droseraceae family grow faster in *in vitro* conditions and accumulate more dry weight in comparison to acclimatized plants. Interestingly, in *D. peltata* plants in *ex vitro* conditions total phenolic content, as well as phenylopropanoids and flavonols production increased about 1.5-fold. Unlike in *D. muscipula* grown in pots, where only phenylopropanoids accumulation increased about 1.4-fold.

Higher phenolic synthesis in carnivorous plants in *ex vitro* conditions can be a consequence of changes in growing conditions during acclimatization process and allocation of carbon skeletons using for primary metabolism (growth) to secondary metabolites pathways production. We conclude, that acclimatization process could induced increased accumulation of secondary metabolites but it requires precise optimalization of acclimatization conditions.

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Taraxacum pieninicum response to sucrose presence in synseeds endosperm during short-term conservation in *in vitro* conditions

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Keywords: plant conservation; slow-growth storage; sucrose; synthetic seeds

Synthetic seeds are artificially encapsulated somatic embryos, shoot buds or any other tissue, that can be used for sowing as a seed and is converted into a whole plant under *in vitro* or *ex vitro* conditions. It was reported that the artificial endosperm should contain both nutrients and carbon source. This is especially evident, when synseeds are stored and sucrose is used as a metabolic substrate acting as an indirect protection during prolonged cold storage. Sucrose, glucose, fructose and oligosaccharides are commonly found in soluble sugars that accumulate along with development of a cold tolerance in higher plants. Sugars also maintain cytoplasm organization and osmotic adjustment during drought conditions. Sugars create condensed glass-like intracellular liquid in the place where crystalline solutes may crystallize when solution becomes concentrated during drying. This process prevents the disintegration of cells by filling the space under water deficiency.

Taraxacum pieninicum (*Asteraceae*) is listed as critically endangered species on Polish red lists. Classical protection methods of *T. pieninicum* appear to be insufficient due to the number of individuals and limited availability of seeds. It seems to be reasonable to develop a method for *in vitro* storage of plant material, especially without the need of frequent passages to avoid losing the culture due to a contamination.

The aim of this research was to (1) assess the possibilities of short-term cold storage of *T. pieninicum* synthetic seeds in dry Petri dishes and (2) determine the effect of the different concentration of sucrose in artificial endosperm on the survival and regrowth of the synseeds after storage.

For encapsulation of the shoot tips sodium alginate and calcium chloride were used. Three concentrations (3%, 6%, 9%) of sucrose were tested. The synseeds were stored in the darkness, into sterile and dry Petri dishes without medium, tightly sealed with Parafilm and wrapped in aluminum foil, at 4°C for 4–24 weeks. Synthetic seeds after cold storage for re-growth were transferred onto MS medium supplemented with 1.11 μM BA, 0.14 μM NAA, 3% sucrose, and solidified with 0.8% agar. The culture was maintained under continuous white fluorescent light at 26 ± 1°C. Whole synseeds or shoot tips mechanically removed from their alginate coating were transferred on proliferation medium. Regrown shoots were analyzed in terms of proliferation rate, chlorophyll content, proline, H_2O_2 and sugar accumulation.

Results indicated that sucrose inside synseeds structure enables plant material to be stored for prolonged time. Conversion of synseeds stored in presence of sucrose was observed even after 24 weeks, while control without sucrose remained viable only for 12 weeks in 4˚C. Concentration of sucrose did not significantly affect proliferation rate, proline, H_2O_2, sugars and chlorophyll content in regrown shoots.

In vitro rhizogenesis of *Euphorbia milii* L. in context of latex composition evaluation

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Keywords: *Euphorbia milii*; *in vitro* rhizogenesis; latex; laticifers; TEM; FT-Raman spectroscopy

Euphorbia milii L. is a plant originating from Madagascar and widespread in Africa, where its latex is used as an alternative to niclosamide, a drug to combat schistosomiasis, the neglected tropical diseases (NTD) [1]. Beyond this property, *E. milii* latex poses many other valuable substances (e.g. anti-inflammatory, antiviral and anti-leukemic), as well as extremely stable serine protease and lipophilic compounds that can be used for biofuel production. In plant, latex is produced in special cells – laticifers, that reach into shoots, leaves and roots in *E. milii*. Among 60% of medicinal plants, the roots are the main reservoir of medicinal substances and 90% of these plants are collected from natural sites causing populations degradation. Therefore *in vitro* rhizogenesis can be an alternative source of valuable substances gathering in laboratory. In the present study the efficient rhizogenesis of *E. milii* was obtained from leaf blades explants (14.7 roots/explant). It was shown that cytokinins 2iP, BAP and KIN, in contrast to TDZ, do not inhibit root formation and the presence of auxin NAA in the medium was necessary for rhizogenesis induction. Using light microscopy, NIC and TEM, the occurrence of laticifers in 7-day old in *in vitro* roots as well as in older roots from mother plant was stated, near the vascular bundles, what indicates their probable origin from vascular cambium. The anatomy of laticifers showed the presence of non-articulated elongated cells, which branched out during they invasive growth in tissues. TEM analysis revealed that latex was formed in the cytoplasm and then transported to vacuole to protect plant cells against metabolic disorders caused by its components, in roots from mother's plant and from *in vitro* conditions. Chemical analysis of latex compound and explant tissues made by using FT-Raman spectroscopy, indicated a decrease of flavonoids and carotenoids content in explants after 4 weeks of *in vitro* culture compared to mother plant.

Does cycloheximide affect the efficiency of photosynthesis and respiration in *Chlamydomonas reinhardtii* cells?

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Keywords: cycloheximide; *Chlamydomonas* phytotoxicity; photosynthesis; dark respiration

Cycloheximide (CHX) is an inhibitor of eukaryotic translation, commonly used in laboratories to stop protein biosynthesis in the cytoplasm and to arrest the cell cycle in both animal and plant cells. The mechanism of CHX interaction with eukaryotic ribosomes is well described but the problem of its influence on "non-target" physiological and biochemical processes, such as photosynthesis or respiration, is rarely considered in literature. Although translation processes in chloroplasts and mitochondria are not blocked by CHX, both of these organelles are not fully autonomous. Most of the proteins present in the chloroplast and mitochondria are encoded by the nuclear genome, synthesized in the cytoplasm and transported to the proper organelle. Due to the use of CHX as a specific translation inhibitor in plant cell research, it is necessary to be aware of its possible side effects on the physiological and biochemical parameters of the cells treated with this antibiotic. Thus, the aim of this study was to estimate the impact of CHX on the efficiency of photosynthesis and respiration in the model green alga *Chlamydomonas reinhardtii*.

Chlamydomonas reinhardtii CC-1690 (Chlamydomonas Resource Center, USA) was cultured in high salt medium (HSM) under continuous fluorescent light ≈125 µmol photons/m^2 s^{-1}, 30° C, bubbled with sterilized air with 2.5% (v/v) CO_2. The cells were exposed to CHX for 24 hours and EC toxicological values were estimated on the basis of population growth inhibition. CHX influence (EC_{50} = 0.169 mg/L) on photosynthetic and respiratory parameters of *C. reinhardtii* were examined, including the photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) content, oxygen evolution (photosynthesis intensity) and consumption (dark respiration intensity) and chlorophyll *a* fluorescence *in vivo*.

CHX inhibited cells multiplication but not their growth. The mean volume of single cell treated with CHX amounted to about 130% of control. Thus, values of physiological parameters were calculated per unit of cell volume, not per one cell, to avoid the possible misvaluation. It was found, that nor photosynthesis neither respiration were inhibited by CHX. On the contrary, photosynthetic oxygen evolution and dark oxygen consumption increased in CHX cells by approximately 50%. Further, the quantum efficiency of photosynthesis was higher (by 10–15%) in CHX-treated cells as compared to the control ones. Also chlorophyll *a* and carotenoids pigment content was raised in CHX-treated cells (about 120% of control). The exact reasons of stimulatory effects of CHX on the physiological parameters of the cells are not clear. In few papers that describe the effects of CHX on photosynthesis or respiration both inhibitory and stimulatory effects are reported. Because of such inconclusive results further investigations of CHX effects on plant and algae cells are needed.

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NO/H2O2 ratio shift as a tool for the microalgae growth control

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Keywords: nitric oxide; hydrogen peroxide; microalgae; growth control

Development of algal mass-culture based biotechnology requires the tools for control and modification of the population growth. Among many factors, reactive oxygen species (ROS) as an important signal molecules, are involved in regulation of algal cell metabolism, growth and division. Two of them: H_2O_2 and NO and their interaction are believed to play a key role in the regulation of plant cell development. We have recently shown that the physiological level of NO and H_2O_2 changes in a characteristic way during the cell cycle of a model green alga *Chlamydomonas reinhardtii*. Continuation of this research is the work presented here, demonstrating that exogenous application of H_2O_2, leading to the changes in H_2O_2/NO ratio, can be an efficient and low-cost method for the control of algal cells growth in the synchronous cultures.

Cells of *C. reinhardtii* (wild type CC-1690) were synchronized by alternating the light/ /dark (10/14 h) regimen. H_2O_2 was added to cells suspensions, to obtain two-fold lower NO/H_2O_2 ratio as compared with the control cells. Four selected time-points of cell cycle (developmental stages) were chosen for analyses: (a) young progeny cells held in the dark, (b) cells of the highest photosynthesis efficiency, (c) cells with the highest H_2O_2 level (prior to cytokinesis), (d) mature mother cells, where the cytokinesis and chloroplast division began.

It was demonstrated that externally induced, mild modification of redox homeostasis (NO/H_2O_2 ratio shift) may lead, depending of the cell cycle moment, to: (a) occurrence of additional replication round, thus increase in the number of progeny cells released from one mother cell' (b) increase of progeny cells biomass and volume, (c) cell's better adaptation to dark/light shift-induced stress, (d) earlier progeny cells release from the mother cells. Our results may form the basis to create a tool, that would allow to control the development of microalgae population and to obtain cultures of desirable features, such as increased biomass, elevated number of cells in the population or cells that possess elevated tolerance to stress.

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Symplast and apoplast as a supracellular systems of information exchange

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Keywords: Arabidopsis; callose; cell wall; symplasmic izolation; totipotency

Cell-to-cell signaling is a major mechanism controlling plant morphogenesis. In recent years, special attention has been paid to the role of symplasmic communication in plant morphogenesis, as it has shown that molecules that regulate cell differentiation move through plasmodsmata (PD). It is also postulated that modifications of the chemical composition of cell walls may be a marker of changes in the direction of cell differentiation.

Transport of signaling molecules through PD is one way in which plants promote or restrict intercellular signaling over short-distances. The role of PD and symplasmic communication in the establishment of plant cell totipotency/pluripotency was studied during somatic embryo induction from Arabidopsis explants as a model system. Cell-to-cell communication was evaluated using fluorescent tracers, supplemented with histological and ultrastructural analysis, and correlated with expression of a *WOX2* embryo reporter. We show that embryogenic cells are isolated symplasmically from non-embryogenic cells. Callose deposition in PD preceded *WOX2* expression in future sites of somatic embryo development. Treatment of explants with the callose biosynthesis inhibitor 2-deoxy-D-glucose suppressed somatic embryo formation, supporting the idea that callose deposition at PD is required for symplasmic isolation and establishment of plant cell totipotency.

The occurrence of selected pectic and AGP epitopes in Arabidopsis explant cells that display different phenotypes during somatic embryogenesis was examined. Compared to an explant at the initial stage, an embryogenic and meristematic cells were characterized by a decrease in the presence of AGP epitopes and an increase of analyzed epitopes was detected in the callus cells. Totipotent cells could be distinguished from pluripotent cells by the presence of the LM2 epitope in the latest one, the appearance of the JIM16 epitope in totipotent cells, and the more abundant presence of the JIM7 epitope in the totipotent cells. The LM5 epitope characterized the wall of the cells that were localized within the mass of embryogenic domain. The JIM8, JIM13 and JIM16 AGP epitopes appeared to be the most specific for the callus cells. The results indicate a relationship between the cell phenotype and the chemical composition of the cell walls.

Molecular cloning, characterization, expression and guanylyl cyclase activity of pathogen peptide receptor (BdPepR2) from *Brachypodium distachyon*

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Keywords: cyclic nucleotide; guanylyl cyclase; *Brachypodium distachyon*

Guanosine 3′,5′-cyclic monophosphate (cGMP) is important signaling molecule which controls a different physiological responses and processes in numerous prokaryotes and all eukaryotes. A concentration of cyclic GMP is regulated by guanylyl cyclases (GCs) that catalyse the formation of cGMP from GTP and phosphodiesterases (PDEs) that hydrolyze cGMP to a non-cyclic 5′-guanosine monophosphate (GMP). Recent studies of plant GCs are focused on identification and functional analysis of a new family of membrane proteins called "moonlighting kinases with GC activity" with guanylyl cyclase catalytic center encapsulated within intracellular kinase domain. The new group of plant GCs included several receptor kinases such as brassinosteroid receptor (BRI1), phytosulfokine receptor 1 (PSKR1), pathogen peptide 1 receptor (PEPR1) and a wall associated receptor kinase 10 (WAKL10). Here we report enzymatic activity of plasma membrane receptor of peptide signaling molecules – BdPepR2 in *Brachypodium distachyon*.

As a result of recent studies, we provide experimental evidence to show that the partial, intracellular domain of BdPepR2 can generate cGMP *in vitro*. Firstly, the partial ORF of *BdPepR2* was amplify and introduced into the pGEX-6P-2 expression vector. The *E. coli* BL21 strain, transformed with the resulting construct, was used to produce the recombinant GST-tagged protein. The affinity chromatography enabled the purification of GST-BdPepR2 fusion protein as a clear main 66 kDa band. Removing the GST tag from fusion protein by digestion with PreScission protease resulted with appearance of one main, approximately 36 kDa protein, which correspond well with the predicted *in silico* molecular mass of 35.3 kDa for truncated BdPepR2 protein. The purified domain of BdPepR2 was further tested for its ability to convert GTP substrate to cGMP and cofactor specificity. The maximum stimulation of BdPepR2 activity was reached at 1 mM GTP after 20 minutes at 30°C in the presence of magnesium and manganese ions. It is surprising that, the highest enzymatic activity was observed with the addition of calcium ions to the reaction mixture.

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DNA de-methylation changes the rye (*Secale cereale* L.) anther protein profiles during androgenesis induction

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Keywords: rye; androgenesis; *in vitro* cultures; protein profiles

Androgenesis is an alternative pathway of microspores (immature pollen grains) development under *in vitro* conditions. As a result the haploid embryo-like structures are formed, which are able to regenerate haploids/doubled haploids (DHs) plants. Being totally homozygous, DHs are highly valued in many research areas and breeding practice. It is known that androgenic development is controlled by numerous factors but the knowledge concerning the molecular and physiological background behind this process is still fragmentary.

Rye (*Secale cereale* L.) is considered as the crop specimen recalcitrant to androgenesis induction. Resistance to this process could be caused by low ability to cope with stress (e.g. low temperature), which is prerequisite for androgenesis initiation. Our previous research revealed that pre-treatment of rye tillers with mannitol and glutathione is the most effective way to induce the androgenesis. Smooth balance between opposite effects of mannitol (MN, in high concentrations imitates drought stress inducing oxidative stress) and reduced glutathione (GSH an antioxidant) is crucial for stimulating microspores vitality and capability for divisions. As DNA hypomethylation (pre-treatment with 5-azacytidine, AC) could also increase androgenesis effectiveness (Nowicka et al. and Krzewska et al., both *in preparation*) we proposed to investigate the effect of MN, GSH and AC treatments on androgenesis induction effectiveness in rye with a special focus to the anthers protein profiles.

Two Polish rye breeding lines, described previously (Zieliński et al. *in preparation*) as extremely different in androgenesis responsiveness, were under investigation. Protein profiles were analysed in anthers isolated from tillers treated with MN and GSH (3 weeks at 4°C) and MN, GSH and AC (5 µM, the last 4 days of pre-treatment). The proteins were extracted and resolved by two-dimensional electrophoresis. Protein expression was evaluated by PDQuest software and compared among studied lines and treatments. Simultaneously, anthers were cultured *in vitro* according to Immonen and Tenhola-Roininen (2003).

AC treatment improved induction effectiveness in the highly responsive rye line. Its effect on the low responsive cultivar was not significant. The comparison of protein profiles among studied lines after different treatments revealed significant (>2-fold) changes in 120 protein spots. AC significantly changed protein abundance in both studied lines, 59 and 61 protein spots in highly and low responsive line, respectively.

To summarize, induced DNA hypomethylation was reflected in significant anther protein profiles changes. Probably such modifications were important for androgenesis effectiveness improvement, but only in the responsive genotype.

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Molecular and biochemical characterization of a novel phosphodiesterase with adenylyl cyclase domain from *Physcomitrella patnes*

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Keywords: cyclic nucleotides; phosphodiesterase; adenylyl cyclase

The ability of cells to receive signals depends on the presence of protein receptors that binds with the ligand, trigger a cascade of events called signal transduction, leading to an appropriate physiological responses. Signaling pathways in plant cells include both simple molecules such as calcium ions (Ca^{2+}), nitric oxide (NO) or cyclic nucleotides (cGMP, cAMP) as well as molecules with more complex structure, like enzymatic or structural proteins.

Cyclic nucleotides, referred as secondary signal relays, result from the interaction of cyclases, the enzymes responsible for their synthesis, and 3', 5'-cyclic nucleotide phosphodiesterases that catalyze degradation of the cyclic nucleotide molecule to its monophosphate equivalent.

Although for a long time, due to their very low, femtomolar concentration, the presence of cyclic nucleotides in plants has been questioned, now their role in the processes taking place in plant cells is indisputable (it raises no doubts) and has already been quite well known. Cyclic nucleotides participate in such processes as light signal transduction, hormonal signaling or plant responses to stressors. Although the enzymes responsible for cyclic nucleotides metabolism in animals and prokaryotes are well known and described, very little is known about their plant counterparts. Only a few representatives of adenylate (AC) and guanylate cyclases (GC) have been known so far, but there are no reports describing plant phosphodiesterases (PDE).

Hence we have attempted a molecular and biochemical characterization of recombinant phosphodiesterase from *Physcomitrella patnes*. The bioinformatics analysis of the cDNA of the gene has shown that the encoded protein has two domains: adenylate cyclase at the carbonyl end and cyclic nucleotides phosphodiesterase at the hydroxyl terminus. RNA isolation and cDNA transcription were performed using commercial kit. The 3' and 5' specific primers were designed for the catalytic domains of both cyclase and phosphodiesterase. The resulting sequences were then cloned into the pGEX-6p-2 vector containing the GST affinity tag and their correctness was verified by sequencing. Overexpressed fusion proteins were purified by affinity chromatography and their quality assessed using Western blot technique with anti-GST tag antibodies.

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Biochemical characterization of two potential adenylyl cyclases (ACs) from *Arabidopsis thaliana*

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Keywords: adenylyl cyclases; *Arabidopsis thaliana*; cyclic nucleotides; plant signalling

Adenylyl cyclases (ACs) are the enzymes which catalyse the conversion of adenosine 5′-triphosphate (ATP) to 3',5'-cyclic adenosine monophosphate (cAMP) and pyrophosphate. The occurrence of cAMP in plants has been established, however in contrast to the well-documented situation in the animal kingdom, the knowledge about structure and physiological role of ACs in higher plants is quite obscure. It is known that these proteins have very low sequence, structural and biochemical homology with their bacterial and animal counterparts. However, new bioinformatics algorithms provide opportunities to broaden our currently limited understanding of adenylyl cyclases in plants. The most promising method is to use highly conserved catalytic centre motifs to identify new plant purine nucleotide cyclases. Using guanylyl cyclase (GC) catalytic centre search motif, modified for specificity for ATP rather than GTP binding, several ACs candidates in *Arabidopsis thaliana* genome were identified. Two of them, a clathrin assembly protein (accession no NP_001323408) and protein belonging to F-box family (accession no NP_001118726) were biochemically analysed during our studies. *In silico* analysis revealed that both of them, include a 14-amino acid long highly conserved motif characteristic for plant ACs.

In order to experimentally confirm hypothetical adenylyl cyclase activity of these proteins, open reading frames (ORFs) of clathrin assembly and F-box proteins were cloned into pGEX-6P-2 expression vector. The *Escherichia coli* BL21 strain, transformed with the resulting constructs, was used to produce the GST-tagged recombinant proteins. The homogeneity and purity of eluted protein fractions were analysed using SDS-PAGE electrophoresis followed by the Western blot.

Additionally, the complementation test that was performed to analyze the ability of both genes to compensate for the AC deficiency in the *Escherichia coli* SP850 strain revealed that two studied proteins function as an adenylyl cyclase and produce cyclic AMP (deep purple colonies were found on MacConkey medium). The screen test was confirmed by *in vitro* enzymatic analysis in which the pure proteins (without GST tag) were able to form cAMP from ATP.

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Droseraceae secondary metabolites as component of poultry feed additives

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Keywords: seconadary metabolites; feed additives; *Droseraceae*

1. Introduction. Plant secondary metabolites are extensively used in medicine and industry. Large amounts of high-quality plant material can be obtained by *in vitro* cultures. The main active compound for *Droseraceae* is plumbagin. Plumbagin and other secondary metabolites can be used as substances which support the development of physiological gut microflora and as compounds that promote the healthy growth of domestic fowl. This work aims to analyse quantitatively and qualitatively *Droseraceae* ethanolic extracts and to assess the suitability of the *Drosera* plants as a potential feed additive.

2. Materials and Methods. Plant cultures were cultivated on solid medium with active carbon (1/2 MS, pH 5,5; saccharose concentration 2%). Cultures were cultivated for four months. After plants' growth material was collected, cleaned from medium and stored in -20˚C. Next 100 mg of frozen plant material was extracted in 1ml of 95% ethanol for 24 h at room temperature. After this time extracts were collected and analyzed. The analysis was performed with the use of HPLC system Ultimate 3000 (Dionex, USA) with C18 column modified with phenyl groups Zorbax SB-Phenyl 3,5 µm, 4,6 mm x 150 mm (Agilent, USA), UV-VIS detector at wavelength 254 nm and gradient elution program.

3. Results. Among the tested species, the major compound in them was plumbagin. All tested species synthesize ellagic acid (amounts range 0,29–0,66 mg/g). The highest production of hyperoside occurred in *D. madagascariensis* (1 mg/g). *D. dielsiana* and *D. ramentacea* also produce hyperoside in high quantities. However, they are smaller than compound amounts in *D. madagascariensis* (*D. dielsiana* 0,72 mg/g, *D. ramentacea* 0,63 mg/g). *D. ramentacea* contains ramentaceone, not plumbagin.

4. Discussion. Plant extracts are rich in biologically active compounds. They have great antimicrobial potential. They can be used in combination with other substances such as antimicrobial agents or natural feed additives that support the development of farm animals beneficial microflora. Culture conditions for species producing the highest concentrations of plumbagin and ellagic acid should be further investigated. Appropriate selection of parameters promoting growth and secondary metabolism will enable broadening the scale of production and introducing a plant feed additive to the market.

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Facilitated penetration of biologically active compounds into crop plants by use of simple organic compounds

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Keywords: herbicides; acetals; nanoparticles; *Zea mays* L.; *Solanum lycopersicum* L.

One of the recent trends in biological research is the use of various types of nanoparticles, for instance carbon nanotubes or gold nanoparticles. Since they are not degraded and may accumulate in the plant or environment, they can have harmful impact on human and animals health. Therefore, their use in agriculture is still controversial.

In this study, acetals as nanoparticles were used. Assuming that this kind of nanoparticle is composed of solly three basic biogenic elements: carbon, oxygen and hydrogen, it can be suggested it would be metabolized by plant and embedded into its structure. In order to verify whether the tested compounds facilitate the penetration of various types of biologically active compounds like herbicides, the Roundup was chosen as the most popular and controversial.

The experiments were carried out using two common crop plants: *Zea mays* L. cv Kosmo and *Solanum lycopersicum* L. cv Moneymaker growing in hydroponic culture. It was found that acetals facilitate the foliar penetration of the herbicide and in consequence increase its concentration in the plant. Results of this project may in consequence reduce the amount of commercial used herbicides in agriculture.

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A novel plant ethylene carbonate fluorescence *in situ* hybridization (EC-FISH) technique – advantages and limitations

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Keywords: chromosomes; ethylene carbonate fluorescence *in situ* hybridization (EC-FISH); plants

Fluorescence *in situ* hybridization (FISH) with DNA probes is a potent and broadly employed technique in biotechnology and medicine. However, its disadvantage is the usage of highly toxic formaldehyde and formamide, time-consumption (at least overnight hybridization) and requirement for experimentally determined heat denaturation of chromosomes at high temperatures – all easily resulting in heat-induced deterioration of chromosomal structural details.

Recently, a novel rapid FISH technique has been developed for molecular cytogenetics (Matthiesen and Hansen 2012, Golczyk 2019) with ethylene carbonate (EC) as the formamide substitute. Here I show the advantages and limitations of this technique in an array of plants: *Allium*, *Nigella*, *Oenothera*, *Rumex*, *Tradescantia*, *Vicia*. The method was tested both for double stranded probes (26S rDNA, 5S rDNA, species-specific DNA repeats, PCR-generated telomeric probe) as well as for single stranded ones (synthetic oligos). Briefly, different types of squash preparations were tested, from thin ones – deprived of any cytoplasm, to thick – containing cytoplasm; subjected or not subjected to microwave treatment. For cytoplasm-free preparations, the technique allowed getting rid of formaldehyde and RNA-se A treatment, giving hybridization signals in the absence of heat denaturation of both the probe and the target prior to hybridization. Denaturation and hybridization could be proceeded simultaneously in the same solution – overnight or rapidly – for 2–3 h at moderate temperatures. Additionally, the technique preserved well chromosomal morphology and DAPI-banding. Interestingly, although the procedure did not give satisfying FISH signals in thick preparations, microwave treatment could circumvent this, by increasing cytoplasm permeability for successful hybridization. However, this, in turn, had negative effects on chromosome DAPI-banding. The usefulness of the described results for improving gene-mapping in plants in diverse cytogenetic contexts was discussed.

■■■

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Raman spectroscopy and AFM applied to studies of primary cell walls of Arabidopsis sepal epidermis

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Keywords: Arabidopsis; sepal; Raman spectroscopy; AFM

Cell wall composition is a crucial factor in regulation of plant development and growth. Raman spectroscopy, a technique based on measurements of inelastic photon scattering, has often been applied to studies of secondary cell walls but less so to the primary walls. This technique can be associated with a confocal setup, allowing one to target specific areas of a tissue. Raman spectra contain information about the chemical bonds of the components that are analyzed. These chemical bonds can be associated with specific components of the cell wall, therefore allowing one to quantify their relative abundance. We used this tool to investigate cell wall composition of the abaxial epidermis of *Arabidopsis thaliana* sepal. Several scales were analyzed to assess heterogeneity in cell wall composition (within an individual cell and within an individual sepal) and to compare sepals representing different developmental stages. Another important factor in growth regulation is mechanical anisotropy of the wall. This anisotropy comes mainly from a specific arrangement of cellulose fibrils. We studied the fibrils arrangement on the wall surface facing the protoplast using Atomic Force Microscopy (AFM), the contact-based microscopy. AFM uses a probe to scan the surface of a sample and reveals the topology of the system. We developed a method to expose the wall surface facing the protoplast, in order to analyze the cellulose fibrillary system.

We focused on the wild type (Col-0) of *Arabidopsis thaliana* and compared it to the cellulose synthase interactive protein 1 mutant (*csi1*), in which the complex responsible for the deposition of cellulose fibrils along the cortical microtubules has been impaired. Our results show that the composition of the cell wall in the *csi1* is changed. However, not only the content of cellulose is affected but also that of other wall components. This may be a compensation effect related to a cell response to defects in cellulose fibrils arrangement, that would ensure a proper development.

Inactivation of nuclear genomes in selected species of the Apiaceae family

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Keywords: UV irradiation; protoplast; cumin; coriander; parsley

Ultraviolet (UV) radiation is a frequently used factor that inactivates the nuclear genome. This is used, among other things, to receive donors for the production of asymmetric hybrids. The effect of inactivation depends on the dose of UV radiation, therefore the key aspect is the selection of optimal radiation. This dose should be chosen individually for species, even those belonging to one family.

The aim of our study was to determine the lowest lethal dose of UV radiation for protoplasts of three plant species belonging to the Apiaceae family: cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.) and parsley (*Petroselinum crispum*). Protoplasts were isolated from 3-week old plants grown *in vitro*, according to the procedure described by Grzebelus *et al*. (2012). 0.5 ml of protoplast suspension with a density of 8×10^5 ml^{-1} was applied to a Petri dish and irradiated with a defined dose of ultraviolet radiation in the range of 0–3000 Jm^{-2}, using Crosslinker (CL-1000 UV Crosslinker, UVP), to select the lowest lethal UV dose degrading the nuclear genome. A solution of sodium alginate was added to a defined volume of UV-treated protoplast solution in a volume ratio of 1: 1. This suspension was applied to Petri dish with solidified calcium-agar medium and a circular layer was formed by gentle rotation of Petri dish. After solidification the thin layer with embedded cells was transferred to Petri dish with protoplast medium. The culture was carried out in the dark at 26 ± 2°C for 2 months. During the culture, parameters demonstrating the degradation of the nuclear genome were monitored. The protoplast viability was evaluated immediately after irradiation and 24 hours later. Plating efficiency was determined after 20 days of culture and the presence of callus tissue for each UV inactivation variant was verified after 2 months.

The applied UV doses decreased both the protoplast viability and the mitotic activity of protoplast-derived cells. On the basis of the obtained results, and especially the last parameter examined – macroscopic evaluation in 2-month old cultures, it was possible to determine the lowest lethal doses for the examined objects. In the case of cumin, the dose of 2500 Jm^{-2} effectively prevented the formation of callus tissue, for coriander the lowest lethal dose was 1500 Jm^{-2}, while for parsley it was 1000 Jm^{-2}.

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Development of an efficient and stable technique of genetic transformation of *Coccomyxa subellipsoidea* C-169

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Keywords: stable genomic transformation; *Coccomyxa subellipsoidea*; electroporation; biolistic bombardment gun; PEG

The ability to introduce foreign genes or produce deletions/modifications of existing genes will be a powerful and universal tool enabling functional and structural studies of multiple biological aspects in the plants using stable mutant lines. The experimental material was unicellular green alga *Coccomyxa subellipsoidea* C-169, adapted to an extremely difficult Antarctic environment, its optimum growth is around 20°C. Also, it is the first organism from the polar environment with a fully recognized genome sequence, thus becoming an excellent model organism to study the mechanisms of plant adaptation to function in low temperatures. The aim of this work was to develop an efficient and stable transformation of the C-169 nuclear genome.

The first stage of the research was the development of an appropriate method for the selection of green algae transformation mutants. For this purpose, antibiotic tests were carried out to determine the selection marker, several antibiotics have been tested. Finally, hygromycin B was chosen as an effective selection marker.

To increase the chance of efficient gene expression, three plasmids were constructed and examined. First one, contained a hygromycin B resistance gene under the control of the CaMV 35s promoter (Cauliflower mosaic virus), in the second plasmid hygromycin B expression was ensured by regulatory region of the small subunit gene Rubisco from *Coccomyxa subellipsoidea*. The third transformation vector contained selective marker gene with codons optimized to the genus *Coccomyxa* sp. under the control CaMV 35s promoter.

Transformation was performed by three methods: electroporation, PEG mixture and biolistic bombardment gun. It has been shown, that only electroporation is effective transformation method of C-169 cells.

Analysis of deformations of the lateral root primordia in *Arabidopsis thaliana*

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Keywords: lateral root; mechanical stress; root morphogenesis; discrete Fourier transform

Typically, a lateral root (LR) primordium forms a regular dome with elliptical base. In the axial section, surface of a primordium resembles a parabola-like line. However, in numerous primordia specific morphological defects are observed. Most of such cases concern young primordia, that have not emerged over the parent root surface.

The aim of the study was to try classifying various shapes of LR primordia in *Arabidopsis thaliana* with the use of a quantification method. Outlines of the analyzed primordia were represented by curves. Characteristic points of derivatives calculated for the curves indicated three regions of the surface outline refering to structural domains in the developing primordium: the main part (arch), the transitional regions and the outer regions. The main parts were fitted to parabolas using the mean square method.

In case of regularly shaped primordia the arches covered parabolic curves while for irregular primordia only extreme fragments of the arch could be fitted. Discrete Fourier transform application to the arches enabled obtaining an amplitude spectrum for every individual primordium. On this base, using Cluster Analysis, six morphological types of primordia differing in a degree of the surface flattening were proposed. Mechanical energy *U*, a quantity proportional to the sum of amplitudes, was estimated for surfaces of primordia. *U* was shown to increase with the level of deviation from a regular parabola.

A separate analysis performed on primordia at various stages of development revealed that the curves representing outlines of young primordia were well fitted to parabolas and that curvature of the surface increased with the primordium age. The first result suggests that parabolic form may be optimal for developing LR primordium. In the light of the second observation the LR formation resembles of buckling under continuous compressive stress from the sides.

The Fourier transform appeared a good and sensitive tool in quantifying shape and in assessing an extent of possible defects in shape of LR primordia in *Arabidopsis* proving that it could be succesfully applied in morphological study.

Physiological responses of *A. thaliana* seedlings to nanodiamonds

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Keywords: *A. thaliana*; nanodiamonds; uptake; growth; oxidative stress

With development of nanoscience, especially in the field of carbon nanomaterials such as nanodiamonds (ND), increased production and usage may raise many questions concerning their potential toxicity to living organisms. Many biological interactions *in vivo* are discovered in recent years, which provokes to research ND biosafety. Interest in nanodiamonds has been growing lately, due to their unique qualities. They possess various superior characteristics of diamonds, such as chemical stability and extremely high hardness, stiffness and strength. They also have the advantages of nanomaterials, such as small size, large surface area, and high adsorption capacity. These nanoparticles are used in electrochemical coatings, polymer compositions, antifriction coatings, polishing, lubricants and biosensors, imaging probes, implants and drug carriers. In medicine the possibility of using ND in therapy is especially interesting. However, it is possible that ND are toxic both for cellular cultures and whole organisms. The main goal of our study was to investigating the effect of nanodiamonds (ND) on the physiological responses of *A. thaliana* seedlings. It seems to be necessary due to the rising interest of using these substances in many branches of industry. All of the experiments were performed on hydroponically grown *A. thaliana* seedlings expressing plasmalemma-associated LTI6B–GFP. In order to perform a quantitative analysis of nanodiamonds uptake and translocation the fluorescent type of this carbon nanoparticles was used. Transgenic line of *A. thaliana* containing a fusion of a plasmalemma protein (LTI6B) with the green fluorescence protein enables the visualization and tracing of the fluorescence nanodiamonds inside the seedlings tissues. Analysis of physiological responses such as growth of the seedlings, oxidative stress level and efficiency of photosynthesis were also conducted.

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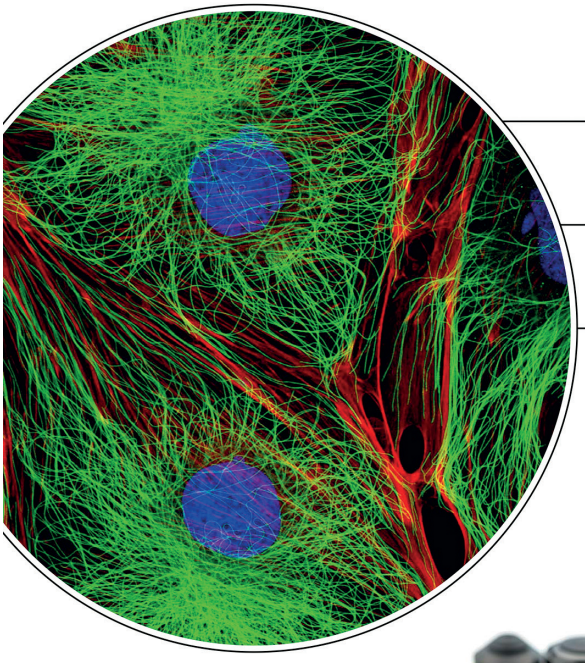

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