Plenary Lecture

PL1.1

On the biological relevance of alternative splicing in plants: dual function of an *Arabidopsis* membrane transporter

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Eukaryotic genes typically contain introns that are removed post-transcriptionally from the precursor mRNA (pre-mRNA) through splicing. The presence of numerous exons per gene enables the splicing machinery to process the same pre-mRNA differently by selectively joining different exons, generating different transcripts from a single gene via a process named alternative splicing. In contrast to transcriptional control, alternative splicing can influence almost all aspects of protein function and has emerged as a key mechanism for generating proteome diversity and functional complexity. Its prevalence in many genomes, including those of higher plants, suggests that alternative splicing plays crucial roles in biological processes, as is emphasized by the fact that its misregulation can lead to many human diseases. However, information on the functional significance of this posttranscriptional regulation mechanism in plant systems is surprisingly scarce. We have identified an *Arabidopsis thaliana* gene, *ZIFL1*, encoding a membrane transporter from the Major Facilitator Superfamily that plays important roles in both root auxin transport and drought stress tolerance. Selection of an alternative 3′ splice site in the *ZIFL1* pre-mRNA generates two splice variants that differ in only two nucleotides. While the longer transcript encodes the full-length transporter, the shorter contains a premature stop codon and codes for a truncated protein lacking the 67 C-terminal amino acids. Sequencing, promoter-reporter gene and fluorescent protein fusion experiments indicate that the full-length protein localizes specifically at the tonoplast of root cells, whereas the C-terminal truncation targets the transporter to the plasma membrane of stomatal guard cells. Using reverse genetics, we show that the root tonoplast-localized transporter regulates various auxin-related processes, while the truncated protein mediates drought tolerance by regulating stomatal closure. Heterologous expression in yeast revealed that the two splice forms share proton-coupled potassium transport activity. Thus, by determining the subcellular and tissue localization of two isoforms, alternative splicing allows the same gene to fulfill two very different but equally relevant roles in the plant.

PL1.2

The microRNA pathway in plants

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MicroRNAs (miRNAs) regulate plant development by post-transcriptional regulation of target genes. In *Arabidopsis thaliana*, DCL1 processes precursors (pri-miRNAs) to miRNA duplexes, which associate with AGO1. Additional proteins act in concert with DCL1 (e.g. HYL1 and SERRATE) or AGO1, respectively, to facilitate efficient and precise pri-miRNA processing and miRNA loading. After a general introduction into processing, mode of action and functions in development of plant miRNAs, I will show our attempts to identify novel components of the plant miRNA pathway. We found that the accumulation of plant microRNAs depends on RECEPTOR FOR ACTIVATED C KINASE 1 (RACK1), a scaffold protein found in all higher eukaryotes. miRNA levels are reduced in *rack1* mutants and our data suggest that RACK1 affects the microRNA pathway via several distinct mechanisms involving direct interactions with known microRNA factors: RACK1 ensures the accumulation and processing accuracy of some pri-miRNAs, directly interacts with SERRATE and is part of an AGO1 complex. As a result, mutations in RACK1 lead to misregulation of miRNA target genes, which is important for ABA responses and phyllotaxy. In conclusion, our study discovered complex functioning of RACK1 proteins in the Arabidopsis miRNA pathway, which are important for miRNA production and therefore plant development.

Oral Presentation

O1.1

Consequences of mitochondrial translation perturbation for the whole cell transcriptome

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We have recently shown that the silencing of the *Arabidopsis RPS10* gene encoding mitochondrial ribosomal S10 disturbs the ratio between small and large mitoribosome subunits (Kwasniak et al., 2013). The altered mitoribosome status had differential effects on mitochondrially produced proteins, suggesting that translation of individual mitochondrial proteins can be regulated by ribosome composition. Here, the whole cell transcriptome of two hemizygous *rps10* phenotypes, P2 and P3, differing by the onset of silencing was examined by microarrays (Affymetrix, ATH1 chips). We found that expression of 1287 and 3661 genes was altered with a change of more than 2-fold as compared to wild type plants, in P2 and P3 phenotypes, respectively. Furthermore, a subset of 1023 genes was similarly affected in both phenotypes. Among them 616 genes were up-regulated and 407 were down-regulated. All of these common genes were categorized according to subcellular localization of their products using the *Arabidopsis* database SUBA. It was revealed that approximately 20% of these gene products were localized in nucleus, 18% in cytosol and 16% in plasma membrane while 14% in chloroplasts and 12% in mitochondria. The affected genes within each compartment were then sorted into functional gene categories. This classification revealed interesting findings concerning genes encoding chloroplast proteins affected in *rps10*. These genes are mainly involved in regulation of transcription (10%), metabolism (9%) biotic and abiotic stresses (7%) as well as transport (6%). The most interesting outcome of these studies was identification of the group of genes involved in chloroplast transcription, which are components of the plastid transcriptionally active chromosome complex (PTAC). Our analysis revealed that the level of the vast majority of PTAC transcripts were significantly decreased in *rps10* compared to wild type plants. Given the fact that PTAC is necessary for chloroplast biogenesis, we speculate that the defect in *rps10* cells probably leads to meaningful changes in chloroplast function. Furthermore, our analysis showed significantly perturbed biosynthesis of hem and chlorophyll in *rps10* mutants, which indicates disturbances in tetrapyrrole metabolism. Consequently, it was shown that genes involved in tetrapyrrole pathway are also down-regulated, which in turn is correlated with a 50% decrease in chlorophyll level in *rps10* mutants. Our data gives strong evidence that the consequences of mitochondrial perturbations affect also the other organelles. For both *rps10* phenotypes, chloroplast-related gene categories were identified as the most responsive. The alterations in expression of genes related with other compartments will be presented on this poster.

O1.2

U12 intron is evolutionary conserved in plant nuclear cap binding protein (CBP20) genes and is required for correct pre-miRNA splicing and proper mRNA/protein level

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Eucaryotic RNA polymerase II transcripts are characterized by the presence of the cap structure and polyA tail at their 5′ and 3′ ends, respectively. Cap Binding Complex (CBC) is a nuclear complex composed of two cap-binding proteins: CBP20 and CBP80. It is known that binding of the (CBC to the 5′ cap is crucial for the proper mRNA maturation and transport. We show that the *CBP20* gene structure is highly conserved across land plants from liverwort

higher plants. The gene contains always seven introns with the fourth intron belonging to U12 class. Additionally the U12 intron divides the gene in two parts: one that encodes the core domain containing RNA recognition motif and the second one that encodes the tail domain containing Nuclear Localization Signal (NLS). In all investigated plants *CBP20* genes first four exons coding the core domain have always the same length whereas exons coding the terminal domain differ considerably in length. To answer the question why the presence and location of the U12 intron in *CBP20* gene is preserved across all plant species we prepared constructs representing *CBP20* mini-genes and its mutated full versions. Mini-gene constructs containing 4^{th} and 5^{th} exons from *A. thaliana CBP20* gene and U12 introns derived from different plants and differing in length (from 134nt to 2733nt) were transfected to tobacco mezophyll protoplasts and splicing was analysed. Our results show that the longer the U12 intron the more efficient splicing was observed. Additionally, transcripts splicing analyses of mini-gene construct containing U2 intron in U12 intron natural position revealed that 37% of mRNAs undergo alternative splicing. Additionally we prepared five constructs containing *A. thaliana CBP20* gene in which (i) U12 intron was removed, (ii) replaced by U2 one, and (iii-v) U12 intron was moved to different locations within the gene body. These constructs were introduced into *A. thaliana cbp20* T-DNA insertion mutant. Our results show that transcripts derived from the mutated *CBP20* genes (in which the U12 intron was moved to the other gene locations) generate additional alternatively spliced isoforms containing retained intron and/or longer/shorter exons (because of alternative 3′ and 5′ splice site selection). Moreover, plants containing mutated *CBP20* gene in which the U12 intron was replaced with U2 one show altered phenotype – changes in leaf size which are larger. In addition we found that the levels of *CBP 20* mRNA and protein are strongly down-regulated and there are changes in selected pri-miRNA expression profiles. All these data suggest that U12 intron in the proper position in plant *CBP20* genes is necessary for correct pre-mRNA splicing and mRNA/protein level.

O1.3

The GUN4 protein plays a regulatory role in tetrapyrrole biosynthesis pathway and is required for chloroplast-to-nucleus signalling in unicellular green algae *Chlamydomonas reinhardtii*

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In aerobic photosynthetic organisms, the GUN4 (GENOME UNCOUPLED 4) protein plays a regulatory role in tetrapyrrole biosynthesis and is involved in chloroplast-to-nucleus signalling. GUN4 binds protoporphyrin IX (ProtoIX) and MgProtoIX and interacts with the CHLH subunit of Mg chelatase. Using the *C. reinhardtii gun4* knock-out mutant we explored the role of GUN4 for activities in the Mg branch and the flux-limiting step of 5-aminolevulinic acid (ALA) synthesis, as well as on nuclear gene expression. The *gun4* strain displays a higher sensitivity to moderate light intensities and accumulates 40% of the wild-type chlorophyll level under these light conditions. When higher photosynthetic rates are enforced on media without reduced carbon source, even low-light intensities are lethal for *gun4*. In the dark wild-type-like levels of chlorophyll are observed in *gun4.* However, light and dark-grown *gun4* accumulates high amounts of ProtoIX and generates1O2 upon light illumination. Accumulated 1O2 is harmful, but it is also involved in chloroplast-to-nucleus signalling. Despite accumulating ProtoIX and induced oxidative stress, surprisingly *gun4* also up-regulates transcription of nuclear genes encoding enzymes of tetrapyrrole biosynthesis and the photosynthetic light-harvesting complex. Interestingly, similar enhanced nuclear gene expression was observed in *gun4* after exogenously applied Rose Bengal which acts as a strong photosensitizer generating 1O2. We propose that GUN4 functions in the control of balanced steady state levels of ProtoIX and MgProtoIX and is required for the transmission of 1O2-mediated retrograde signalling triggered by photosensitizing tetrapyrrole intermediates. By these means, GUN4 contributes to changes in nuclear gene expression triggered by signals originating in the chloroplast.

O1.4

Arabidopsis thaliana wall associated kinase-like protein 22 (WAKL22) takes part in development and/or management of syncytium induced by *Heterodera schachtii*

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Beet cyst nematode (*Heterodera schachtii*) induces development of multinuclear feeding structures – syncytia in the roots of *Arabidopsis thaliana*. Syncytium is transcriptionally, ultrastructurally and metabolically different from other plant cells. It is formed by local degradation of plant cell walls followed by fusion of neighbouring cell protoplasts. Syncytium is the only source of nutrients for the nematode. Wall associated kinase (*WAK*) and kinase-like (*WAKL*) genes encode transmembrane proteins, which physically link cytoplasm and extracellular matrix thus possibly taking part in signalling across plasma membrane. WAK and WAKL proteins are involved in plant stress responses and regulation of developmental processes. Semi-quantitative RT-PCR analysis showed higher level of *WAKL22* gene expression in root segments containing syncytia at 3, 7 and 15 days post inoculation with *H. schachtii*. The phenotype of plants with knocked-out *WAKL22* gene was not different from the phenotype of wild type Col-0 *Arabidopsis* plants. However, mutation in *WAKL22* gene reduced the number of developing nematodes, suggesting that WAKL22 is involved in development and/or management of nematode feeding site.

O1.5

MiR396 involvement in regulation of somatic embryogenesis in *Arabidopsis*

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MicroRNAs (miRNAs), products of non-protein coding *MIRNA* genes, are key regulators of gene expression in many eukaryotic organisms. In plants, miRNAs were indicated to control hormone signaling, plant responses to environmental stresses, pathogen invasions and numerous processes related to plant growth and development, including zygotic embryogenesis. Thus, it is believed that miRNAs are also essential for somatic embryogenesis (SE), the embryogenic pathway of development inducted under *in vitro* culture in somatic cells. In support, the study on SE induced *in vitro* in culture of Arabidopsis indicated that: 1) lost of DICER LIKE1 (DCL1) activity, required for miRNAs biogenesis in *dcl1* mutant, results in total inhibition of SE-induction 2) numerous *MIRNA* genes, including *MIR396a*, are differentially expressed during SE. *MIR396a* gene was found up-regulated (150 folds) in SE-induction stage.The aim of study was to verify that miR396 is involved in the regulation of SE in Arabidopsis. To this end, the 35S::MIR396b transgenic line overexpressing *MIR396b* was analysed. The analysis of SE induced in Arabidopsis indicated that overexpression of *MIR396b* results in a significant increase in production of somatic embryos. Moreover, to reveal the role of miR396 particles in SE, we aimed at identification of their targets in embryogenic culture. Accordingly, the activity of putative genes controlled by miR396 and encoding *GROWTH-REGULATING FACTORs* (*GRFs*) transcription factors were analysed in SE. GRFs are believed to control cell proliferation during leaf development in Arabidopsis. With the use of Real-Time qPCR four *GRF* genes (*GRF1, GRF4, GRF8* and *GRF9*) were indicated to display differential expression pattern in embryogenic culture. To indicate *GRFs* regulated by miR396, the expression levels of its putative targets were compared in cultures of Col-0 (WT) and the transgenic 35S::MIR396b line. The analysis indicated distinctly reduced expression level of *GRF1, GRF8* and *GRF9* in embryogenic culture overexpressing miR396 than in WT once. In conclusion, the results suggest that miR396: 1) is essential for efficient induction of SE, 2) three of *GRFs* (*GRF1, GRF8* and *GRF9*) are negatively regulated by miR396 during SE. Further analysis is in progress to reveal other genetic components of miR396-mediated control of embryogenic induction in somatic cells.

O1.6

Poly(A) RNA accumulation/storage in Cajal bodies in the plant cells

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The Cajal bodies (CBs) are common structure of eukaryotic nucleus. CBs have been implicated in RNA-related metabolic processes such as snRNPs biogenesis, maturation and recycling, histone mRNA processing and telomere maintenance. There are some differences between plants and animals in the composition and function of CBs. Plant Cajal bodies participate for example in the siRNA biogenesis and recently mRNA was found in the Cajal bodies of meiotic cells. In the present study, we examined if mRNA occurrence in the CBs in the plants somatic cells. To answer those questions, in situ hybridization techniques and immunocytochemical methods either reticular (*Allium cepa*) or chromocentric (*Lupinus luteus*) nuclei were used. Using a double-labeling technique U2B′ and U2snRNA with RNA poly(A) detection we found occurrence mRNA in the Cajal bodies of chromocentric and reticular plant cell. In situ hybridization and electron microscopy analysis confirmed this result. Additionally our preliminary experiment indicated stronger accumulation mRNA in CBs in the inhibition transcription and hypoxia-treated cells. These results show that mRNA accumulation/storage in CBs is common and occurs in the reticular and chromocentric nuclei plant cells.

Posters

P1.1

Identification and analysis of cDNA *LlTIR1* – the miR393 target gene in *Lupinus luteus*

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Plant hormones, auxin, have been implicated in almost every aspects of plant development. In the past few years, it was shown that TIR1 (TRANSPORT INHIBITOR RESPONSE1) and some close homologs act as auxin receptors in *Arabidopsis thaliana*. TIR1 is an integral component of the SKP1/CULLIN/F-BOX PROTEIN (SCF)TIR1 complex that mediates the degradation of AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) proteins. AUX/IAAs are bound by TIR1 in the TIR1 pocket via their domain II, and auxin acts as molecular glue to enhance this interaction. Proteolysis of AUX/IAA allows ARF (AUXIN RESPONSE FACTOR) transcription factors to activate the promoters of primary auxin-responsive genes. Moreover, recently studies have shown that the expression of *TIR1* gene is regulated post-transcriptionally by the microRNA miR393. Micro RNAs about 21 nt in length are a major part of the complex responsible for gene silencing at posttranscriptional level, usually by cutting the target mRNA strand. The aim of this study was to identify and analyze cDNA of *TIR1* gene homologue in yellow lupine (*Lupinus luteus* L.), Mister and Taper cultivars. Identification was made using the degenerate primers, complementary to the consensus sequence present in the *TIR1* genes in other species of plants. Sequences identified in both lupine cultivars are identical and indicate a high sequence similarity to *TIR1* cDNA genes from other plant species. In addition, *LlTIR1* contain sequence complementary to miR393. The work was supported by the Multi-Year Programme of the Polish Ministry of Agriculture and Rural Development, No. 149/2011.

P1.2

Gene-to-metabolite network for coumarins biosynthesis in natural accessions of *Arabidopsis thaliana*

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Coumarins are a group of secondary metabolites that provide plants antimicrobial and antioxidative activities. As phytoalexins, they are produced in response to different biotic and abiotic stresses. Importantly, coumarins have many biological activities and as a consequence their multi-pharmacological properties are widely used in medical applications and cosmetics industry. Recently, it was shown that some coumarins (scopoletin, scopolin, skimmine and esculin) are produced in roots and shoots of model organism *Arabidopsis thaliana*. Accessions of Arabidopsis originating from different habitats exhibit natural variation in a range of traits including secondary metabolites accumulation. This great phenotypic variation reflects selection for adaptation to a wide distribution of Arabidopsis through the north hemisphere, providing genetic variation in responses to local environmental factors. Nowadays, natural genetic variation is becoming a very powerful tool in plant biology and is currently being used in the fields of genomics, metabolomics and transcriptomics. It was shown in our group that a significant natural variation in the accumulation of coumarins is present among 28 Arabidopsis accessions. In the present work, we have tested the expression level of genes involved in coumarins biosynthesis using real-time qPCR in 3 Arabidopsis accessions and quantified the level of selected secondary metabolites using UPLC. These accessions were selected according to the contrasted accumulation of scopoletin as was shown previously in our group. The expression profiling of coumarins biosynthetic genes performed by qPCR have revealed the presence of interesting differences in gene expression level linked with the contrasting coumarins accumulation in tested accessions. Additionally, we have observed that some of the coumarins biosynthetic genes were particularly induced when plants were grown in *in vitro* cultures as compared with plants grown in soil mixes. This research is supported by grants from the National Science Centre (6815/B/P01/2011/40) and by European Union under the European Social Fund “Kształcimy Najlepszych – kompleksowy program rozwoju doktorantów, młodych doktorów oraz akademickiej kadry dydaktycznej Uniwarsytetu Gdańskiego – Zad. 2. Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS)”.

P1.3

Comprehensive studies on biogenesis and function of tRNA-derived small noncoding RNAs in *Arabidopsis thaliana*

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Small noncoding RNAs (18-35 nt sRNAs) have emerged as important factors in regulation of gene expression. They have been reported to play a significant role in regulation of plant growth and development, and are increasingly recognized as important players in responses to environmental stresses. The sRNAs act by silencing gene expression on post-transcriptonal and transcriptonal level. Besides extensively studied microRNAs and siRNAs, other small functional RNAs have been identified in various organisms by using high-throughput sequencing techniques. Recent reports indicate that abundant, noncoding RNAs, like rRNA, tRNA and snoRNA may, aside of their primary function, be a source of small regulatory RNAs. We are interested in tRNA-derived small RNAs species. Initial screening of publicly available data from deep sequencing experiments allowed us to select four 20-22nt long sRNAs

derived from tRNAs. Their physical presence in the Arabidopsis transcriptome was experimentally verified by using Northern hybridization. Positive signals in the expected range of 20-30 nt may implicate a precise processing and stability of the fragments as well as indicate their potential regulatory capabilities. Moreover, we showed that precursor of one of the seven Gln-tRNA^{TTG} can, aside of being source of small RNA molecule, fold into an alternative secondary structure that is similar to and could function as a miRNA precursor. Northern hybridization technique revealed that the level of this tRF (tRNA-derived fragment) is SE, HYL1 and DCL1-dependant. By using interdisciplinary, whole genome approach based on bioinformatics and molecular biology techniques, we plan to identify sRNAs derived from tRNAs, study molecular mechanisms involved in their biogenesis as well as determine their potential regulatory function in a model plant *Arabidopsis thaliana*. The initial phase of our research includes identification of molecular mechanisms involved in biogenesis of tRNA-derived short RNA molecules. By using high-throughput sequencing techniques (Illumina, SOLEXA) we plan to analyze changes in identified tRNA-derived sRNAs expression profiles in a set of representative *Arabidopsis* mutants selected based on the present knowledge about biogenesis and degradation of tRNA and microRNA/siRNA transcripts. We assume that sRNAs resulting from tRNA cleavages can represent a byproduct of tRNA maturation and/or degradation machinery or alternatively, can be processed by factors involved in siRNA/microRNA biogenesis.

P1.4

Introns of plant pri-miRNAs enhance miRNA biogenesis

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MicroRNAs (miRNAs) are 20-22-nt-long small RNAs that regulate the expression of genes involved in critical developmental programmes or in response to specific environmental conditions. While animal miRNAs are generally embedded into introns of protein-coding genes, plant miRNAs are encoded by independent MIR genes that are transcribed by Pol II to yield long primary miRNA precursors (pri-miRNAs). Processing of the pri-miRNAs occurs in two steps by the RNAse III enzyme DICER-LIKE1 (DCL1) and its main double-strand RNA-binding partner DRB1/HYL1. The first cut generates the intermediate hairpin-containing pre-miRNAs, whereas the second cut releases the miRNA/miRNA* duplexes. The miRNA strand is then incorporated into ARGONAUTE (AGO) effector complexes to guide RNA cleavage or translation inhibition. We have recently shown that plant pri-miRNAs are unexpectedly long and contain one or more introns located usually in their 3′ regions downstream of the hairpin folds that encode the miRNA duplexes. These pri-miRNAs are bound, similarly to pre-mRNAs, by the nuclear cap-binding complex (CBC) proteins, CBP80 and CBP20, to promote their splicing in a step that involves the C2H2 zinc finger protein SERRATE, which is proposed to bridge the CBC and the spliceosome. For two miRNA genes *MIR163*, and *MIR161*, we show that introns are crucial for the accumulation of proper levels of mature miRNA. Removal of the intron in both cases led to a dropoff in the level of mature miRNAs. We demonstrate that the stimulating effects of the intron mostly reside in the 5′ss rather than on a genuine splicing event. Our findings are biologically significant since the presence of functional splice sites in the gene appears mandatory for pathogen-triggered accumulation of miR163 and proper regulation of at least one of its targets.

P1.5

The crosstalk between splicing of protein-coding genes and processing of microRNAs located within their introns

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MicroRNAs are small non-coding RNAs of about 21 nt in length, which take part in a wide variety of physiological and cellular processes. MiRNAs act by regulating expression of many genes. The target mRNAs are recognized by miRNAs in a sequence-specific manner and subsequently cleaved or blocked inhibiting translation. In plants, miRNAs are encoded mostly by independent transcriptional units and are transcribed into long non-coding primary transcripts (pri-miRNAs), containing miRNA sequences. We have already identified the structure of 44 *Arabidopsis MIR* genes. It has been also reported that 11 plant miRNAs are embedded within intron of protein-coding genes. Our further bioinformatic analyses revealed the existence of additional 18 intronic microRNAs. The 5′ RLM RACE experiments showed that most of intronic miRNAs are within protein-coding transcriptional units and their transcription starts from host-gene promoters. The mechanism of intronic microRNAs biogenesis is still unknown. There are two major players in this process – miRNA-biogenesis proteins and splicing machinery, but the role of both of them remains unclear. By now three pathways are proposed: 1) pre-splicing, 2) co-splicing or 3) post-splicing microRNA cleavage, but none of them is experimentally confirmed. To outline the role of microRNA biogenesis and splicing in intronic miRNAs maturation we selected miR400 and miR402, both of them located within first intron of protein coding host genes – At1g32583 and At1g77230, respectively. To test the importance of microRNA biogenesis proteins (HYL1, SE and CBC) the expression level of chosen miRNAs was examined in *Arabidopsis thaliana* mutants. The significant changes of the miR400 and miR402 level in *hyl1-2* and *se1* mutants clearly showed the great impact of these two proteins on intronic miRNA biogenesis, but no effect was visible in case of *cbc* mutant. This might suggest that CBC-protein complex is less important during their biogenesis. To evaluate the role of splicing machinery in microRNA maturation we generated constructs containing host-genes under control of the 35S promoter and transiently expressed them in *N. benthamiana*. In both cases the conservative and alternative splice sites were mutated in the following combinations: 1) only 5′SS; 2) only 3′SS; 3) only 5′ASS; 4) 5′SS + 5′ASS; 5) 5′SS + 3′SS; 6) 5′ASS + 3′SS; 7) 5′SS + 5′ASS + 3′SS. Using real-time PCR technique we analyzed changes of splicing pattern of microRNA-containing introns and the accumulation of both pri-miRNAs in each transgenic line. The expression level of mature miR400 and miR402 was checked by Northern Blot technique. The results will be presented and discussed in the poster.

P1.6

Experimental validation of novel microRNA candidates in the liverwort *Pellia endiviifolia*

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MicroRNAs are short (18-24 nt) sequence-specific regulatory molecules of eukaryotic gene expression. Until now there are no publicly available data on any liverwort microtranscriptome. With the use of high-throughput sequencing techniques (SOLEXA, Illumina) we sequenced small RNAs from the dioecious liverwort *Pellia endiviifolia* species B. 246 conservative miRNA species belonging to 62 miRNA families that are identical to *Physcomitrella patens* or higher plants miRNAs were identified. The presence of selected conservative miRNAs identified in *P. endiviifolia* was confirmed by northern hybridization. Moreover, three miRNAs with one nucleotide substitution

to known, conservative plant miRNAs and more than 50 with two nucleotide substitutions to miRNAs from other plants were found. With the use of bioinformatic approaches we study novel *Pellia endiviifolia* miRNA candidates which have not been previously described. A novel algorithm was implemented for the identification of new functional sRNAs. The annotation procedure involved clustering of sequence reads, size and expression profiling of cluster components as well as identification of functional sRNA features characteristic for already described sequences from other plants. Using northern hybridization and splinted ligation technique we evidenced the presence of forty one ~21-nt long stable, small RNAs, which can represent novel, unique liverwort miRNAs. Analysis of *P. endiviifolia* transcriptome revealed the presence of at least twenty miRNA putative precursors. Several of them were already verified using experimental approaches like RACE and genome walking. In the case of conservative miRNAs data obtained from these analyses show pre-miRNA structure conservation between liverworts and mosses. However, the sequence conservation is restricted only to mature miRNA and miRNA* region. Using bioinformatics tools we selected putative targets for conservative and novel miRNAs. We are now validating selected targets using 5′RACE-obtained results strongly suggest an existing regulation of target mRNA levels for chosen miRNA species.

P1.7

The expression of *UVR3* and two putative photolyases, *PHR2* and *At4g25290*, is regulated by light

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UVB is known to induce a formation of dimers between adjacent pyrimidines in a DNA strand. Such photoproducts block the progress of DNA and RNA polymerases along the DNA strand during replication and transcription. Thus, they may be both mutagenic and cytotoxic. Pyrimidine dimers in plant DNA are removed via highly efficient light repair (photoreactivation repair) and/or relatively inefficient dark repair mechanisms (excision repair pathways). Photoreactivation repair is catalysed by photolyases, enzymes using blue light to excise DNA lesions. According to The *Arabidopsis* Information Resource database five gene loci encoding proteins with photolyase activity were found in *Arabidopsis* genome: i) *AT1G12370* (*PHR1* photolyase1, *UVR2*- UV resistance2); ii) *AT3G15620* (*UVR3*); iii) *AT2G47590* (*PHR2*); iv) *AT4G25290* and v) *AT5G24850* (*CRY3* cryptochrome3). Two genes, *AtPHR2* and *At4G25290*, are classified as photolyases only by *in silico* analysis of their sequences. Their activities have never been confirmed either *in vitro* or *in vivo*. It has been shown that expression of *PHR1* is up-regulated strongly by white light, UVA, UVB and UVB + white light and very weakly by blue light. White light-regulation of *UVR3* has been found neither in leaves from plants at the eight-leaf stage nor in seedlings. *AtCRY3* mRNA level is up-regulated by red light via phyA in etiolated *Arabidopsis* seedlings. In our study we tested the influence of light on the mRNA levels of three genes, *UVR3*, *PHR2* and *At4g25290*. We used leaves from 6 week-old wild type *Arabidopsis* dark-adapted overnight and then illuminated with white, blue or red light. The steady state levels of both *PHR2* and *At4g25290* mRNAs increased significantly after 3 hours illumination with white light. In contrast, the expression of *UVR3* was strongly down-regulated under the light regime tested. This regulation was very weakly dependent on the used light intensity in the range 100-500 $\mu molm^{-2}s^{-1}$.We analyzed time-course of white light regulation of gene expression and found that for both *PHR2* and *AT4G25290* mRNA the extent of up-regulation increased with time, reaching maximum after 24 hours of irradiation. The down-regulation of *UVR3* was highest after 6 hours of irradiation. In order to identify mechanism underlying this regulation we tested the influence of light of specific wavelengths, blue and red. For all examined genes, both blue and red light affected their expression in the same manner as white light. We analyzed the expression under different light regimes in numerous photoreceptor mutants to deeper understand this phenomenon. We found that the expression of *AtUVR3*, *AtPHR2* and *At4g25290* is regulated by light in a complex manner. The study was supported by Polish National Science Centre, a grant no. UMO-2011/03/D/NZ3/00210.

P1.8

Preliminary expression analysis of *FCA* gene from the flowering autonomous pathway of *Lupinus luteus*

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The flowering time is one of the factors providing reproductive success. At least four genetically defined pathways have been described that control flowering: vernalization, photoperiod, gibberellin or hormonal and autonomous pathways. All pathways control of flowering cooperate regulation of key flowering genes which name "integrator genes" or "central floral pathway integrators". The autonomous pathway of flowering induction genes includes at least 7 genes. All these genes are negative regulators of *FLOWERING LOCUS C* (*FLC*) – main inhibitor of generative development. RNA BINDING/FLOWERING TIME CONTROL PROTEIN FCA ALPHA (FCA) contains two conserve domains RNA recognition motifs (RRM) and a WW domain. FCA participates in posttranscriptional *FLC* RNA modifications. *FCA* pre-mRNA is alternatively spliced. *FCA* gene has several alternative versions of different length mRNAs in various tissues it follows that activity of FCA protein is connection with kind of mRNA matrix used for translation. In likely model, FCA acts in binding with FLOWERING TIME CONTROL PROTEIN (FY) to repress *FLC*. In this study, expression of *LlFCA* gene was quantitative used real time PCR technique. *Lupinus luteus* were cultivated in a growth chamber at a temperature of 22 ± 1°C under long day conditions. RNA was isolated from leaves and roots which were collected in 21th, 33th and 45th days of cultivation. Before collected plants were sprayed aqueous solutions of abscisic acid or gibberellins. Control plants were sprayed water. Plants were collected after one hour after sprayed. Preliminary results obtained here will enable us to determine *LlFCA* expression pattern in vegetative organs of *L. luteus* – the agricultural valuable species in Poland. It will also facilitate to characterize the role of these genes in the regulation of development of *L. luteus* crops. This research was supported by the Ministry of Agriculture and Rural Development (Poland) grants No 149/2011 and the National Science Centre (Poland) grants No 2011/01/B/NZ9/03819.

P1.9

LlGA20ox1 and *LlGA20ox2* – the *Lupinus luteus* homologues of *Arabidopsis* genes involved in gibberellin biosynthesis pathway

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The hormone-mediated control of plant growth and development involves both synthesis and response. Due to the fact that previously we cloned the genes encoding proteins responsible for the proper conduct of the gibberellin signal transduction pathway in yellow lupine (*Lupinus luteus* L.), now we identified and characterized genes associated with the phytohormone metabolism. Mainly in *Arabidopsis thaliana*, *Oryza sativa* and cereals is known that gibberellin 20-oxidases (GA20oxs) are important in determining GA concentration, because they catalyse the final steps in the biosynthesis pathway. Precisely GA20oxs are responsible for the sequential oxidation of C-20, including the loss of C-20 as CO_2 and the formation of γ-lactone. Thus, enzymes produce C_{19}-GAs using C_{20}-GAs as substrates. All of them belong to the large class of Fe(II) and 2-oxoglutarate dependent dioxygenases encoded by multiple genes. At present, in *A. thaliana* five *GA20ox* genes (*AtGA20ox1-5*) and in *O. sativa* four genes (*OsGA20ox1-4*) were identi-

fied. In our study we cloned two cDNA fragments of *GA20ox* genes, defined as *LlGA20ox1* and *LlGA20ox2*, in two different cultivars of yellow lupine – mister (traditional form) and taper (epigonal form). These results were obtained using RT-PCR method with degenerate primers, designed based on the sequences of closely related species. Subsequently, available on-line computer programs enabled translation of the nucleotide sequences (521 nt *LlGA20ox1* and 206 nt *LlGA20ox2*) to the predicted amino acid sequences (173 aa LlGA20ox1 and 68 aa LlGA20ox2). The presence of the highly conserved N- and C-terminal region with 2-oxoglutarate/Fe(II) dependent dioxygenase activity (DIOX_N/2OG-Fe(II)_0xy) and PLN03176 flavanone-3-hydroxylase domain indicate with a high probability that they are part of the functional enzyme proteins. Further analysis showed a high level of identity and similarity of nucleotide/amino acid sequences between yellow lupine cultivars. Recognized sequences exhibit also high degree of identity and similarity to other plant species, which indicates their conservatism during the evolution and may suggest potential role of LlGA20ox proteins in regulation such processes as its homologues in different plant species. Obtained results will lead us to better understanding of the GA biosynthesis pathway and determine the mechanism of yellow lupine flowering. This work is supported by the Ministry of Agriculture and Rural Development of Poland Grant No 149/2011.

P1.10

TSD1 (*TUMORUS SHOOT DEVELOPMENT1*) and *TSD2* genes related with cell wall function are involved in somatic embryogenesis induced *in vitro* in *Arabidopsis*

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The *Arabidopsis TUMORUS SHOOT DEVELOPMENT1* (*TSD1*) gene encodes a membrane-bound endo-1,4-beta-D-glucanase, involved in cellulose biosynthesis, while the *TUMORUS SHOOT DEVELOPMENT2* (*TSD2*) encodes a putative methyltransferase with an essential role in cell adhesion and coordinated plant development. Function of the both genes was related to cell wall prosperities. It was also found that *tsd1* and *tsd2* mutants exhibit non-coordinated shoot development resulting in disorganized tumor-like growth under *in vitro* culture on hormone-free medium. *TSD1* and *TSD2* were found recently to be required for maintaining a correct meristematic pattern, organ growth and hormone response in *Arabidopsis*. Thus, we hypothesized on the involvement of *TSD1* and *TSD2* genes in plant morphogenesis induced *in vitro*. To verify this hypothesis *TSD1* and *TSD2* expression patterns were studied in alternative morphogenic pathways induced in culture of *Arabidopsis* explants. Accordingly, somatic embryogenesis (SE), shoot organogenesis (ORG) and normal seedling development (E0) were induced in culture of immature zygotic embryo explants of Col-0. Moreover, the *tsd1* and *tsd2* mutants were analysed in terms of their capacity for morphogenic processes induced *in vitro*. Real Time qPCR analysis indicated the activity of *TSD1* and *TSD2* during SE process and *TSD1* transcripts were found to be accumulated in embryogenic culture at higher level (up to 5-times) than *TSD2* once. In contrast to SE culture, the expression of both genes was distinctly lower during shoot organogenesis and seedling development. This observation suggests SE-specific activity of *TSD1* and *TSD2* transcripts. In support for the involvement of *TSD1* and *TSD2* in SE, both mutants (*tsd1* and *tsd2*), displayed a decreased embryogenic capacity under *in vitro* culture in terms of significantly reduced SE efficiency and productivity. The obtained results indicate that *TSD1* and *TSD2* genes are necessary for efficient and productive SE induction under *in vitro* culture.

P1.11

NO-induced alleviation of dormancy of apple embryos is related to modification of phytohormonal signaling pathways

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NO pre-treatment eliminates symptoms of delayed germination and morphological abnormalities, typical for dormant apple embryos. Seed dormancy is maintained by abscisic acid (ABA), while germination is controlled by gibberellins (GA). Jasmonates (JA) are also key players during apple embryo dormancy alleviation and germination. We analyzed the expression of genes of main components of transduction pathway of i) ABA (*PYL1, PYL2, PYL4, RCAR1, RCAR3* – encoding proteins, function as co-receptors; *ABI1, ABI2, PP2C* – encoding a phosphatase 2C, negative factor of this pathway; *SnRK2*, encoding a protein kinase similar to SNF1 (SnRK2) acting as a positive regulator route; *AREB3* and *ABF* as transcription factors of ABA path) and ii) GA (*GID1, SLY*, coding GA receptors, *DELLA* – repressor of this pathway, *SLP2* – gene response to GA) as well as iii) JA (*COI1,* encoding a JA receptor, *JAZ* – repressor of this path and *MYC2* – as a transcription factor genes response to JA). The expression profiles of genes mentioned above were done in embryonic axes isolated from dormant or NO short (3 h) pre-treated embryos. The analyzed genes were differentially regulated during dormancy alleviation and "*sensu stricto*" germination; the main modifications in transcription level were detected for PP2C, ABF, DELLA, GID1, JAZ12, MYC2. It seems that regulatory role of NO in removal of seed dormancy is associated with modification of hormonal signaling, mainly with induction of GA transduction pathways.

P1.12

The expression profile of galactinol synthase genes (*VhGolS1* and *VhGolS2*) in developing *Vicia hirsute* ([L.] S.F. Gray) seeds

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Many higher plants accumulate soluble carbohydrates during seed development. The most common carbohydrate in seeds is sucrose. Moreover, seeds contain α-D-galactosides: raffinose family oligosaccharides (RFO) and galactosyl cyclitols (Gal-C). Galactosides consists of galactose residues connected by an α-(1→6) glycosidic linkage. *V. hirsuta* seeds contain galactosides of RFO, galactinol, GPA and GPB series. In developing seeds accumulation of RFO and Gal-C is positively correlated with acquisition of desiccation tolerance and longevity of seeds. Oligosaccharides may promote the formation of a vitreous (glassy) state that protects macromolecular structures during desiccation. Besides, they may stabilize membranes directly by replacing water molecules between the polar headgroups of phospholipids. Biosynthesis of α-D-galactosides proceeds by stepwise addition of galactose residues to the acceptor: sucrose, cyclitols, monogalactosides and their higher homologues. The metabolism of RFO and Gal-C may include three types of galactose residues donors: galactinol, UDP-galactose and galactosides themselves. However, in seeds of most plant species, the role of main galactose donor is galactinol. Galactinol synthase (GolS, EC 2.4.1.123) catalyzes the synthesis of galactinol (O-α-D-galactopyranosyl-[1-1]-L-*myo*-inositol) from UDP-galactose and *myo*-inositol. GolS is considered as a key enzyme regulating α-D-galactosides biosynthesis rate. The aim of this study was to determine the expression profile of galactinol synthase genes (*VhGolS1 and VhGolS2*) in developing *V. hirsuta* seeds. Developing seeds, beginning from 16 DAF (day after flowering) to 36 DAF were collected. Then total RNA was isolated and reverse transcribed. Resulting cDNA was used as a template in real-time PCR in order to determine the gene expression changes. Obtained results show differences in expression profiles of *VhGolS1* and *VhGolS2*. Expression of *VhGolS1* rose during seed development. In immature seeds (16-24 DAF) the expression level was very low. In seeds

at 26 DAF expression significantly increased and reached approximately 10-times higher value than in the youngest seeds (16 DAF). Further development of seeds resulted in a linear rise of *VhGolS1* expression and reached 65-times higher value in mature seeds (36 DAF) than in immature (16 DAF). Expression profile of *VhGolS2* in seeds at 16-24 DAF was similar to *VhGolS1*. In 26 DAF seeds *VhGolS2* expression level rose almost 3-times. From 26 DAF to mature seeds, in contrast to *VhGolS1,* changes in expression of *VhGolS2* were not statistically significant. In mature seeds, *VhGolS2* expression level was 10-times lower than *VhGolS1*. This research was supported by Polish Ministry of Science and Higher Education funds (NN 310088137).

P1.13

Transcriptional activity changes of the *SOC1* homologue in vegetative organs in *Lupinus luteus*

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The induction of flowering is the most important stage in the life of the higher plant. A key factor to correct its progress is to change the expression of a small group of genes. These genes are called flowering integrators. One of them is SOC1. It is controlled by all the flower induction pathways that determine the time of flower development. One of the major proteins that affect the transcription of this gene is the *FT* member photoperiod pathway. Negatively impact on the activity *SOC1* described for FLC, flowering inhibitor belongs to autonomous and vernalization pathways. However, the SOC1 is also regulated independently of these pathways by the age of the plant. Using qRT-PCR technique a pattern of transcriptional activity of this gene in vegetative organs was established. In this work, we compared the expression identified in yellow lupine SOC1 homologue in plants grown under natural and artificial conditions. Transcriptional activity of studied gene was determined in vegetative organs develop in the subsequent weeks. The results of these experiments were shown increased expression *LlSOC1-like* in both roots and leaves. The highest activity of the studied gene was shown after 33 day growth that might indicate the initiation of flowering in plants at this development stage. The work was supported by the Multi-Year Programme of the Polish Ministry of Agriculture and Rural Development, No. 149/2011 and the National Science Centre (Poland) grants No 2011/01/B/NZ9/03819.

P1.14

Molecular cloning and preliminary expression analysis of complete cDNA homologue *LOX2* gene in *Lupinus luteus*

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LOX2 encodes of plastid-localized enzyme that catalyzes dioxygenation of polyunsaturated fatty acids (18:3 and 16:3) and as a consequence the specific formation of 13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HPOT) and 11(*S*)-hydroperoxy-hexadecatrienoic acid (11-HPHT), respectively. Both compounds can be further metabolized into jasmonates (JAs) – the plant hormones that controls many growth and development processes, e.g. fruit ripening, production of viable pollen, flower development, root growth, plant response to wounding and abiotic stress, defense

against insects and pathogens. In this work, we identified a full length of *LlLOX2* cDNA from *Lupinus luteus*. Analysis of *LlLOX2* sequence revealed that predicted amino acid sequence is similar to that of the lipoxygenase superfamily. The RT-PCR technique enables us to investigate tissue-specific *LlLOX2* expression pattern. Our data obtained in this work indicate that *LlLOX2* regulate similar physiological processes as *LOX2* from other plant species. This research was supported by Ministry of Agriculture and Rural Development grant no 149/2011. Special thanks to Mr Paweł Barzyk from Poznańska Hodowla Roślin Tulce, Wiatrowo for submission of seed material and his helpful advices.

P1.15

Changes in distribution of 5′methylcytosine in male and female gametophyte of *Hyacinthus orientalis* before and after fertilization

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Changes in gene activity and chromatin structure during the maturation of the male and female gametophyte and early stages of seed development in flowering plants are observed. Transcriptional activity could be regulated by epigenetic chromatin modification. One of important epigenetic factors engaged in regulation of transcription is DNA methylation which correlates with genes inactivation. Chromatin of cell engaged in double fertilization process is characterized by changes in its structure and varied transcriptional activity [2, 3]. Based on this data we assumed that different genes activity and chromatin structure of pollen grain and embryo sac cells before and after fertilization are the consequence of chromatin epigenetic modification, especially methylation of DNA. Results of our experiment, carried out by immunocytochemical methods, showed changes in level and distribution of 5meC in the nuclei of both male and female *H. orientalis* gametophyte. In male gametophyte the level of 5meC in the generative nucleus was higher than in the vegetative one. It was positively correlated with their transcriptional activity and chromatin condensation. In female gametophyte before fertilization, both egg and central cell represented level of genome methylation comparable with that of synergids, although their transcriptional activity and chromatin condensation has been different [3]. The fusion with sperm cells caused a change in the methylation level of these nuclei. After the transcriptional activation, the signal observed in the zygote nucleus was slightly lower than the level of 5meC recorded in egg cell nucleus. It was accompanied by chromatin condensation. Unlike chromatin of the central cell, the primary endosperm DNA was almost completely demetylated. Our investigation clearly showed that gene activation following the fertilization accompanied by the demethylation of DNA. It suggest that in the regulation of gene activity and chromatin organization in the cells involved in the double fertilization DNA methylation plays an important role.

P1.16

Immunolocalization of AGO1 in female gametophyte cells of *Hyacinthus orientalis* L. before and after fertilization

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MicroRNAs (miRNAs) are endogenous 21-24 nt RNAs that can down-regulate gene expression by pairing to the messages of protein-coding genes to specify mRNA cleavage or repression of productive translation. They act within the RNA-induced silencing complex (RISC) which contains a member of the ARGONAUTE (AGO) protein family, which comprises 10 members in *Arabidopsis*. AGO1 is the founding component of this group. The aim of the present investigation was to determine the spatial and temporal distribution of AGO1 in the cells of *H. orientalis* embryo sac

before and after fertilization. AGO1 protein was localized by fluorescence and electron microscopy immunocytochemical detection techniques. The obtained results have shown that AGO1 is present in the nucleus and in the cytoplasm of the embryo sac cells during successive stages of its functioning. Before fertilization in the egg cell and central cell the level of the protein was highest. During progamic phase, when pollen tubes have reached about three-quarters of the style length and did not enter the female gametophyte, the level of the labelling in the egg cell was decreased. In the central cell the level of AGO1 was similar to the level which was observed in this cell before pollination. Fertilization induced growth of the level of AGO1 in the zygote. In the forming endosperm the level of AGO1 was still high. In summary, the presence of AGO1 in the embryo sac cells of *H. orientalis* before and after fertilization indicates that in the cells, which are target for sperm cells and in the cells formed after the double fertilization (zygote and endosperm) the processes including noncoding small RNAs take place. The research was supported by the National Science Centre (NCN) grant 2011/03/D/NZ3/00603.

P1.17

Molecular interactions between the microRNA biogenesis complex and the spliceosome

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MicroRNAs (miRNAs) are small non-coding RNAs of about 21 nt in length, which take part in a wide variety of physiological and cellular processes. They act by regulating gene expression by cleaving or inhibiting translation of target mRNAs. In plants, miRNAs are encoded mostly by independent transcriptional units or, less frequently, within introns of protein-coding genes. In addition, the miRNA genes that form independent transcriptional units and are transcribed into long non-coding transcripts, can embed miRNA sequences in both, introns or/and exons of such miRNA primary precursors (pri-mRNAs). Plant miRNA biogenesis, which is still not a fully understood process, occurs inside the cell nucleus, and is performed by a complex compromising of at least five different proteins: DCL1 (an RNase III ribonuclease), HYL1 (a dsRNA-binding domain containing protein), SERRATE (SE) (a zinc-finger-domain protein) and CBC (a nuclear cap-binding protein complex) that is composed of two subunits, CBP20 and CBP80. DCL1 provides nucleolytic activity of the miRNA processing complex, and other factors involved in miRNA maturation are required for the efficient and correct excision of miRNAs from plant pri-miRNAs. Interestingly, SE and CBC are also involved in pre-mRNA splicing. It is unclear, however, how this dual function of SE and CBC is fulfilled. We hypothesized that SE connects CBC with the miRNA processing machinery as it binds DCL1, HYL1, and, on the other hand, it has many functional connections with CBPs. Therefore we decided to characterize the interaction between CBC complex and the SERRATE protein in plants. We also intended to test if CBP20 and/or CBP80 has the ability to bind SERRATE at the same time, or whether these interactions occur independently of each other, in other word whether CBC components interacts with SE together or separately. We performed experiments on the subcellular localization of the *Arabidopsis thaliana* CBC and SERRATE. Both proteins of the CBC complex co-localize with SE in the cell nucleus. Furthermore, we have shown direct interactions between CBP20, CBP80 and the SERRATE protein. The Bimolecular Fluorescence Complementation (BiFC) and *in vitro* pull down assays were used to confirm experimentally CBC/SE interactions. The CBP20/SE and CBP80/SE complexes seem to be localized exclusively in the nucleus where they are mainly accumulated in specific subnuclear bodies. Moreover, *in vitro* analyses have shown stronger interactions of overexpressed in *E. coli* MBP-SERRATE with the whole CBC complex, obtained by *in vitro* translation, in comparison to the single interaction between SE and CBP20 or SE and CBP80. These results indicate that SE, in order to fulfill its role in the cell, binds both CBC proteins, CBP20 and CBP80.

P1.18

In silico modeling of an interaction network of genes involved in the cell cycle progression during root morphogenesis in mono- and dicotyledonous plants

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The objective of the presented study is to reach a more comprehensive understanding of the cell cycle genes involvement in the root system development in monocotyledonous and dicotyledonous plants. The vast majority of studies on the genetic control of root system development are carried mainly on the well-studied dicotyledonous model species, such as *Arabidopsis thaliana*. The current state of knowledge on mechanisms of regulation of analogous processes in monocots, which include cereals, remains incomplete. Applied research strategy is based on a comparative *in silico* analysis of the course of genetically conserved cell cycle regulatory pathways that are also involved in the morphogenesis of the root system. It will allow for the verification of the convergence of these processes within mono-and dicotyledonous plants. Conducted *in silico* analyses were aimed at the identification of a defined set of genes that possess an overlapping function in cell cycle regulation and root system development. The initial screening of core regulating factors consisted in a database mining process. Determined subset of core genes was afterwards subjected to analysis of intra- and interindividual interaction with a use of bioinformatics tools and characterized among the biological context based on the Gene Ontology (GO) annotation. Target genes expression profiles regarding spatial and temporal expression during plant growth and development were evaluated depending on databases derived repository. Gathered data served as an input data for the construction of a conceptual model of key factors interaction within differential cell cycle progression during root system development of mono- and dicotyledonous plants. This will allow for a comparison of the function and redundancy of analyzed regulatory pathways involved in the course of morphogenesis of the root system, which architecture significantly differs between the analyzed groups. The idea behind the proposed study is to create an interactive model of the regulation of plant root system morphogenesis occurring in the course of cell cycle progression. The analyses enabled the assessment of redundancy and existing homology within processes of morphogenesis in plants root of mono- and dicotyledonous plants. Created model will provide a useful tool to support further detailed functional analysis of selected regulatory genes controlling investigated processes, with particular emphasis on the differences the advancement of the processes within mono- and dicotyledonous plants. Presented resources, can also be used in the selection of specific involved in the regulation of morphogenetic processes in the development of the root system of candidate genes for which the identification of new alleles would be highly desirable in breeding programs of cultivated plants.

P1.19

Enzymatic activity and arginase gene expression in *Arabidopsis* plants infected with a cyst-forming nematode

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The nematode *Heterodera schachtii* is a sedentary endoparasite of sugar beet and many Brassicaceous plants. Its second-stage juveniles penetrate host roots and induce permanent feeding site (a syncytium) being the sole source of nutrients for the developing nematode. Increased contents of many amino acids (including proline) in syncytia induced in *Arabidopsis thaliana* roots were found. Proline and polyamines are important for cell protection and repair processes. They are synthesized from ornithine, the product of arginase-catalyzed reaction. Arginase

(EC 3.5.3.1) is an important enzyme for nitrogen metabolism as it produces urea aside from ornithine. Recently, the role of arginase in plant defense has attracted attention, as the arginase gene expression is induced as a result of viral or microorganism infections, as well as after wounding. Because our experiments have shown an elevated activity of arginase in shoots of *A. thaliana* infected with *H. schachtii*, we studied arginase gene expression in syncytia, roots and shoots of the nematode-infected plants. Using semi-quantitative RT-PCR we showed the presence of arginase1 and arginase2 RNAs in shoots of *A. thaliana,* whereas only arginase 1 was expressed in roots. In plants collected on the third and seventh day after infection, expression of arginase1 both in roots and shoots was lower than in uninfected plants. Similarly, the arginase2 expression was strongly inhibited in shoots on the seventh day after nematode infection. However, fifteen days after infection, a higher expression of arginase1 was found in infected plants than in appropriate control shoots. Thus, we infer that cell wall damage and/or metabolic changes caused by invading nematodes influence the profile of arginase expression in *H. schachtii*-infected *Arabidopsis* plants.

P1.20

Molecular characterization and expression of a new calreticulin gene involved in pistil transmitting tract maturation and progamic phase in *Petunia hybrida*

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Calreticulin (CRT) is a highly conserved and ubiquitously expressed Ca^{2+}-binding protein in multicellular eukaryotes. In animals, CRT is involved in many different intra- and extracellular processes, such as Ca^{2+} storage and signaling, molecular chaperone activity in the endoplasmic reticulum (ER), regulation of gene expression, control of cell adhesion and migration, immune regulation, apoptosis, and pathogenesis. Plant CRT has the same molecular structure as the animal protein and shares its chaperone and Ca^{2+} binding activities. In higher plants, the CRT family consists of three members, which are classified into two distinct subclasses: CRT1/CRT2 (also designated CRT1a/CRT1b) and CRT3. Sequence homology of plant CRTs suggests that CRT1 and CRT2 are similar to each other, whereas the plant-specific *CRT3* genes are more highly conserved across species. *CRT's* expression pattern suggests that it could play a role in regulation of Ca^{2+} homeostasis during pollen-pistil interactions and thus contribute to successful fertilization. To address this possibility, we cloned and characterized the full-length cDNA of a new *CRT* gene (*PhCRT*) from *Petunia*. The deduced amino-acid sequence of *Ph*CRT shares homology with other known plant CRTs, and phylogenetic analysis indicates that the *PhCRT* cDNA clone belongs to the CRT1/CRT2 subclass. Northern blot analysis was used to assess *PhCRT* gene expression in different parts of the pistil before pollination and during subsequent stages of the progamic phase. The highest level of *PhCRT* mRNA was detected in the stigma-style part of the unpollinated pistil one day before anthesis and during the early stage of the progamic phase, when pollen is germinated and tubes outgrow on the stigma. In the ovary, *PhCRT* mRNA was most abundant after pollination and reached maximum at the late stage of the progamic phase, when pollen tubes grow into the ovules. From these results, we suggest that *PhCRT* is expressed during multiple steps of plant reproduction: pistil transmitting tract maturation, pollen germination and tube outgrowth, and pollen tube growth into the ovule. We speculate that CRT's molecular chaperone and Ca^{2+}-buffering activities facilitate these processes, which require high rates of protein synthesis and careful regulation of Ca^{2+} homeostasis. This project was supported by the Ministry of Science and Higher Education in Poland, grant N303 023 32/1034 (to ML) and founds provided by Nicolaus Copernicus University for the research program of the Laboratory of Developmental Biology.

P1.21

Copy number variation in *Arabidopsis thaliana* genome

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Copy number variants (CNVs) are genomic rearrangements resulting from gains or losses of DNA segments. Typically the term refers to rearrangements of sequences larger than 1 kb. This type of polymorphism has recently been shown to be a key contributor to intra-species genetic variation, along with single-nucleotide polymorphisms and short insertion-deletion polymorphisms. Although a great proportion of CNVs has been localized in non-coding regions of the genomes, a lot of variants that affect genes have been identified as well. Gene-overlapping CNVs may cause alterations in gene structure or dosage and therefore affect phenotypes. It has been shown that in humans, individual CNVs have been linked to risks for severe diseases. At the same time, exploration of the extent and role of CNVs in plants is at its beginning. The 1001 Genomes Project provides whole-genome sequence data of multiple *Arabidopsis thaliana* accessions, allowing for CNVs discovery and analysis in this plant. We used the next generation sequencing data of 80 A. thaliana accessions from eight different geographic locations (MPICao2010 dataset) for CNV inference in this species. By combination of read depth and paired-end mapping methods we were able to identify over 1000 CNVs, each at least 1kb large. We used MLPA and PCR genotyping approaches for validation of selected gene-overlapping CNVs. Our results indicate that copy number variation affects a substantial part of *A. thaliana* genome. This work was supported by the National Science Centre (Poland) grant No 2011/01/B/NZ2/04816.

P1.22

Elastin-like polypeptides-alternative way of recombinant protein purification

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The development of molecular biology and biotechnology enables the production of a wide range of protein using variety expression platforms. At present, several technologies utilizing highly efficient protein expression systems allow produce almost any protein of our interest. However, in many cases relatively high costs of protein extraction and purification methods are limiting factor. One of the very promising solutions of this problem in molecular biology (including plant systems) could be application of elastin-like polypeptide tag (ELP) in the nonchromatographic recombinant protein purification method. ELP are biopolymers containing repeats of Val-Pro-Gly-Xaa-Gly (VPGXG) pentapeptide, in which Xaa (the guest residue) can be any amino acid except proline. These polypeptides have a distinctive and very useful feature, which is the ability to transition from a soluble to insoluble form in aqueous solution, in response to changing environmental conditions (Inverse Transition Cycing). Genes for ELPylated fusion proteins can be easily synthesized using standard methods of molecular biology. Separation of ELPylated target proteins can be achieved by raising the temperature above so called transition temperature (T_t) when ELP aggregate and can be separated by centrifugation.

P1.23

Using immunobloting and chemiluminescence methods for identification immunoreactive fractions of wheat storage proteins

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Wheat storage proteins – gliadins and glutenins – are a very special group of food allergens. They are highly polymorphic proteins and their polymorphism is determined genetically. Gliadins are coded by six complex loci on chromosomes (1A, 1B, 1D, 6A, 6B, 6D) of wheat genome. The aim of the experiment was to characterize specificity of immunological reaction of IgE class anti-gluten antibodies present in sera of chosen patients with particular subunits and fractions of gluten proteins identified in analyzed wheat genotypes. Protein loci were identified using electrophoretic methods (A-PAGE, SDS-PAGE and 2D electrophoresis) and reversed phase high performance liquid chromatography (RP-HPLC). The obtained results showed that: 1) polymorphism of wheat gluten proteins is closely related to differentiation of immunoreactive properties of this group of food allergens; 2) patients with different symptoms of wheat allergy show specific characteristics of IgE binding with different subunits and fractions of gluten proteins and 3) the main allergens for analyzed patients among identified gluten proteins are ω-gliadins, D-type LMW glutenin, gliadin like LMW glutenin and α/β type gliadin of molecular weight 43 and 50 kDa.

P1.24

Analysis of expression profiles of barley, medicago and soybean galactinol synthase through microarray data integration

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Current knowledge and capabilities in integration of microarray data from different experiments is restricted, especially in plants, by lack of particular annotations. Data integration based on microarray experiments enables inter alia: utilization of data from already executed research without collecting new samples, but with views update, at the same time. High popularity of human and mice studies is incomparable with average popularity of plant studies. The solution is next generation sequencing technology which is efficient way to gain new references and annotations for genomes of recondite plant species. Integration of different experiments data is not used as standard research, however recently few bioinformatics tools such as: inSilicoMerging and VirtualArray, have appeared. The goal of analysis was to show expression profiles of galactinol synthase genes and point out sequences with similarities in expression profiles. Galactinol synthase gene (GolS) belongs to glycosil transferase 8 (GT8) gene family. GolS proteins are relatively small class of this family. *Eukaryotic galactinol synthase play* a significant role in regulation of the biosynthesis of the *raffinose family* oligosaccharides. Analysis was based on data from microarray data repository – Gene Expression Omnibus (GEO). Research involved three organisms: *Hordeum vulgare, Medicago truncatula* and *Glycine max.* There were 30 experiments and over 500 microarray samples used in data integration in total. In this research R programming environment were used, especially libraries of Bioconductor project, such as: *gplots, affty, GEOquery, heatplus, affyPLM and inSilicoMerging.* Experiments and accession numbers of galactinol synthase sequence samples were localized through PLEXdb database. Samples were collected from different plant pieces, thus comparison of all data to one reference was not feasible. In this case in every microarray experiment fold change coefficient were calculated and then expression profiles have been determined based on fold change coefficient. Expression profiles have shown underexpression of galatcinol synthase genes during drought, cold and salinity stress. Reduced expression has shown in mannitol presence. Expression profiles did not differ considerably during plant response on pathogen presence. Only tissues treated with powdery mildew have shown reduced genes expression

but not in genes strongly associated with galactinol synthase. Data integration provides opportunity to compare methodologically different experiments and what is more important, draw new additional conclusions which was not the aim of original research.

P1.25

The preliminary stage of SCAR markers development for *Ranunculus subgen. Batrachium*

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Batrachium is a group of aquatic plants classified within the genus *Ranunculus* (*Ranunculaeceae*). One of the adaptations to the aquatic environment is their plastic vegetation response to changing environmental conditions, manifested in their morphological variability. This phenotypic variability is taxonomically insignificant but impedes the identification of individual species. The rapid development of molecular techniques provides an opportunity to develop specific DNA markers for identifying species based on small, vegetative parts of plants, regardless of its state of preservation or manifested morphological features. The aim of the project is the development of SCAR (Sequence Characterized Amplified Region) markers. Those molecular markers will allow an easy and quick way to identify distinctive taxa within the genus *Ranunculus*. Although amplification and direct sequencing of ITS (Internal Transcribed Spacers) allow reliable way to determine the species, but this method is relatively time-consuming and expensive. As an alternative to sequencing, RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeats) are chosen as cheap and simple methods, which do not require an information sequence and it is effective in obtaining the characteristic DNA profile. The main drawback of these methods is low repeatability. Conversion RAPD markers to the SCAR increase the specificity and stability, allowing for convenient and fast way to identify groups. For the analysis, there were selected 11 species of the genus *Ranunculus*. In each species, a different number of samples were taken – from the two, as in the case of *R. trichopyllus* type II to 8 samples as in the case of *R. fluitans*. The differences in numbers of samples were determined by the availability of previously defined species based on the analysis of ITS sequences. Samples were combined and treated as a single sample during the primers test. From 26 tested RAPD primers 17 were selected for further analysis. The next step was to identify RAPD profiles showing the specific products for each species and tested whether specific products are present in individual samples within a given species. Selected 18 products were chosen to be cloned into plasmid and sequenced. Based on yet obtained nine sequences for six species there were designed a pair of primers for each sequence. Two pairs of SCAR markers gave products for all tested samples within the particular species moreover didn't give products where were not expected. The effectiveness of these two markers gives hope for obtaining SCAR markers for all tested species. These markers will give the possibility to examine a much larger number of samples than it would be possible by direct sequencing. This can lead to obtain knowledge of distribution of particular groups within *Ranunculus*, as well as provide new information about their habitat preferences and morphological variation.