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Poster session

Poster 1

Blue-light-activated phototropin2 trafficking from the cytoplasm to Golgi/post-Golgi vesicles

C. AGGARWAL[1,2], A.K. BANAS[2], A. KASPROWICZ-MALUSKI[1], H. GABRYS[2]

[1] Department of Gene Expression, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Phototropins are plasma membrane localized UVA/blue-light photoreceptors which mediate phototropism, inhibition of primary hypocotyl elongation, leaf positioning, chloroplast movements and stomatal opening. Blue light irradiation activates the C-terminal serine/threonine kinase domain of phototropin which autophosphorylates the receptor. *Arabidopsis thaliana* encodes two phototropins, *PHOT1* and *PHOT2*. In response to blue light PHOT1 moves from the plasma membrane into the cytosol and PHOT2 translocates to the Golgi complex. Our work show the molecular mechanism and route of blue-light-induced PHOT2 trafficking. It is shown that AtPHOT2 behaves in a similar manner when expressed transiently under 35S or its native promoter. The PHOT2-kinase domain but not blue light-mediated autophosphorylation is required for the receptor translocation. Using co-localization and western blotting, the receptor was shown to move from the cytoplasm to the Golgi complex, and then to the post-Golgi structures. The results were confirmed by brefeldin A (an inhibitor of the secretory pathway) which disrupted PHOT2 trafficking. An association was observed between PHOT2 and the light chain2 of CLATHRIN via bimolecular fluorescence complementation. The fluorescence was observed at the plasma membrane. The results were confirmed using co-immunoprecipitation. However, tyrphostin23, (an inhibitor of CLATHRIN-mediated endocytosis) and wortmannin (a suppressor of receptor endocytosis) were not able to block PHOT2 trafficking indicating no involvement of receptor endocytosis in the formation of PHOT2 punctuate-structures. Protein turnover studies indicated that the receptor was continuously degraded in both darkness and blue light. The degradation of PHOT2 proceeded via a different transport route than translocation to the Golgi complex.

Poster 2

Deep: non-reference based method for functional sRNA identification and first bioinformatics analysis of liverwort *Pellia endiviifolia* microtranscriptome

S. ALABA[1], P. PISZCZALKA[2], Z. SZWEYKOWSKA-KULINSKA[1,2], W. KARLOWSKI[1]

[1] Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

[2] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

Small RNA (sRNA) are short 18-26nt sequences generated by RNA endonucleases and functionally associated with Argonaute protein family members to regulate gene expression level via complementarity. Recently, thanks to decreasing Next Generation Sequencing costs, sRNA world started to grow rapidly. Nevertheless, discovery of novel

sRNA classes is mainly restricted to single sRNA types from model organisms with known genomic sequences. This is a result of two major disadvantages of currently used sRNA identification methods: 1) focus on best described sRNA class – microRNA (miRNA) only and 2) usage of mandatory reference sequence. To overcome known software limitations we have developed Deep – a novel non-reference based method for functional sRNA discovery. Our approach is based on three criteria focused on features related to sRNA processing: 1) 5′-end cleavage accuracy, 2) sequence length, and 3) abundance. Small RNA clusters built from sRNAs are encoded as position-specific feature profiles that allows sequence-free identification of novel short RNAs. Resulting sequence lists created by grouping similar profiles and ranked by sRNA abundance, allow simple sRNA candidate selection. For learning step, algorithm requires as input only raw sRNA data and any user-specifed known sRNA sequences. Small RNA data from *Pellia endiviifolia* – a liverwort representing a group of first land plants was selected to test our approach. No genomic sequence is available for *P. endiviifolia*. Five samples of *P. endiviifolia* sRNA data from male and female thalli with or without sex organs, were analyzed. First, all sequences homologous to plant miRNA were identified representing 311 miRNA families with up to 2 substitutions. Northern hybridization performed on selected candidates confirmed its presence in *Pellia* thalli. Small RNAs annotated as plant miRNA without substitutions were then used for algorithm learning step. By applying the new approach we were able to select 69 positive candidates representing putative novel liverwort specific short RNAs. Using northern hybridization, 41 of them were confirmed as stable sRNA products. For further validation of 41 candidate sequences we performed transcriptome and degradome sequencing. Genome Walking technique confirmed 10 miRNA genes identified *in silico*. Analysis of degradome data revealed 13 mRNA targets cleaved precisely at 9-11 position of sRNA:mRNA duplex. One out of 41 sRNA candidates represented *Pellia* specific miR* sequence of miR408 family which expression was experimentally confirmed.

Poster 3

Novel proteins interacting with the SERRATE protein in *Arabidopsis thaliana*

M. Bajczyk, Z. Szweykowska-Kulinska, A. Jarmolowski

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

In plants the SERRATE protein (SE) is involved in two important pathways of RNA metabolism: microRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in microRNA biogenesis, where together with DCL1 (DICER-LIKE 1) and HYL1 (HYPONASTIC LEAVES 1) form a core of the plant microprocessor. In this complex SE influences the cleavage accuracy of pri-miRNAs by DCL1, interacting directly with DCL1 and some other miRNA biogenesis factors. Interestingly, the Arabidopsis *se* null mutant is embryonic lethal what proves the key role of SE in plant development and growth. SE has been also found to be involved in splicing of pre-mRNA. Moreover, its role in splicing is connected with interaction of SE with both subunits of the nuclear cap-binding complex (CBC): CBP20 and CBP80. Thus, CBC and SE have dual functions: in pre-mRNA splicing as well as in processing of pri-miRNA. We suggest that SE can be a link in the crosstalk between biogenesis of miRNA, pri-miRNA splicing and transcription of *MIR* genes. In order to understand better such key role of SE, we decided to search for proteins that interact with the *Arabidopsis* SERRATE protein. To this end, we constructed the *Arabidopsis thaliana* transgenic line, in which in the genetic background of the *se-1* mutant the FLAG-tagged version of the SE gene was integrated into the genome. Next, we carried out immunoprecipitation against the FLAG epitop of the expressed fusion protein to find SE protein partners using mass spectrometry. First of all, we optimized the method. Using this protocol we performed co-immunoprecipitation experiments which gave us a list of potential SE interactors. Surprisingly, a big set of proteins interacting with SE are factors involved in transcription and chro-

matine structure remodeling. Our data suggest that a zinc finger protein SERRATE can be involved in the regulation of transcription carried out by RNAPII as well as RNA-directed DNA methylation. Currently, we are confirming the interactions using the yeast two hybrid system and microscopic methods (BiFC and FRET).

Poster 4

Comparison of expression *LlFCA*, *LlFY* and *LlFLD* genes from the flowering autonomous pathway of *Lupinus luteus*

M. Banach, W. Wojciechowski, J. Kesy, P. Glazinska, E. Wilmowicz, A. Kucko, K. Marciniak, J. Kopcewicz, A. Tretyn

Chair of Plant Physiology and Biotechnology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Torun, Poland

The flowering time is one of the factors providing reproductive success. At least four genetically defined pathways have been identified 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'. The autonomous pathway includes at least 7 of flowering induction genes. All these genes are negative regulators of *FLOWERING LOCUS C* (*FLC*) – main inhibitor of flowering induction. RNA BINDING/FLOWERING TIME CONTROL PROTEIN FCA ALPHA (FCA) is a nuclear protein mediates the floral transition. This protein contains two conserve domains RNA recognition motifs (RRM) and a WW domain. The RRM domain is involved in selection of polyadenylation site, chromatin silencing of single and low copy genes, interaction with small interfering RNA-directed DNA methylation pathway for regulating common targets. FCA participates in post-transcriptional *FLC* mRNA 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 used for translation. FCA plays important roles in RNA 3 processing and transcription termination, thus limiting intergenic transcription. FLOWERING TIME CONTROL PROTEIN (FY) plays dual roles in FLC regulation. FY is an RNA 3′ end-processing factor. FY interacts with FCA (FY/FCA) by two proline-rich (PPLPP) motifs in the C-terminus end of FY. FY/FCA interaction shares in the autoregulation of FCA expression and the selection polyadenylation site in the *FLC* pre-mRNA. FCA/FY is the key factor in regulating the RNA processing machinery. In likely model, FY acts in conjunction with FCA to repress FLC, but also has an FLC-promoting activity that is FCA independent. FLOWERING LOCUS D (FLD) regulate *FLC* expression by chromatin remodeling. FLD regulates FLC by preventing hyperacetylation of the locus. FLD is histone demethylase. In likely model, FLD might participate in the deacetylation of FLC chromatin as a component of a histone deacetylase (HDAC) complex. In this study, expression of *FCA*, *FY* and *FLD* 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. The research materials were vegetative organs. Before collected plants were sprayed aqueous solutions of auxin, 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 *FCA*, *FY* and *FLD* expression pattern in vegetative organs of *L. luteus* cultivars – 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 in different growth conditions. Acknowledgements: 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.

Poster 5

RNAi as a tool for a functional analysis of ABC transporters in the model legume plant *Medicago truncatula*

J. Banasiak[1], W. Biala[2], K. Jarzyniak[2], M. Jasinski[1,2]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

[2] Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poznan, Poland

Nowadays genomic projects provide researches with numerous genes with unknown functions. To solve this problem the strategy called reverse genetic has been established. It is based on phenotype analysis of plants in which expression of a particular gene is switched off. This approach exploits mutagenesis or gene silencing. The latter can be exemplified by RNA interference (RNAi) triggered through double-stranded RNA (dsRNA) which inhibits gene expression in a sequence-specific manner. This post-transcriptional gene silencing (PTGS) is still the method of choice to gain information about function of any chosen target gene. Especially when mutants collection of a given plant is scarce or knockouts are lethal. We have used PTGS in the functional studies of ABC (ATP-binding cassette) transporters in the model legume plant *Medicago truncatula*. Transgenic hairy roots and root cultures with silenced *MtABCG10* have been obtained by *Agrobacterium rhizogenes*-mediated RNAi. To suppress *MtABCG10* expression, a 139 bp fragment from the coding region was introduced into the pK7GWIWG2(II)-p35S::DsRED binary vector. Presence of DsRED marker allowed for a quick and efficient inspection of the chimeric nature of *A. rhizogenes* transformed roots and selection of material for proper phenotyping. By usage of this construct we were able to silenced *MtABCG10* expression what was confirmed at the mRNA and protein level by Real-Time PCR and Western Blot respectively. Selected transgenic hairy root clones have been used for metabolomic analysis and biological assays (pathogen infection and nodulation efficiency).

Poster 6

Novel *Arabidopsis thaliana* miRNAs responsive to different abiotic stress conditions

M. Barciszewska-Pacak, K. Skorupa, D. Bielewicz, J. Dolata, A. Jarmolowski, Z. Szweykowska-Kulinska

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

MicroRNAs are key regulators of eukaryotic gene expression via post-transcriptional inhibition and cleavage. They play crucial roles in plant development and response to various abiotic and biotic stresses. However, the expression of MIR genes themselves is a subject of careful control. A high throughput real-time PCR platform (mirEX) has been developed to discriminate nearly all individual known primary miRNA precursors (pri-miRNAs) and to analyze reliably their individual expression patterns (Bielewicz et al. 2012, http://comgen.pl/mirex). In progressive drought experiment, the stress was applied to Arabidopsis plants at 1.10 growth stage (Boyes et al. 2001) by water withholding, and continued until the soil moisture level reached 30% field capacity (FC) (2 days before wilting) and 20% FC (wilting stage), both for different plant batches. The experiment was monitored by leaf relative water content (LRWC) measurements. 14 day-old Arabidopsis seedlings were subjected independently to 24h salinity, sulfur deficiency, copper deficiency, copper excess, cadmium excess and 12h heat stresses. The polyA+ RNA isolated from plants subjected to 30% and 20% FC drought conditions and the other abiotic stresses was analyzed by mirEX

platform for pri-miRNA and by Northern hybridizations for mature miRNA expression profiles, respectively. The analyses revealed altogether 33 known and novel mature miRNAs responsive to different abiotic stresses. With regard to the applied 20%FC and 30%FC drought stresses and salinity stress 13 new mature drought-responsive miRNAs and 9 new salinity-responsive miRNAs were identified. The progressive drought analysis showed also different responsiveness of pri- and mature miRNAs in the time course. For metal toxicity stresses the responsiveness of 25 miRNAs was shown and can be compared to the data known only from metal toxicity experiments done in rice, *Medicago truncatula* and *Brassica napus*. For Arabidopsis sulfur deficiency responsive miRNAs, new data have been obtained for 8 novel and 9 generally known stress responsive miRNAs, what can be compared to *Brassica napus* published data. The changes of the mature miRNA levels were analyzed for correlation with the changes of their cognate precursors in the applied abiotic stresses. Using available data for single gene miRNA family members that were detected as new Arabidopsis stress responsive miRNAs, we noted potential 7 transcriptional (correlated pri- and mature miRNAs changes) and 9 post-transcriptional (uncorrelated pri- and mature miRNAs changes) miRNA genes expression regulations. For drought stress 2 transcriptional and 3 post-transcriptional gene expression regulations can be postulated, meanwhile only 3 transcriptional gene regulations are potential under salt stress. Under copper as well as cadmium stresses, out of 6 analyzed 5 post-transcriptional gene regulations can occur. The work was supported by the NCN Harmonia funding scheme UMO-2012/04/M/NZ2/00127: "The regulation of *Arabidopsis thaliana* microRNA genes expression in response to selected abiotic stresses: the role of transcription and splicing factors in microRNA biogenesis".

Poster 7

Translational regulation during root developmental adaptation to phosphate starvation

J. Bazin[1,2], M. Crespi[2], J. Bailey-Serres[1]

[1] Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, California, USA

[2] Institut des Sciences du Vegetal (ISV), Centre National de la Recherche Scientifique (CNRS), Saclay Plant Sciences, Gif-sur-Yvette, France

The sessile lifestyle of plants requires them to adapt their growth and development to environmental variation. This implies a tight spatiotemporal regulation of gene expression to control developmental responses. Translational control, the regulation of the initiation, elongation or termination of ribosomes on an mRNA, provides cells additional mechanisms to rapidly control gene expression in a reversible manner, in response to environmental and developmental cues. It is known that non-coding RNA (ncRNA) can modify spatiotemporal gene expression patterns, and play a key role in developmental plasticity. Their role in the regulation of initiation of mRNA translation have been described in animals and hinted in plants, where mechanistic details are limited. Improving tolerance to nutrient deficiency is a major goal of agricultural research. Improvement of inorganic phosphate (Pi) starvation has been the target of classical breeding and genetic engineering. ncRNAs (i.e. miRNA399) have been shown to regulate root development during Pi starvation in roots of the model plant *Arabidopsis thaliana*. The three objectives of this project are to a) analyze the role of translational control at the genomic level in response to Pi starvation using state-of-the-art methods that resolve ribosome activity to the codon level; b) to assess the role of diverse classes of ncRNA in translational regulation in response to Pi starvation; and c) to delve into the mechanistic role of selected ncRNAs in translational regulation during Pi starvation. Experimental approaches used to fulfill these objectives will be presented. This will include the use of tissue-specific ribosome footprint sequencing and mRNA-seq of Arabidopsis roots cells to study translation regulation.

Poster 8

mirEX2: a new version of RT-qPCR and sRNA NGS platform for comparative exploration of plant miRNA expression data

D. Bielewicz[1], J. Dolata[1], K. Kruszka[1], A. Zielezinski[2], S. Alaba[2], A. Pacak[1], A. Swida-Barteczka[1], K. Skorupa[1], A. Stepien[1], P. Piszczalka[1], H. Pietrykowska[1], A. Piasecka[1], A. Jarmolowski[1], W.M. Karlowski[2], Z Szweykowska-Kulinska[1,2]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

[2] Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

mirEX is a comprehensive RT-qPCR platform for comparative analysis of microRNA expression data. In the new version of mirEX we have combined data from RT-qPCR-based gene expression profiles of pri-miRNAs and data from NGS-based expression profiles of mature miRNAs from three plant species: *Arabidopsis thaliana*, *Hordeum vulgare*, and liverworth *Pellia endiviifolia*. Currently, the mirEX2 integrates information about the expression profile of all (299) *Arabidopsis thaliana* pri-miRNAs in thirteen different developmental stages: seeds, seedlings and various organs of mature plants. Additionally, for two-week-old seedlings we analyzed the expression level of pri-miRNA for three microRNA biogenesis mutants (*hyl1-2, se-1,* and *cbp20xcbp80*) an for three-week-old plant we performed analyses of pri-miRNAs for four mutants (*dcl1-7, hyl1-2, se-1,* and *cbp20xcbp80*). Pri-miRNA analyses for barley were carried out for five developmental stages: 1-,2-,3-,6-week-old plants and from 68-day-old plants when kernels reach milk ripeness. Pri-miRNA expression analyses for dioecious *Pellia* were performed for female thalli producing archegonia, male thalli producing antherida or for female or male thalli without sex organs. One of the main new features of the mirEX2 database is integration of information about expression level of mature microRNAs. NGS data will cover two developmental stages in *Arabidopsis thaliana.* There will be data from wild type, *hyl1-2*, *se-1*, *cbp20xcbp80* from two-week old seedlings and data from 35-day-old wild type plants. For *Hordeum vulgare* deep sequencing was carried out for all (5) developmental stages and for *Pellia endiviifolia* NGS sequencing was performed separately for female and male thalli with or without sex organs. The possibility of correlation of pri-miRNAs expression level with expression level of mature miRNAs gives users more detailed information of microRNA gene expression regulation. All data are stored in a universal and expandable database scheme and wrapped by an intuitive user-friendly interface. A new way of accessing gene expression data in mirEX includes a simple mouse operated querying system and dynamic graphs for data mining analyses. In contrast to other publicly available databases, the mirEX interface allows a simultaneous comparison of expression levels between various microRNA genes in diverse organs and developmental stages. Additionally, by providing RNA structural models, publicly available deep sequencing results, experimental procedure details and careful selection of auxiliary data in the form of web links, mirEX can function as a one-step solution for microRNA information in *Arabidopsis*, *Hordeum* and *Pellia*. A web-based mirEX interface can be accessed at http://bioinfo.amu.edu.pl/mirex.

Poster 9

ARP4 – the busiest protein in the plant nucleus?

T. Bieluszewski, L. Galganski, M. Abram, A. Bieluszewska, P. Ziolkowski, J. Sadowski

Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

ARP4 belongs to the actin related protein (ARP) family which is represented by cytoplasmic and nuclear proteins in all eukaryotes. Nuclear ARPs are subunits of several protein complexes responsible for chromatin remodeling and

histone acetylation. Among nuclear ARPs, ARP4 is the most evolutionary conserved and occurs in the greatest variety of complexes. In human, ARP4 is a subunit of the chromatin remodeling complexes SWI/SNF, INO80 and SRCAP as well as the Tip60-p400 complex which has a dual role of a H2A-H2A.Z histone-exchange complex and a histone H4 acetyltransferase. Our results suggest that ARP4 is a subunit of at least seven different chromatin modifying complexes in the model plant *Arabidopsis thaliana*, including plant analogs of SWI/SNF and SWR1 complexes which are known as important regulators of transcription in plant development. Here we focus on two less well studied complexes, INO80 and NuA4 and their possible roles in *Arabidopsis*.

Poster 10

AtNTR1 is required for histone methyltransferase activity

G. Brzyzek, Y. Guo, S. Swiezewski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

In higher eukaryotes messenger RNA splicing is required for gene expression as most of the nuclear-encoded genes harbor multiple introns. Splicing of pre-mRNA is carried out by spliceosome, a multi-megadalton ribonucleoprotein complex in a complex multistep process. Most of splicing is believed to take place co-transcriptionally and splicing has been shown to feed back on transcription. NTR1 is one of splicosomal accessory factors, highly conserved within all eukaryotes, required for disassembly of splicing complex and completion of the splicosomal cycle. We have analyzed the AtNTR1 interacting proteins using a complementing tagged version of AtNTR1. One of the interactors was a putative histone methyltransferase. We have confirmed this interaction by Y2H and BiFC assay. *In vitro* biochemical assay with purified putative methyltransferase failed to show biochemical activity. However when this putative methyl transferase was combined with purified AtNTR1 we could observe a specific methyltrasferase activity towards H3K36. Our data suggest a direct link between splicing and histone posttranslational modification that could potentially provide a mechanistic explanation for spliceosome mediated feedback on chromatin.

Poster 11

An evidence for reversible DNA methylation changes in drought stress-responsive genes in barley

K. Chwialkowska, I. Szarejko, M. Kwasniewski

Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

Plants are constantly challenged by biotic and abiotic stresses and, thus, have developed a number of strategies allowing for a rapid adaptation to unfavorable environmental conditions. DNA methylation is one of the epigenetic phenomena that play a crucial role in regulation of gene expression in response to environmental stimuli. In the presented study, the effect of drought stress on DNA methylation level, pattern and its correlation with gene expression in *Hordeum vulgare* L. were assessed. The global evaluation of changes in the level and pattern of cytosine methylation under drought stress was carried out using methylation sensitive amplification polymorphism (MSAP) technique on leaf and root samples of plants exposed to drought stress, after re-watering and unstressed controls. Moreover, we modified the MSAP method by replacing of the conventional separation of MSAP amplicons on polyacrylamide gels with their direct sequencing using Next Generation Sequencing methods and automated data analysis (MSAP-Seq). The identified DNA methylation changes in specific genomic loci were confirmed using methylation-sensitive restriction enzyme digestion coupled with quantitative PCR (MSRE-qPCR). Our study revealed that

the overall methylation level within 5′-CCGG-′3 sequences in barley genome was at a high and comparable level in both leaves and roots (about 70%). Drought stress induced global-wide changes of DNA methylation pattern in barley genome and most of them consisted of new methylation profiles. There were more methylation alterations induced by drought in leaves than in roots and most of them went back to their initial status after re-watering. Detailed MSAP-Seq identification of loci undergoing DNA methylation changes revealed that most of new methylation profiles were established in genes, whereas demethylation occurred mainly within repeated sequences, especially in regions of transposable elements. Establishment of new methylation profiles under drought was observed among genes involved in basic metabolic processes such as oxido-reductive processes, chromatin condensation regulation, DNA repair, translation, RNA metabolism, as well as in stress-responsive genes, for example: response to jasmonic acid, pathogen resistance, anthocyanin biosynthesis and signal transduction. Interestingly, genic demethylation of cytosine was identified within similar group of stress-related genes, which suggests that stress triggers dynamic methylation/demetylation events preferentially in stress-responsive genes. Single-loci analysis using MSRE-qPCR confirmed that a large fraction of identified genomic loci was subjected to methylation changes under drought and, in addition, revealed that they all went back to their initial level under re-watering. Simultaneous gene expression profiling with RT-qPCR indicated that most of these genes underwent transcriptional down-regulation under drought and also returned to the basic expression level after recovery from water deficit. Thus, our results demonstrate a strong correlation of DNA methylation changes and gene expression modulation under drought and indicate their coupled reversibility.

Poster 12

Wheat (*Triticum aestivum* L.) gibberellins biosynthesis genes expression alteration during growth regulator application

K. Dudziak, M. Zapalska, J. Lesniowska-Nowak, M. Nowak, K. Kowalczyk

Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Lublin, Poland

Gibberellins (GAs) are plant hormones which mediate plant growth and development. Production of biologically active GAs is a complex process involving number of enzymes. Two of them: oxidases GA20 and GA3 play crucial role in bioactive GAs biosynthesis and have been tested in this study. Additionally, level of bioactive gibberellins is also regulated by GA2 oxidase, which transform bioactive gibberellins into biologically inactive forms. Last studies indicate that some of cereals dwarfing genes encode modified enzymes of gibberellins biosynthesis pathway. The purpose of our study was determination of the influence of plant growth regulator application on transcription alteration of three main gibberellins biosynthesis pathway genes (*GA20OX*, *GA3OX* and *GA2OX*) in common wheat (*Triticum aestivum* L.) Bezostaya 1 isogenic lines containing *RHT-B1b* and *RHT12* dwarfing genes. As a control form tall isogenic line without dwarfing genes was used. In presented study seven-day-old seedlings were treated with etephon in concentration suggested for commercial application. After three days plant material was harvested, and total genomic RNA was extracted by means of Trizol reagent method. For determination of analyzed dwarfing genes transcript level in plant tissue qPCR method based on SYBR Green dye was applied. For quantitative PCR cDNA obtained from reverse transcription of total RNA was used as template. For amplification reaction the sequence specific primers for selected dwarfing genes developed previously were used. Obtained results showed, that etephone application caused alteration of analyzed genes expression in tested wheat lines. After growth regulator treatment the level of expression for *GA20OX* gene was lower for both dwarfing genes containing lines, whereas for tall line an increase of transcript level was noticed. The level of *GA3OX* gene expression decreased in response to etephone activity in all analyzed lines. For *GA2OX* gene, responsible for biosynthesis of enzyme involved in degradation of bioactive gibberellins forms, increase of transcript level in all three analyzed lines was observed. Obtained results

confirm, that changes in gibberellins biosynthesis pathway genes expression is a part of complex plant response to application of growth regulation. Etephone treatment caused decrease of expression in genes encoding enzymes responsible for bioactive gibberellins biosynthesis (*GA20OX, GA3OX*). In the same time the level of transcription of *GA2OX* gene increase, what suggests intensification of bioactive gibberellins degradation process in tissue. Presented results were obtained as a part of the project #N N310 774140 granted by Polish National Science Centre.

Poster 13

New tools to monitor PBs and SGs dynamic in response to heat stress in *Arabidopsis*

J.-J. Favory[1,2], J. Descombin[1,2], J.-M. Deragon[1,2], C. Bousquet-Antonelli[2,1]

[1] Laboratoire Genome et Developpement des Plantes, Universite de Perpignan Via Domitia, Perpignan, France

[2] Laboratoire Genome et Developpement des Plantes, Centre National de la Recherche Scientifique, Perpignan, France

For each living organism it is of prime importance to be able to adapt to changes of environmental conditions. For plants, as sessile organisms, the fine tuning of the gene expression in an ever changing environment is even more vital. Post-transcriptional regulations play a crucial role in gene expression control. In animals two types of cytosolic ribonucleoprotein (RNP) structures have been described in response to translation repression: stress granules (SGs) and processing bodies (PBs). SGs are dynamic aggregates of untranslated mRNAs in association with translation initiation factors and PBs are RNA protein aggregates containing untranslated mRNAs associated with the mRNA decapping and 5′- decay machineries. Presently, only few components involved in PBs and SGs formation are identified in plants. In addition, recent studies showed that some key factors are common to plants and animals and can thus be used to identify more partners, as well as makers of these granules. These aggregates are visible as cytoplasmic foci which can be followed using fluorescent microscopy approaches. To study the dynamics of these RNP granules in *Arabidopsis*, stable transgenic lines expressing YFP and RFP tagged version of AtDCP1 and AtPAB2 under control of their endogenous promoters were selected to study PBs and SGs respectively. Our experiments conducted in young seedlings root tips indicate that both structures response to selected stresses with different dynamics. These first studies show that these selected lines provide useful cytosolic RNP granules dynamic reporter lines and stress sensors in *Arabidopsis*. Our team's main interest focuses on the understanding of the reprogramming of translation and cytoplasmic mRNA stability in response to heat stress. We are in particular aiming at understanding the dynamics of SG and PB formation and dissociation in various heat stress regimes. We will present our latest data about the dynamics of PB and SG along a short-term acquired thermotolerance (SAT) process.

Poster 14

Post-transcriptional regulation of an *Arabidopsis* protein involved in the heat stress response

N. Fernandez Bautista, L. Fernandez-Calvino, A. Munoz, M.M. Castellano

Centre for Plant Biotechnology and Genomics (CBGP) INIA-UPM, Pozuelo de Alarcon, Madrid, Spain

High temperature is one of the most deleterious environmental factors affecting plants. It seriously constrains plant development and growth, not only by reducing crop yield and quality, but also by limiting the arable land area and the use of varieties to crop. Therefore, elucidating the heat stress response is an important goal to understand

how plants can adapt to this physical parameter. During the last years our lab has developed different genomic approaches, analyzing transcriptional and translational changes during the survival process of plants to the increase of temperatures. This research has allowed identifying a group of proteins which functions in heat stress response have not been studied before in plants. One of these proteins is the protein 3P. The transcriptional changes in the 3P gene during the heat shock treatment has been studied and compared with other already known proteins involved in heat stress response. These analyses show that its expression is highly increased during the heat stress, but actively degraded under normal conditions. The 3P protein is localized in the cytoplasm under non stress conditions and at the early stages during the heat stress response. However, it changes its localization to cytoplasmic isolated foci and to the nucleus later on under stress conditions. All these results suggest that 3P is a highly regulated protein whose function during the plant response to heat stress is currently been evaluated.

Poster 15

Changes in the chromatin state and gene transcription in response to salinity stress in T87 *Arabidopsis thaliana* cells

A. Fogtman[1], A. Kwiatkowska[3], A. Palusinski[2], R. Iwanicka-Nowicka[1,2], A. Pacek[2], A. Macioszek[4], B. Wilczynski[4], M. Kotlinski[1,2], J. Dolata[5], A. Jarmolowski[5], A. Jerzmanowski[1,2], M. Koblowska[1,2]

[1] Corelab, Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland

[2] Laboratory of Systems Biology, Faculty of Biology, University of Warsaw, Warsaw, Poland

[3] Department of Botany, University of Rzeszow, Rzeszow, Poland

[4] Institute of Informatics, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Warsaw, Poland

[5] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

Chromatin is a nucleoprotein complex encompassing DNA and core histones in the nucleus of an eukaryotic cell. Stability of chromatin structure is crucial for the proper regulation of all nuclear DNA-templated processes – transcription, replication, repair and recombination. Emerging studies show that various cell signaling pathways trigger changes in the chromatin state and thus incoming signals can regulate gene expression through chromatin reorganization. Plants as sessile organisms developed diverse mechanisms allowing for quick response and adaptation to abiotic stress conditions. One of the first levels of the plant cell response to stress is induction of transcription of different gene classes. Defining how changing environmental conditions influence the chromatin state is essential for understanding regulation of plant gene transcription critical for improvement the resistance of cultivated plant to environmental stress. We studied in parallel the quick changes at both levels: transcription and chromatin structure in response to salt stress. As a model we chose T87 *Arabidopsis thaliana* cell line grown in suspension. Analysis of transcriptional changes was performed on Affymetrix GeneChip ATH1 microarrays. We carried out microarray experiments of treated with 250 mM NaCl T87 cells in 6 time points (0′, 20′, 40′, 60′, 80′ and 100′). A number of known (*COR15a*, *DREB2A*) and unknown genes were identified to have changed transcription levels in response to salinity, which was confirmed by qPCR analysis. Our transcriptomic analysis identified new genes potentially crucial for plant adaptation to salinity stress. Chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) with the use of antibodies against characteristic for transcriptional regulation core histone modifications revealed global changes in chromatin state under salt stress conditions, which correlated with changed expression of early responsive (ER) genes. Our results show the relations between cell signaling, chromatin state and gene regulation in response to environmental stress in plants.

Poster 16

Functional characterization of *Arabidopsis* LUC7 proteins, components of the U1 snRNP

M. DE FRANCISCO AMORIM, S. LAUBINGER

Center for Plant Molecular Biology (ZMBP), University of Tubingen, Tubingen,Germany
and Chemical Genomics Centre (CGC), Max Planck Institute of Molecular Physiology, Dortmund, Germany

Splicing, which is the removal of intronic regions from mRNAs transcripts followed by the exons joining, is an essential RNA processing step in all eukaryotes. Splicing is carried out by a macromolecular complex, the spliceosome, which consists of 5 so-called small nuclear ribonucleoproteins particules (snRNP) sub-complexes (for instance, U1, U2, U4, U5 and U6 snRNP for the major spliceosome). Each snRNP is assembled of a common core set of proteins (SM or LSM proteins), snRNP specific proteins and specific small nuclear RNA (snRNA). Splicing of introns proximal to the cap, which is a covalent modification attached to polymerase II derived transcripts, is assisted by additional proteins, among them the nuclear cap-binding complex (CBC) and SERRATE (SE). The CBC is an heterodimeric complex formed by CBP20 and ABH1/CBP80 that serves as a platform for binding of different proteins dictating the RNA fate. The CBC directly interacts with the zinc-finger protein SE. This interaction is not only required for efficient splicing, but also important for pri-miRNA processing and possibly other RNA processing events. While the role of SE/CBC in the miRNA biogenesis has been extensively studied, little is known about how this complex regulates splicing. In a yeast two-hybrid screen using SE as bait, we identified several components of the spliceosome as potential SE interactors. These findings suggest that SE/CBC physically interact with the spliceosome to facilitate splicing of cap-proximal introns. The main focus of this work is a protein called LUC7, which is part of the U1 snRNP and is encoded by a small gene family (LUC7A, LUC7B and LUC7RL) in *Arabidopsis*. While a mutation in LUC7A has been reported to impair fertilization in plants, we were able to identify hypomorphic alleles with only slightly compromised functions. A hypomorphic *luc7* triple mutant exhibited severe developmental defects: it is dwarf, flowers late and display loss of the apical dominance. Complementation analysis revealed that all the three LUC7 proteins act redundantly to control plant development. Splicing analyses, localization studies and protein-protein interaction data will be discussed.

Poster 17

The new RNAi approaches as tools in functional analysis of genes in polypolid cereals – introduction to research

S. GASPARIS[1], A. NADOLSKA-ORCZYK[1], W. ORCZYK[2]

[1] Department of Functional Genomics, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

[2] Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

In the last decade a significant progress has been made in studying molecular mechanism of RNA interference (RNAi). RNAi is now routinely used in model plant species to characterize gene function or to alter the phenotype. Because of some limitations this approach has not been commonly used in case of polyploid species, however it has the same great research potential. The recent studies performed by our team have proved the high effectiveness of post-transcriptional gene silencing (PTGS) in polyploid cereals. This method was successfully used for silencing the expression of genes encoding important agronomic and quality traits i.e. puroindoline genes in wheat and *HvCKX* genes in barley. Here we present the background of our new research in which another two RNAi pathways have

been studied – transcriptional gene silencing (TGS) and silencing by artificial microRNA (amiRNA). Both methods were applied for silencing of puroindoline genes *PINa* and *PINb* in allohexaploid wheat and amiRNAs were also used for silencing of secaloindoline genes in triticale – the orthologs of wheat puroindolines. Grain hardness determined by puroindoline genes is one of the most important technological trait in wheat. In the first bioinformatical approach the proper RNAi constructs were designed for TGS and amiRNA experiments. For TGS, the silencing cassettes of hpRNA type were constructed. Promoters of *PINa* and *PINb* genes were sequenced and analyzed to find the fragments containing regulatory elements. These fragments were cloned into RNAi vector pMCG161 in a sense and antisense orientation. amiRNAs for *PINa* and *PINb* genes were designed using special algorithm. 21 nt amiRNA fragments were then inserted into precursor miRNA from wheat – Tae-miR164. This construct was cloned into overexpression vector pBract214. Finally, the TGS and amiRNA constructs were used for *Agrobacterium* -mediated transformation of wheat and triticale and transgenic plants were obtained. In the next part of the research the molecular and phenotypic analysis will be performed to determine the effectiveness and preciseness of gene silencing with RNAi pathways used. We believe that the results of our studies will be helpful in choosing the most suitable and efficient method for either functional analysis of agronomically important genes or for genetic engineering of polyploid cereals. This research is supported by the National Science Center grant UMO-2011/03/B/NZ9/01383.

Poster 18

Identification and analysis of miRNA precursors in yellow lupine based on the cDNA sequences submitted in Sequence Read Archives NCBI

P. GLAZINSKA[1,2], M. GRZECA[1,2], W. WOJCIECHOWSKI[1,2], E. WILMOWICZ[1,2], K. MARCINIAK[1,2], J. KESY[1,2], J. KOPCEWICZ[1]

[1] Chair of Plant Physiology and Biotechnology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

[2] Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Torun, Poland

Yellow lupin (*Lupinus luteus* L.) is an important legume crop characterized by high content of seed proteins. The crucial stage for lupine productivity, the formation and development of flowers and seeds, is often associated with the abscission of flowers. Plant miRNAs are approximately 21-nt-long small regulatory RNAs that recognize their mRNA targets based on imperfect sequence complementarity, thereby suppress expression of the target gene by guiding degradation and/or translational repression of the cognate mRNA target, which are involved in the regulation of plants growth and development. MiRNAs are produced from either their own genes or from introns and then processed from single-stranded precursors that form hairpin structures, with the miRNAs residing in one arm of the stems. In contrary to mature sequences, much less evolutionary conserved precursors of miRNA hinder the identification of pre-miRNA homologous in other plant species. Using the fact that, plant pri-miRNAs are primarily transcribed by RNA polymerase II to produce mRNA-like structure with 5′-end cap and 3′- end polyA tails, we decided to identify miRNA precursors using data from 454 sequencing cDNA *Lupinus luteus* L. libraries submitted in Sequence Read Archives NCBI (SRP014198). In the present study, we have used seven miRNAs sequences (from miRBase) that play important role in generative development of another plant species. As the result, we have found that SRA data base contains homology sequences to five known miRNAs of seven used in the study. Part of them form hairpin structures and contain the mature miRNA sequence in one arm of the stems. Identified putative precursors are very similar to soybean and *Medicago* with minor difference in the hairpin structure outside of the highly conserved miRNA/miRNA* region. The occurrence of the identified pre-miRNA in yellow lupine have been confirmed by PCR and sequencing. This is the first step to study the putative miRNAs involving in flower morphogenesis and abscission in *Lupinus luteus* L.

Poster 19

mRNA decay factors contribute to ABA singaling in *Arabidopsis thaliana*

A. GOLISZ, I. WAWER, A. SULKOWSKA, J. KUFEL

Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Sm-like (Lsm) proteins have been identified in all organisms and function in RNA metabolism. They form two distinct heptameric complexes: the nuclear Lsm2-8 complex is a core component of the U6 snRNP and is involved in mRNA splicing and the cytoplasmic Lsm1-7 is engaged in 5′-3′ mRNA degradation. We have recently confirmed that similar LSM8 and LSM1 complexes exist also in *Arabidopsis* and are involved in mRNA splicing and decay, respectively. Interestingly, a mutant in the *SAD1/LSM5* gene, was shown to be hypersensitive to the plant hormone abscisic acid (ABA), salt and drought, and displays altered expression of some stress related genes, whereas *lsm4* plants are hypersensitive to salt and ABA. To further investigate the link between RNA decay and plant hormone response we have analyzed *Arabidopsis lsm1* mutant, which similarly to *sad1* shows growth sensitivity to ABA. As *lsm1* transcriptome profiles revealed accumulation of some mRNAs encoding core components of ABA signaling we have tested mRNA level for chosen genes, including PYL/PYR/RCAR ABA receptors, PP2C protein phosphatases and SnRK2 kinases. Notably, *PYR1* and *PYL5* transcripts were upregulated, with *PYR1* mRNA stabilized in *lsm1* plants, which shows that it is a direct subtrate of the LSM1 decay pathway. Consistently, the activity of ABA-dependent SnRK2 kinases were enhanced, and mRNA level of some ABA-induced and SnRK2-regulated PP2C phosphatases were upregulated in this mutant. mRNAs changed in cytoplasmic *lsm* mutants partially overlap with substrates of the cytoplasmic 5′-3′ exonuclease AtXRN4, the decapping enzyme AtDCP2 as well as NMD effectors AtUPF1 and AtUPF2. In addition, mutants in these factors are sensitive to ABA. We have therefore tested the level of ABA receptor mRNAs and it transpired that in *upf1* and *dcp5* mutants, but not in *xrn4*, *PYR1* and *PYL5* behave alike as in *lsm1* plants. Also, SnRK2 kinase activity is increased in the absence of AtUPF1. These results indicate that LSM1 complex, and possibly other components of the cytoplasmic mRNA decay pathway, contribute to the regulation of stress- and hormone-related processes via their role in mRNA metabolism.

Poster 20

Alternative splicing events in two maize lines under herbicide stress conditions

J. GRACZ[1], A. TYCZEWSKA[1], A. HOFFA[2], A. ZMIENKO[1], A. SWIERCZ[1,2], J. BLAZEWICZ[1,2], T. TWARDOWSKI[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

[2] Institute of Computing Science, Poznan University of Technology, Poznan, Poland

Plants, as sessile organisms, must adapt their growth and metabolic style to a changing environment. Splicing is one of the mechanisms which play an important role in plant adaptation and is an additional element of fitness benefit adjusted to the limited capacity of genome size. Studies of splicing and its role in diverse aspect of cell biology, pathology and stress response, has remained undescribed for many plant species, including maize. Through the mechanism of alternative splicing, exons from primary transcripts (pre-mRNA) with multiple introns may undergo ligation in many different ways generating multiple proteins from single gene. This process can affect mRNA stability and translation efficiency as well as activity, cellular localization, regulation and stability of coding protein. For better

characterization of alternative splicing role in plant herbicide stress response, we sequenced transcriptomes of two maize breed lines – sensitive and tolerant to herbicide RoundUp. We used Illumina next-generation sequencer Genome Analyzer IIx and we conducted pair-end sequencing. As a result we obtained 35 to 76 mln 50nt reads per sample. Using bioinformatics tools such as BowTie, TopHat, Cufflinks, Cuffdiff and CummRbund we managed to identify between sensitive and tolerant maize line. We also managed to identify different types of splicing events with java script. Funding acknowledgement: Ministry of Science and Higher Education 3098/B/P01/2010/39; National Science Center DEC-2011/01/N/NZ9/02900

Poster 21

Transcriptional gene silencing induced by VIGS-BSMV system in rye for functional analysis of *ScBx1*

J. Groszyk[1], M. Rakoczy-Trojanowska[2], W. Orczyk[1]

[1]Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

[2]Department of Plant Genetics, Breeding, and Biotechnology, Warsaw University of Life Sciences, Warsaw, Poland

Benzoxazinoids (BXs) are defensive compounds of secondary metabolism that have been found in several species of *Poaceae*, including the major agricultural crops maize (*Zea mays*), wheat (*Triticum aestivum*) and rye (*Secale cereale*) and wild barley (*Hordeum lechleri*). The compounds are considered as important factors involved in allelophatic interactions and biotic and abiotic stress tolerance. The most important BX synthesized in rye is DIBOA (2,4-dihydroxy-1,4-benzoxazinon-3-one). The compound is the final product of benzoxazinoid biosynthetic pathway catalyzed by enzymes encoded by *Bx1* ÷ *Bx5* genes. The goal of this project was to experimentally verify the biological function of *ScBx1* gene encoding indole-3-glycerol phosphate lyase, probably the first specific enzyme of BIBOA biosynthesis in rye. The experimental VIGS (virus induced gene silencing) system was selected for functional analysis of this gene. The genomic sequence of the analyzed *ScBx1* gene including the sequence of promoter region was obtained as a result of this project. Selected fragments of promoter regions were cloned into cDNA of modified BSMV (*Barley stripe mosaic virus*) β and γ units. The resultant plasmids pT7_BSMV: α, pT7_BSMV: β_prom-*ScBx1* and pT7_BSMV: γ_prom-*ScBx1* were used as the templates for *in vitro* transcription. The mixture of α, β_ prom-*ScBx1* and γ_prom-*ScBx1* transcripts were used for inoculation of rye seedlings cultivar Konto F1. Leaves with symptoms of BSMV infection were collected 14, 21 and 99 days post inoculation (dpi) and used for: i) analysis of *ScBx1* transcript level, ii) detection of CG methylation in promoter region selected and cloned into VIGS vectors and iii) analysis of total amount of DIBOA. The analysis of gene expression and CG methylation of target region was compared with control plants i.e. the plants inoculated with the 'empty' BSMV: α, BSMV: β and BSMV: γ RNA mixture. *ScBx1* transcript level in experimental plants ranged from 0.03 to 0.09 14dpi, from 0.14 to 1.33 21dpi and from 0.02 to 0.67 99dpi compared with transcript level in control plants. CG methylation of target *ScBx1* promoter in plants with lowered expression was from 1.31% to 1.96% 14 dpi, from 3.06% to 34.87% 21 dpi and from 12.16% to 33.92% 99 dpi. CG methylation of corresponding region in control plants was 1.55% and it was similar in all six control plants in three (14, 21, 99 dpi) experimental time-points. The results indicate that VIGS-BSMV system can be used as efficient experimental tool for directed CG methylation of selected genomic DNA regions. We conclude the observed lowered transcript level was the result of methylation-induced transcriptional silencing. Total DIBOA content and its correlation with *ScBx1* transcript level will be investigated. The research has been financed by The National Centre for Research and Development grant nr PBS1/A8/12/2012.

Poster 22

Evidence for alternative splicing mechanism for meadow fescue (*Festuca pratensis*) and perennial ryegrass (*Lolium perenne*) *RcaA* gene

B. Jurczyk, M. Rapacz

Department of Plant Physiology, Faculty of Agriculture and Economics, University of Agriculture in Krakow, Cracow, Poland

RuBisCO ACTIVASE (RCA) catalyzes the activation of RuBisCO. In several plant species two RCA isoforms are evidenced as an effect of alternative splicing of pre-mRNA. On the other hand two isoforms may be also products of separate genes. The aim of the study was to confirm, that two isoform of RCA in *Lolium perenne* and *Festuca pratensis* are products of alternative splicing of *RCAa* gene. 3′ RACE (rapid amplification of cDNA ends) has been performed for meadow fescue and perennial ryegrass *RCAa*. In both species PCR products of about 1200 bp was obtained and sequenced after isolation from agarose gels. As confirmed by ClustalW alignment, this fragment represented the majority of the coding sequence of *RCAa*. A very high homology in coding sequence has been observed between *Lolium perenne* and *Festuca pratensis*. PCR primers flanking the orthologous gene region, where splicing has been observed in barley, were designed. Two PCR products differing 48 bp in length were evidenced. Both products was isolated from agarose gel and sequenced to confirm that they represent two splicing variants of *RCAa*. The insertion contains an early stop codon, in the same position as observed in barley *RCAa2* mRNA. The presence of two RCA isoforms was additionally confirmed by Western Blot.

Poster 23

Apple miRNAs and their role in fire blight resistance

E. Kaja[1], T. McNellis[2], M. Szczesniak[1], I. Makalowska[1]

[1] Laboratory of Bioinformatics, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Department of Plant Pathology, Penn State University, Pennsylvania, USA

Micro RNAs (miRNAs) are small, single stranded RNA molecules, which are involved in post-transcriptional gene silencing in plant and animal cells. To date, it has been reported that plant miRNAs, by targeting many regulatory genes, play an important role in such processes as: plant development, hormone signaling or biotic and abiotic stress response. Although, many interesting facts have already been discovered about miRNA nature and way of action, those molecules are still surprising and not fully understood. In this research we characterized miRNAs, which are specific for Gala apple scions grafted on four different rootstocks: B.9, G.30, M.27 and M.111, presenting diverse fire blight resistance. Our previous studies (Jensen et al., 2009) showed, that those rootstocks also induce a different gene expression pattern in the apple scion as well as they determine tree size. Although the mechanism of this regulation is not known yet, we suggest that miRNAs might play a crucial role in it. In order to identify miRNA species, as well as their expression levels in selected trees, SOLiD sequencing of small RNAs has been performed. All the reads have been mapped to the apple genome (http://www.rosaceae.org/projects/apple_genome) and searched for conserved and apple-specific miRNAs. Performed analyses allowed us to extend the apple miRNA repertoire by 38 conserved and 78 novel, apple specific, miRNA as well as verify 143 miRNAs from previous studies. We confirmed five of new miRNAs using qPCR or RT-PCR. We also identified miRNAs with significantly changed expression among analyzed rootstocks. In addition, we searched for potential miRNA targets using psRNATarget focusing on transcripts with significantly higher expression in fire blight resistant trees.

Poster 24

Links between F-box proteins and ARGONAUTE1 N domain

S. KAUSIKA, P. BRODERSEN

School of Biology, University of Copenhagen, Copenhagen, Denmark

ARGONAUTE (AGO) proteins are the core components of RNA induced silencing complex (RISC) that cause post-transcriptional gene silencing (PTGS) guided by small RNAs. Four domains, the N, PAZ, MID and Piwi are common to all AGOs, and have key functions in small RNA binding and target mRNA repression. Our interest is in the function of the poorly understood N domain. It has been shown that membrane association of AGO is important for function and our previous data shows that a mutation in the N domain of *Arabidopsis* AGO1 alleviates membrane association. In this study, we used the N domain of AGO1 as bait in a yeast two-hybrid screen to identify interactors. We found two F-box proteins and a ubiquitin-like protein as candidates. F-box proteins are core components of the Skp1-Cullin1-Fbox (SCF)-type E3 ubiquitin ligases that recognize specific target substrates and catalyze their ubiquitination. The two AGO1-interacting F-box proteins require two distinct set of amino acids in AGO1, potentially identifying important interaction sites in AGO1. Point mutants in amino acid residues predicted to be surface-exposed at these sites led to severe phenotypes, suggesting that these sites are crucial for AGO1 function *in planta*. Knockout of either F-box protein led to appreciable increases in AGO1 protein levels, suggesting that the F-box proteins could be involved in of AGO1 in regulated proteolysis of AGO1 *in planta*.

Poster 25

Can we design PPR proteins to bind user-defined RNA targets?

P. KINDGREN, A. YAP, I. SMALL

Plant Energy Biology, Australian Research Council Centre of Excellence, University of Western Australia, Crawley, Australia

Pentatricopeptide repeat (PPR) proteins bind RNA and determine a wide range of RNA processing events required to prepare plant organellar RNA for translation, such as stability, splicing and editing. PPR motifs are capable of sequence-specific interaction with the target RNA via interactions involving 2-3 amino acids in each motif that recognise individual nucleotides in the RNA target. The aim of this project is to obtain the knowledge needed to design and construct proteins capable of binding user-defined RNA sequences. By systematically mutating the RNA-interacting amino acids in CLB19, which edits *rpoA* and *clpP* in plastids, and the nucleotides in its target RNAs, we have confirmed the exact binding sites and the residues that determine target specificity. We have shown that it is possible to predictably alter the binding preferences of RNA editing factors *in vitro* and *in vivo*. To investigate if it is possible to make more drastic changes, synthetic proteins have been created where the PPR tract from another editing factor, YS1, have replaced the PPR tract of CLB19. The specific role of individual PPR motifs along a tandem PPR tract and their involvement in RNA recognition will be discussed.

Poster 26

The role of small RNAs in the tomato root response to cyst nematode infection

M.D. KOTER[1], M. SWIECICKA[1], A. PACAK[2], M. FILIPECKI[1]

[1] Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences, Warsaw, Poland

[2] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

Plant cyst nematodes (PCN) infect roots and induce formation of multinuclear syncytium, a specialized structure becoming a sole food source for developing larvae and adults. The formation of syncytium is accompanied with active suppression of defense response as well as substantial reprogramming of development and metabolism of incorporated cells. Such process are likely to engage different mechanisms of gene expression regulation including those mediated by small RNAs. Since down-regulation of genes upon plant parasitic nematode infection is a common phenomenon concerning a third part of regulated genes we decided to purify and sequence the sRNA fraction of infected root transcriptome. The tomato roots were grown in vitro and infected with PCN. Root fragments with syncytia were collected and RNA was isolated and fractionated. The indexed sRNA libraries were sequenced using Illumina MiSeq genome sequencer. Resulting sequences were analyzed using UEA sWorbench 3.1 and potential target genes were identified using psRNATarget server. The results show many changes in composition of sRNA profiles. 54 known miRNAs have been found with 6 of them manifesting over 2-fold induction/suppression as compared to control samples. Their predicted target genes in tomato genome are transcription factors (GRAS, MYB, NAM), proteins involved in signal transduction (glucose/ribitol dehydrogenase, phosphatidate cytidyltransferase) and LRR receptor-like serine/threonine-protein kinase. Substantial portion of target candidates are likely to participate in other stress responses. Several miRNA homologues identified, such as mir1446, mir164 and mir399 are up-regulated in infected tissues showing the potential mechanism of plant response suppression by cyst nematodes.

Poster 27

Heat stress-regulated microRNAs in barley

K. KRUSZKA[1], A. PACAK[1], A. SWIDA-BARTECZKA[1], P. NUC[1],
S. ALABA[2], W. KARLOWSKI[2], A. JARMOLOWSKI[1], Z. SZWEYKOWSKA-KULINSKA[1,2]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Heat stress is one of the major abiotic factors that can induce severe plant damages leading to a decrease in crop plant productivity. Despite barley being a cereal of great economic importance, little data is available concerning its thermotolerance mechanisms. Here we investigated miRNAs involved in heat stress response in barley. Using northern hybridization we found out that four mature miRNAs: miR160a, 166a, 167h and 5157a were up-regulated under heat stress in barley. Our studies also revealed that the level of their pri-miRNAs was affected under the heat stress conditions. Surprisingly, the splicing of the intron-containing precursors pri-miR160a and pri-miR5175a was induced by heat, suggesting the post-trancriptional regulation of miRNA precursor processing. Furthermore, we experimentally identified conserved (HD-Zip transcription factors and auxin response factors) as well as novel target genes (*HOX9, ACC oxidase* and *Nek5-like kinase*) of the heat-responsive barley miRNAs using the degradome

analysis and 5′RACE approach. The observed induction of the mature miRNAs expression was correlated with the down-regulation of the expression level of their corresponding target genes. The identified target genes of barley heat-regulated miRNAs are involved in the regulation of a leaf morphology and polarity, shoot morphology, flower development, adventitious root formation, microtubule function and ethylene biosynthesis. These morphological and physiological features were often reported to be affected under heat stress conditions leading to a significant decrease in plant height and biomass reduction or the arrest of the root elongation and branching that frequently resulted in a decrease of crop yield. Our findings showed that barley miRNAs, together with target genes function in a complex regulatory network that barley plants developed to cope with stressful conditions. This work was supported by POLAPGEN-BD project no. UDA.POIG.01.03.01-00-101/08 "Biotechnological tools for breeding cereals with increased resistance to drought", subject 20: "The role of microRNA in regulation of mechanisms leading to drought adaptation in plants", executed within Innovative Economy Programme 2007-2013, subject "Biological progress in agriculture and environment protection".

Poster 28

Transcriptomic and proteomic study of seeds priming and post-priming germination emphasize the importance of mRNA translation regulation and post-translational processing in priming-induced improvement of seeds germination

S. Kubala[1], M. Quinet[2], L. Wojtyla[1], A. Kosmala[3], S. Lutts[2], M. Garnczarska[1]

[1] Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Groupe de Recherche en Physiologie Vegetale (GRPV), Earth and Life Institute – Agronomy (ELI-A), Universite catholique de Louvain, Louvain-la-Neuve, Belgium

[3] Department of Environmental Stress Biology, Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

Osmopriming is a pre-sowing treatment that exposes seeds to a low external water potential that allows partial hydration but prevents germination. Priming improves seed germination performance as well as stress tolerance of germinating seeds and seedlings. In this work, rape (*Brassica napus* L.) seeds were osmoprimed with −1.2 MPa polyethylene glycol (PEG 6000) for 7 days. A global expression profiling method was used to compare transcriptomic and proteomic data for osmoprimed seeds at the crucial phases of priming procedure (soaking, drying), whole priming process and subsequent germination. Total number of 952 genes and 75 proteins were affected during the main phases of priming and post-priming germination. In general, more genes were up-regulated during osmopriming treatment and post-priming germination. Regarding the different phases of priming, most of genes down-regulated during PEG soaking were affected in opposite way during drying. There were more proteins which abundance decreased in response to PEG soaking and seed drying while most of the proteins analysed during complete osmopriming and post-priming germination showed an increase of their abundance during these processes. Progress towards germination in primed seeds was associated with an increase in protein synthesis potential, post-translational processing capacity and targeted proteolysis. Higher expression of genes involved in regulation of transcription, water transport, cell wall modification, cytoskeletal organization and cell division was also linked to the advanced germination of primed seeds. Moreover, improved germination of primed seeds was associated with higher genes expression and abundance of proteins involved in the management of oxidative stress during post-priming germination. The differences between transcriptome and proteome data set (the match between genes and proteins was limited to only 12 gene-protein pairs) reinforce the importance of the regulation of mRNA translation and post-translational

processing during priming and post-priming germination. This weak correspondence between mRNA levels and protein abundance is due to the existence of complex post-transcriptional processes such as transcript de/stabilization, translation, posttranslational modifications and protein degradation which determine and modulate the quality and quantity of expressed proteins. This work was supported by grant no. 2011/03/B/NZ9/00068 from the National Science Centre given to MG. SK obtained financial support for the best PhD students in PO KL 8.2.2. program editions: 2011/2012 and 2012/2013 and from the National Science Center DEC-2013/08/T/NZ9/01019. SK is a scholarship holder of The Adam Mickiewicz University Foundation in Poznan in 2013/2014.

Poster 29

Participation of genes encoding biosynthesis and metabolism compound of jasmonates in flower morphogenesis of *Ipomoea nil*

A. KUCKO[1,2], E. WILMOWICZ[1,2], K. FRANKOWSKI[1], K. MARCINIAK[1,2], J. KESY[1,2], J. KOPCEWICZ[1]

[1] Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Torun, Poland

[2] Centre for modern interdisciplinary technologies, Nicolaus Copernicus University, Torun, Poland

Jasmonates, with representative jasmonic acid (JA), are lipid-derived phytohormones with diverse functions, ranging from the initiation of biotic and abiotic stress responses, to the regulation of plant growth and development. It has been shown that bioactivity of jasmonates is not restricted to the free acid, but also some of its precursors and derivatives are activators of plant responses. JA biosynthesis originates from LIPOXYGENASE (LOX) - catalyzed oxidation of polyunsaturated fatty acids in chloroplast, which are subsequently converted by ALLENE OXIDE SYNTHASE (AOS) and ALLENE OXIDE CYCLASE (AOC) to 12-oxophytodienoic acid. Cyclopentanone ring structure of jasmonates is established by OPDA REDUCTASE (OPDR) activity in peroxisome. Cytosol is the compartment of JA biochemical diversification, e.g. formation of predominant amino acid conjugate with isoleucine (JA-Ile) catalyzed by JAR (JASMONATE RESISTANT) or MeJA (JA-methyl ester) synthesis performed by JMT (JA CARBOXYL METHYLTRANSFERASE), which has been recognized as highly active in bioassays for JA activity. In this study we reported the expression profile of enzymes involved in biosynthesis (*InLOX*, *InAOS*, *InOPR3*) and metabolism (*InJMT*, *InJAR*) of JA in vegetative (roots, hypocotyls, cotyledons, apexes and leaves) and generative (stamens, pistils and petals) organs of *Ipomoea nil*. Experiments have been performed by Real-Time PCR (qRT-PCR) with gene-specific primers and UPL probes, with actin as a reference endogenous control. *InLOX* transcript accumulation was greater in generative than in vegetative tissues. There was no significant difference between *InAOS* and *InOPR3*. The highest transcripts level of these genes in hypocotyls and petals was observed. The activity of genes involved in metabolism of JA in cotyledons, hypocotyls and leaves was similar. *InJMT* strongly accumulated in petals, whereas *InJAR* in pistils, almost at all stages of their development. The level of mRNA *InLOX* and *InOPR3* increased during pistils and stamens development. We suggest that that differential expression of JAs biosynthesis and metabolism genes during vegetative and generative development, allows plants to maintain specific JAs homeostasis, necessary for proper plant development.

Poster 30

Model expansion: gene expression under binary metal treatment, hydroponics vs. peat pots

A. KUTROWSKA, A. MALECKA, A. PIECHALAK, B. TOMASZEWSKA

Department of Biochemistry, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Trace metals present in the environment in excess cause adverse effects on plant yield and biomass quality. Impact of the elements depends on the soil pH, its redox state, organic content, ion capacity and many other factors. Despite this, most of the fundamental research on metal treatment performed in the laboratories is limited to the semi-ideal environment created with the use of hydroponics or perlite foundation. This approach, although burdened with many disadvantages, presents unique opportunity to isolate and closely examine interesting phenomena. In our study we aimed to enrich the standard model of metal uptake by examining the simultaneous treatments with binary metal combinations in a hydroponic culture (with Hoagland solution). We then performed additional experiments replicating our approach but on the commercially available peat pots instead of hydroponic medium. Our model involved the use of 3-weeks-old seedlings of Indian mustard (*Brassica juncea*), one of the known metal hyperaccumulators. Careful examination of different metal treatments resulted in the following final metal concentrations: for individual treatments of Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} – 50 μM of each metal in hydroponics and 100 μM for the peat pots; for binary combinations (CuZn, CuPb, CuCd, CdPb, ZnCd, ZnPb) – 25 μM of each metal in hydroponics and 50 μM of each metal for the peat pots. Double concentration of metals was used for the peat pots due to the lowered metal mobility in this medium. Additionally, plants showed similar levels of stress (measured by the antioxidant enzyme activity and reactive oxygen generation, among other) on these separate mediums, when treated with selected concentrations. For the gene expression analysis, we decided to measure the short response to metal stress, thus limiting ourselves to following time points: 0′, 4′, 8′ hours of metal treatment for roots and 0′, 8′, 24′ hours for the aboveground tissues. Relative levels of transcription for genes encoding superoxide dismutase (*Mn-SOD*, *Cu,Zn-SOD*), gamma-glutamylcysteine synthetase (*γ-ECS*) and glutathione reductase (*GR*) were determined with the use of RT-PCR and qPCR. Generally, gene expression was induced mostly by the binary combinations. Genes encoding antioxidant genes (SOD) responded to a lower extent to metals than the genes encoding detoxicative enzymes (ECS, GR). We observed different patterns of gene expression on hydroponics and peat pots, suggesting that though the plant stress symptoms on the higher level (RFT, antioxidants) were similar for these media, gene expression levels could not have been easily translated. This work was partially supported by NCN grant no 3811/ B/P01/2010/38.

Poster 31

In vitro biochemical characterization of *Arabidopsis thaliana* DXO1 protein

A. KWASNIK, K. STEPNIAK, A. GOZDEK, J. KUFEL

Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

The correct formation of mRNA 5′ end is a crucial component of the regulation of gene expression and is therefore subjected to surveillance mechanisms that detect molecules containing aberrant 5′ end structures and target these dysfunctional transcripts for degradation. Yeast and human members of the RAI1/DXO1 family were recently shown to participate in these processes as they exhibit phosphodiesterase (PPE) activity to remove incomplete,

unmethylated caps and may additionally act as pirophosphohydrolase (PPH) or 5′-3′ exoribonuclease towards uncapped mRNAs containing 5′ end triphosphates and monophosphates, respectively. Here we describe the *in vitro* biochemical activity of a putative RAI1/DXO1 homologue from *Arabidopsis thaliana.* This protein displays high conservation of active site amino acid sequence with other RAI1/DXO1 proteins, but it additionally contains unique N-terminal unstructured domain that may affect its biochemical properties. The fact that *Arabidopsis dxo1* insertion mutant lines show severe growth inhibition, sterility and several defects in molecular phenotypes underlines the importance of this protein for RNA metabolism in plants. We performed a series of *in vitro* assays using purified AtDXO1 and its catalytically inactive or N-terminally truncated variants to test for the PPE, PPH and 5′-3′ exoribonuclease activities towards oligoribonucleotide substrates containing methylated or unmetylated cap, as well as triphosphate or monophosphate group at their 5′ ends. These experiments demonstrated that AtDXO1 exhibited all three activities, with enzymatic properties somewhat different than those of human DXO. Our data suggest that AtDXO1 activities are probably employed to remove abnormal cap structures and degrade improperly capped or uncapped RNA molecules.

Poster 32

Calcium-dependent phosphoproteins – a multiprotein hub for stress signal transduction

A. Ludwikow[1], F. Mitula[1], M. Tajdel[1], A. Ciesla[1,2], L.H. Misztal[1], J. Sadowski[1,2]

[1] Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Institute of Plant Genetics of the Polish Academy of Sciences, Poznan, Poland

Calcium-dependent protein kinases (CPKs) comprise a large family of structurally conserved serine/threonine kinases involved multiple biological processes. A key challenge in CPK research is to understand the structural basis for activation of CPK. Here, we implemented advanced proteomic approaches to identify and analyze the drought-induced CPKs in *Hordeum vulgare.* To facilitate the analysis we raised antibodies against active site of kinase domain and used them to immunoprecipitate protein complexes containing CPKs from drought tolerant and drought sensitive barley genotypes. Subsequent LC-MS/MS analysis allowed identification of two putative barley CPKs involved in regulation of drought stress response in stress tolerant genotype. Interestingly, identified putative barley CPK shows substantial similarity to Arabidopsis CPK17 and CPK34. To further investigate the function of CPKs an initial insight into cellular localization was achieved. In the Arabidopsis and barley protoplasts *HvCPK* fused to the *GFP* reporter was targeted to the plasma membrane localization. To test mechanism of HvCPKs activation we purified recombinant HvCPKs and analyzed kinase activity using *in vitro* and in gel assay methods. Together, we show evidence for the role of barley CPKs in the regulation of drought stress response.

Poster 33

High-throughput sequencing identification of novel and conserved miRNAs in the *Brassica oleracea* leaves

A. Lukasik[1], H. Pietrykowska[2], L. Paczek[1,3], Z. Szweykowska-Kulinska[2], P. Zielenkiewicz[1,4]

[1] Institute of Biophysics and Biochemistry, Polish Academy of Sciences, Warsaw, Poland

[2] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[3] Department of Immunology, Transplant Medicine and Internal Medicine, Medical University of Warsaw, Warsaw, Poland

[4] Department of Plant Molecular Biology, Institute of Experimental Plant Biology and Biotechnology, University of Warsaw, Warsaw, Poland

Plant microRNAs are short (~21 nt) non-coding molecules that regulate gene expression by targeting the mRNA cleavage or protein translation inhibition. In this manner, they play many important roles in the cells of living organisms. One of the plant species in which the entire set of miRNAs has not been yet completely identified is *Brassica oleracea* var. *capitata* (cabbage). For this reason and for the economic and nutritional importance of this food crop, high-throughput small RNAs sequencing has been performed to discover the novel and conserved miRNAs in mature cabbage leaves. In this study, raw reads generated from three small RNA libraries were bioinformatically processed and further analyzed to select sequences homologous to known B. oleracea and other plant miRNAs. As a result of this analysis, 261 conserved miRNAs (belonging to 62 families) have been discovered. MIR169, MIR167 and MIR166 were the largest miRNA families, while the highest abundance molecules were miR167, miR166, miR168c and miR157a. Among the generated sequencing reads, miRNAs* were also found, such as the miR162c*, miR160a* and miR157a*. The unannotated tags were used in the prediction and evaluation of novel miRNAs, which resulted in the 26 potential miRNAs proposal. The expressions of 13 selected miRNAs were analyzed by northern blot hybridization. The target prediction and annotation for identified miRNAs were performed, according to which discovered molecules may target mRNAs encoding several potential proteins – e.g., transcription factors, polypeptides that regulate hormone stimuli and abiotic stress response, and molecules participating in transport and cell communication. Additionally, KEGG maps analysis suggested that the miRNAs in cabbage are involved in important processing pathways, including glycolysis, glycerolipid metabolism, flavonoid biosynthesis and oxidative phosphorylation. Conclusively, for the first time, the large set of miRNAs was identified in mature cabbage leaves. Potential targets designation for these miRNAs may suggest their essential role in many plants primary biological processes. Presented study not only supplements the knowledge about B. oleracea miRNAs, but additionally it may be used in other research concerning the improvement of the cabbage cultivation.

Poster 34

smRNAome sequencing to identify conserved and novel microRNAs in *Stevia rebaudiana* Bertoni

Vibha Mandhan, Kashmir Singh

Department of Biotechnology, Panjab University, Chandigarh, India

MicroRNAs (miRNAs) constitute a family of small RNA (sRNA) species that regulates the gene expression and plays an important role in plant development, metabolism, signal transduction and stress response. Extensive studies of miRNAs have been performed in different plants such as *Arabidopsis thaliana*, *Oryza sativa* and volume of the

miRNA database, mirBASE has been increasing on day to day basis. *Stevia rebaudiana* Bertoni is an important perennial herb which accumulates high concentrations of diterpene steviol glycosides which contributes to its high indexed sweetening property with no calorific value. Several studies has been carried out on understanding molecular mechanism of biosynthesis of these glycosides, however, information about miRNAs has been lacking in *S. rebaudiana*. Deep sequencing of small RNAs combined with transcriptomic data is a powerful tool for identifying conserved and novel miRNAs irrespective of availability of genome sequence data.To indentify miRNAs in *S. rebaudiana*, sRNA library was constructed and sequenced using Illumina genome analyzer II. A total of 30,472,534 reads representing 2509190 distinct sequences were obtained from sRNA library. Based on sequence similarity, we identified 100 miRNAs belonging to 34 highly conserved families. Also, we identified 12 novel miRNAs whose precursors were potentially generated from stevia EST and nucleotide sequences. All novel sequences have not been earlier described in other plant species. Putative target genes were predicted for most conserved and novel miRNAs. The predicted targets are mainly mRNA encoding enzymes regulating essential plant metabolic and signaling pathways. This study led to the identification of 34 highly conserved miRNA families and 12 novel potential miRNAs indicating that specific miRNAs exist in stevia species.Our results provided information on stevia miRNAs and their targets building a foundation for future studies to understand their roles in key stevia traits.

Poster 35

LlGAMyb gene expression profile in generative organs of yellow lupine (*Lupinus luteus* L.)

K. Marciniak, P. Glazinska, E. Wilmowicz, A. Kucko, W. Wojciechowski, M. Banach, J. Kopcewicz

Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Torun, Poland

GAMyb, a positive regulator involved in the GA signalling pathway, has been known to act as an important downstream component in the degradation of DELLA proteins. *GAMyb* gene was first identified in barley (*Hordeum vulgare*) aleurone cells, where its expression is upregulated by GA treatment. The synthesized protein can bind specifically to GA-response elements in promoter regions of an α-amylase gene and many other GA-regulated genes, for example *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*) and *LFY* (*LEAFY*).Therefore, the GAMyb has been demonstrated to play an important role in such processes as seed and flower development. In the first step of our work, the *GAMyb* homologue in yellow lupine (*Lupinus luteus* L.) cultivar Taper was identified (Marciniak et al., 2013). As a consequence, the transcriptional activity of *LlGAMyb* at different development stages of inflorescence, flowers and pods was determined using RT-qPCR technique. During the growth of the whole inflorescence (8 phases), a decrease of mRNA level by about five times between the first and last phase was observed. In turn, in the six individual flower whorls of fully mature inflorescence were no significant differences in the content of the *LlGAMyb* transcripts. At the later stage of flowers withering and pods forming, rapid increase in the transcriptional activity of investigated gene was recorded, especially in the two upper whorls of all six. In plants with fully mature pods in each whorl (3-6), we observed a similar level of gene expression as in plants with fully developed flowers. During single flower development slight upward tendency of mRNA accumulation was observed, whereas during single pod development (from 1 to 6 seeds) was downward tendency. The results indicate the specific expression pattern of the studied gene in different generative organs of *Lupinus luteus* L. Due to the highest level of transcriptional activity in the examined variants, the *LlGAMyb* gene may be involved mainly in the early stage of pod development. It should be added, that presented results are an introduction to the extensive research that in the near future will determine the precise mechanism of the flower and pod development. This in turn can lead to improved yields of yellow lupine. This work was supported by the Ministry of Agriculture and Rural Development of Poland Grant No 149/2011.

Poster 36

Natural variation of transposon silencing in *Arabidopsis thaliana* is caused by transposition and regulation by *MUR1*

T. MEYER, I. BAURLE

Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

The silencing of DNA transposons and retroelements is essential for all eukaryotic organisms to maintain genome stability and integrity and suppress their mutagenic potential. Otherwise active mobile elements can destroy functional genes by insertion, alter gene expression or cause a rapid growth of the genome size. A large number of interacting pathways, including DNA methylation, small RNA-based silencing and histone modifications have evolved to ensure transposon silencing. While it is accepted that transposon activity over millions of years has contributed to the evolution of species, it is currently unclear how much variation in transposon activity exists at a smaller timescale, for example on an intraspecific level. As a model case for this question, we have chosen to study the DNA transposon *AtMu1* of *Arabidopsis thaliana*, which is characterized by an over 100fold expression difference between two studied ecotypes. Using eQTL mapping we were able to identify the genomic region responsible for this large expression difference. A single trans-QTL on the upper arm of chromosome 1, narrowed to a 100 kb interval by fine mapping, is responsible for the variation. Within this interval, we identified a new copy of *AtMu1* inserted in the 3′UTR of a protein coding gene. Furthermore, in the mapped region a previously uncharacterized gene (*MUR1*) was found, which has a role in *AtMu1* activation. Taken together, our work identifies the genetic basis for natural variation in the silencing of a mutagenic DNA transposon and it also demonstrates the existence of host factors that positively regulate transposon expression.

Poster 37

Novel algorithm for designing plant artificial miRNA

A. MICKIEWICZ[1], A. RYBARCZYK[1,2], A. HOJKA-OSINSKA[1], P. JACKOWIAK[1], J. BLAZEWICZ[1,2], M. FIGLEROWICZ[1,2]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

[2] Institute of Computing Science, Poznan University of Technology, Poznan, Poland

MicroRNAs (miRNA) are small non-coding RNAs found in most eukaryotic organisms. They are involved in post-transcriptional control of gene expression in a sequence specific manner. The miRNAs are generated from endogenous transcripts synthesized mainly by RNA polymerase II. These precursors contain stem-loop structures, which harbor a ca. 21-nucleotide long miRNA. There are differences in the miRNA biogenesis and mode of function in plants and animals. Animal miRNA precursors are about 80 nt and two different proteins are involved in their processing. The first step occurs in the nucleus and the second yielding miRNA/miRNA* duplex occurs in the cytoplasm. Plant miRNA precursors are significantly longer, up to 600 nt, display structural diversity and are digested to miRNA/miRNA* by a nuclear DICER-LIKE 1 protein. Both in animals and plants, the miRNA (guide strand) is loaded onto the ARGONAUTE protein component of the RNA-induced silencing complex while the miRNA* is destroyed. The miRNA then directs the cleavage of mRNA or represses translation. Animal miRNAs harbor “seed” region: 2-8 nucleotides of 5 ends complementary to ca. 7 nt of target. Plant miRNAs usually display perfect or near-perfect pai-

ring with their mRNA target sites. In animals, miRNA target sites are located mostly within 3′untranslated regions whereas plant miRNAs bind both to the coding and untranslated regions. The main concept of the artificial miRNA (amiRNA) is to design a 21 nt RNA molecule that is able to regulate the target gene expression and is incorporated into the backbone that forms a fold similar to miRNA precursor structures. At present, there are few bioinformatics tools capable of designing amiRNA. One of the mostly used tools is Web MicroRNA Designer (WMD3), which is the only one dedicated to plants. Recently developed tools, miR-Synth or AmiRzyn, are designed preferentially for human amiRNA. In this work we present a new approach to design plant amiRNA. Our method is based on the analysis of the decomposed free energy profiles of known miRNA/miRNA* and miRNA/target interactions. These thermodynamic profiles serve as templates for amiRNA design. The major advantage of AmiRNA Designer, as compared to other tools, is a possibility to introduce mismatches within miRNA/miRNA* and amiRNA/target duplexes. This allows designing the regulatory RNA for targets whose expression cannot be regulated by amiRNA obtained with the currently available tools. The use of artificial miRNA is a promising technique for functional genetic studies, cultivable plant improvement, as well as for plant antiviral protection. Thus, it is very probable that this algorithm will turn out to be powerful and broadly applied. Acknowledgments: This work was partially financed by the Polish Ministry of Science and Higher Education grant number IP2012 014972, to PJ, and by grant No. 2012/05/B/ST6/03026 from the National Science Centre, Poland.

Poster 38

RNApathwaysDB – a database of RNA maturation and decay

K. Milanowska[1,2], K. Mikolajczak[2], A. Lukasik[2], M. Skorupski[2], Z. Balcer[2], M.A. Mika[1], K.M. Rother[1,2], J.M. Bujnicki[1,2]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

[2] Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Many RNA molecules undergo complex maturation, involving e.g. excision from the primary transcripts, post-transcriptional modification, splicing, and polyadenylation. The level of mature RNAs in the cell is controlled by degradation, which proceeds via many different reactions including, but not limited to endo- and exonucleolytic cleavage. The systematization of data about RNA metabolic pathways and enzymes taking part in RNA maturation and degradation is essential for the full understanding these processes. RNApathwaysDB (RNA pathways database) is the first database of metabolic pathways involving RNA as the substrate. It presents information about reactions and enzymes (proteins, RNA molecules or complexes) that take part in RNA processing. The database provides also links to other databases and literature information. The current dataset is limited to the maturation and degradation of tRNA, rRNA and mRNA, and describes pathways in three model organisms: *Escherichia coli, Saccharomyces cerevisiae* and *Homo sapiens*. Other RNAs, enzymes and pathways and data for other organisms will be successively added in the future. The database can be queried with keywords or by the name of a pathway, a reaction, an enzymatic complex, a protein or an RNA molecule. Amino acid sequences of protein enzymes involved in pathways included in RNApathwaysDB can be compared to a user-defined query sequence with a BLAST utility. Options for data presentation include graphs of pathways and tabular forms with enzymes and literature data. Structures of macromolecular complexes are presented as “potato models” using DrawBioPath – a new javascript tool. The contents of RNApathwaysDB can be accessed through the World Wide Web at http://genesilico.pl/rnapathwaysdb.

Poster 39

Effects of sugars as endogenous signals and *Fusarium oxysporum* on expression level of isoflavonoids biosynthesis pathway genes in yellow lupine

I. MORKUNAS[1], M. FORMELA[1], D. NAROZNA[2], W. NOWAK[3]

[1] Department of Plant Physiology, Poznan University of Life Sciences, Poznan, Poland

[2] Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poznan, Poland

[3] Laboratory of Molecular Biology Techniques, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Sugars not only function as substrates for growth of sink tissues, which are dependent on the import of carbohydrates, but also affect sugar-sensing systems that initiate changes in genes expression. These genes encode proteins that function in many metabolic pathways and developmental programs. Therefore, sugar-induced signaling pathways will interact with many other signaling pathways to form regulatory webs that allow the integrated response to changing environmental conditions, including invasion of pathogens. The aim of the present study was to examine effects of sucrose and monosaccharides (glucose and fructose) as endogenous signals, and a hemibiotrophic fungus *Fusarium oxysporum* f.sp. lupini on the expression of genes of flavonoid biosynthetic pathway. Real-time PCR analyses of the level of mRNA encoding enzymes involved in the synthesis of isoflavones, revealed post-infection accumulation of mRNA in embryo axes of yellow lupine cv. Juno. In embryo axes infected with *F. oxysporum* cultured *in vitro* on the medium with sucrose, glucose and fructose (+Si, +Gi, +Fi) the level of mRNA for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI) and isoflavone synthase (IFS) was higher than in non-infected axes (+Sn, +Gn, +Fn) in the period from 0 to 96 h. Moreover, up to 72 h after inoculation in inoculated axes with a sugar deficit (-Si) a higher level of mRNA encoding PAL, CHS, CHI and IFS was observed post infection in relation to non-inoculated axes (-Sn). At the next time point after inoculation, i.e. at 96 h in infected axes cultured at carbohydrate deficit (-Si) a very strong reduction was recorded in the mRNA level, while at 96 h in infected axes with a high level of carbohydrates (+Si, +Gi and +Fi) the level of mRNA encoding the above mentioned enzymes was highest. It needs to be stressed that a very high post-infection level of mRNA was recorded for enzymes of the specific isoflavone synthesis pathway, i.e. chalcone synthase (CHS) and isoflavone synthase (IFS) in embryo axes inoculated with *F. oxysporum*, being much higher than for PAL and CHI. These results suggested that the sensing of carbohydrate levels and the response to pathogen can be interrelated at some level. This study was supported by the Polish Ministry of Science and Higher Education (MNiSW, grant no. N N303 414437).

Poster 40

RNAi in practice – PTGS silencing in functional studies of agronomically important cereal genes

A. NADOLSKA-ORCZYK[1], S. GASPARIS[1], W. ZALEWSKI[1], Y. YANUSHEVSKA[2], W. ORCZYK[2]

[1] Department of Functional Genomics, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

[2] Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

Biotechnology offers a range of tools for functional analysis and for improvement of selected traits such as yield, product quality, resistance to biotic and tolerance to environmental stresses. One of the most promising experimental tools, especially useful for research on allopolyploid cereals, is RNAi-based gene silencing. We proved its

applicability by silencing three groups of genes: *PINa*/ *PINb* in wheat and their orthologs in triticale and, in other line of experiments, *HvCKX1*/ *HvCKX2* and *HvGSK* in barley. The *PIN* genes determine grain hardness in wheat, which is important technological trait. Silencing of the genes in wheat resulted in reduction of the transcript exceeded 90% what caused significant reduction or lack of both puroindoline proteins and increased grain hardness up to the level of *T. turgidum* var. *durum* cultivar, which lacks of the genes (Gasparis et al., 2011). Unexpectedly silencing of *SINa* and *SINb* genes in triticale however resulted in significant reduction of transcripts and secaloindoline proteins, did not prove their role in grain hardness (Gasparis et al., 2013). The second group of silenced genes belongs to the *HvCKX* family of barley. They encode cytokinin dehydrogenase enzymes (CKX), which regulate cytokinin level in different tissues of developing plants. The detailed functions of the genes are not known. We have already documented that silencing of the *HvCKX1*, which expression was the highest in the roots and developing spikes of wild plants, decreased cytokinin dehydrogenase level in these tissues. This leaded to higher plant productivity expressed as the yield, the number of seeds per plant and the 1000 grain weight and greater mass of the roots (Zalewski et al., 2010). Silencing of *HvCKX2* (Zalewski et al. 2012), which expression was the highest in the developing spikes as well as in the young and fully developed leaves of wild plants, decreased transcript and CKX level in these tissues. There was a positive correlation between the low level of transcript in spikes 7 DAP and the higher plant productivity. The data of productivity of modified lines up to T_3 generation will be presented. Current project, and unpublished results, is focused on identification and functional analysis of putative barley homologs of brassinosteroid regulators *OsGSK1* in rice and *BIN2* / *AtSK2* in *Arabidopsis*. Depressing transcript level of the tested gene to 0.09 – 0.22 of the transcript in control plants revealed the clear correlation with elevated salt tolerance of the seedlings. The research has been financed by grants: N302 013 31/1517, 620/N-COST/09/2010, UMO-2011/03/B/NZ9/01383.

Poster 41

Retention of poly(A) RNA in the cell nucleus of roots subjected to the hypoxia treatment

J. Niedojadlo, B. Kalich, E. Kubicka

Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

Flooding of agricultural lands is one of the biggest problems of modern agriculture, because it causes the reduction in yields. The reason for that is the reduced availability of oxygen (hypoxia) for the root system of plants. In this report, distribution and quantity of active RNA polymerase II, poly (A), SR and SUMO proteins were investigated in *Lupinus luteus* root cell nuclei under natural and stress conditions. The stress was induced by flooding the seedlings in water, which imitates the conditions during the flood. After 3 and 6 h of hypoxia treatment, a significant increase in the level of poly (A) in the cell nuclei was observed. In the cytoplasm, stress granule-like poly (A) RNA clusters were also noticed. Since the presence of poly(A) RNA in Cajal bodies (CBs) (Smolinski and Kolowerzo 2012) has recently been demonstrated, the signal level in these structures was also measured. It appeared that in the hypoxia-treated cells, the growth of poly (A) in CBs is even higher than in the whole nucleus. Measurements of the quantity of active RNA polymerase II and distribution of SR proteins revealed a strong termination of transcription during consecutive hours of hypoxia treatment. It can be concluded, that the observed increase in poly (A) is a result of the strong retention in the of nucleus, including Cajal bodies, and not transcription process intensification. One also checked, whether poly(A) RNA accumulated in the nucleus, includes RNA coding proteins. Recently, a relationship between SUMO1proteins accumulation and the retention of poly (A) RNA in the nucleus under two abiotic stress treatments, heat and ethanol treatment respectively, has been shown (Muthuswamy and Meier 2011). Our studies have demonstrated changes in the nuclear distribution of SUMO1 protein in control cells and hypoxia-treated

cells. In addition, during the initial period of hypoxia, SUMO1 protein was accumulated in Cajal bodies. Our preliminary results indicate retention of poly (A) RNA as an important mechanism of gene expression transcribed via RNA polymerase II in hypoxia-treated cells. The obtained results also suggest the involvement of Cajal bodies in the retention of poly (A) RNA in plant cells.

Poster 42

Barley microRNA 444.1 expression is regulated by alternative splicing and affects barley tillering upon heat stress

A. Pacak[1], K. Kruszka[1], A. Swida-Barteczka[1], W. Karlowski[2], A. Jarmolowski[1], Z. Szweykowska-Kulinska[1,2]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

MicroRNAs are key molecules regulating gene expression. Barley microRNAs are encoded by the genes with diverse organization, representing mostly independent transcriptional units with or without introns. The intron – containing miRNA transcripts undergo complex splicing events to generate various spliced isoforms. There are three genes encoding different species of barley microRNA444 (*MIR444.1*, *MIR444.2* and *MIR444.3*). All of them contain long introns that separate microRNA* from microRNA. Functional pre-miRNA444 can be formed only after intron removal. MiRNA 444.1, 444.2 and 444.3 differ from each other by one or two nucleotide substitutions. Recently we have tested barley response to different abiotic stresses and found that heat stress induces significantly expression of miR444. By Northern blotting and small RNA NGS analyses we identified the *MIR444.1* as a heat-induced gene. Transcript of *MIR 444.1* gene undergoes complex alternative splicing events that generate three RNA isoforms: i) fully spliced, functional pri-miR444.1 isoform, ii) alternatively spliced non-functional isoform in which the exon encoding miR444.1* is removed, and iii) non-functional spliced isoform in which the whole region containing exons encoding miR444.1* and miR444.1 together with the intron separating these exons is spliced out. We found that upon heat stress conditions the general level of pri-miR444.1 transcript as well as the level of the functional, spliced pri-miRNA isoform were significantly elevated compared to the control conditions. We observed also an interplay between all spliced isoforms of pri-miR444.1 leading to the increase of the functional pri-miRNA 444.1 isoform when compared to the control conditions. Interestingly, the *MIR444.1* and its target gene – transcription factor belonging to the MADS-box type II gene family are encoded within the same locus at the opposite DNA strands. Barley degradome analysis showed that indeed *MADS.1* is cleaved by microRNA444.1. Exons forming spliced MADS-box TF are found almost exclusively within the introns of the *MIR444.1* gene. Thus spliced pri-miRNA miR444.1 isoforms and the MADS-box TF spliced mRNA represent different in the nucleotide sequence molecules having only a short, about 62 nt long complementary region encompassing binding sites between the miRNA 444.1 and mRNA target site. Accordingly to the elevated level of the mature miR444.1 during the heat stress, we observed dramatic decrease of its target mRNA. We found that plant growth is affected upon heat-stress. We postulate that the miR444.1 increase during the plant response to the heat-stress is responsible for barley tillering inhibition via *MADS.1* downregulation. To validate the involvement of MADS.1 an tillering inhibition we use VIGS approach (Virus Induced Gene Silencing). Using *in vitro* transcribed BSMV (Barley Stripe Mosaic Virus) RNAs: RNAα, RNAβ and modified RNAγ-containing MADS.1 fragment in sense orientation, we silence *MADS.1* to observe tillering phenotype. The work was supported by the European Regional Development Fund through the Innovative Economy for Poland 2007-2013, project WND-POIG.01.03.01-00-101/08 POLAPGEN-BD “Biotechnological tools for breeding cereals with increased resistance to drought”.

Poster 43

Protein interacting with post-translationally modified H3 histone in *Arabidopsis thaliana*

A. PALUSINSKI[1], M. KOTLINSKI[1,2], T. RUBEL[3], D. BUSZEWICZ[2], J. OLEDZKI[4], M. DADLEZ[4], A. JERZMANOWSKI[1,2], M. KOBLOWSKA[1,2]

[1] Laboratory of Systems Biology, University of Warsaw, Warsaw, Poland

[2] Laboratory of Plant Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

[3] Institute of Radioelectronics, Warsaw University of Technology, Warsaw, Poland

[4] Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The post-translational modifications of histones are among the major mechanisms modulating chromatin structure. These modifications are mainly located in N-and C-terminal unstructured domains of core histones (called histone tails), extending beyond the core of nucleosome. The best known modification include methylation of lysine (K) and arginine (R), acetylation of lysine and phosphorylation of serine (S) and threonine (T). One of the most important functions of these modifications is mediating the recruitment of specific nonhistone proteins to chromatin loci. However, presence of post-translational histones modifications may function not only by the recruitment of these proteins, but also act to prevent their binding. Histone-bounding proteins are crucial in the reorganization of chromatin and regulation of numerous processes occurring at the DNA level. Therefore, understanding the roles of histone modifications is of primary importance for the study of gene regulation at the chromatin level. Relatively large numbers of animal proteins that associate with post-translationally modified histones is known. However, data on such interactions in plants is extremely poor. Our studies aim to identify proteins that bind to modified core histone H3 in *Arabidopsis thaliana*. To this end, we adapted for the first time for plant material a technique of peptide pull-down that has been used earlier for analogous experiments in animals. This assay allows to search for protein partners interacting *in vitro* with chemically synthesized peptides that are analogous to core histone tails carrying selected posttranslational modifications. So far, using mass spectrometry technology (Orbitrap Velos or Orbitrap Elite), we successfully identified several proteins binding to modified core histone in *Arabidopsis thaliana*.

Poster 44

U12 intron in *A. thaliana CBP20* gene is necessary for correct pre-mRNA splicing and mRNA/protein level

M. PIECZYNSKI[1], D. BIELEWICZ[1], J. DOLATA[1], M. SZCZESNIAK[2], A. WYRZYKOWSKA[1], W. KARLOWSKI[2], A. JARMOLOWSKI[1], Z. SZWEYKOWSKA-KULINSKA[1]

[1] Department of Gene Expression, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Laboratory of Bioinformatics, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

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 (RRM) and the second one that encodes the tail domain containing nuclear localization signal (NLS). In all investi-

gated 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 the *CBP20* gene is preserved across all plant species we prepared constructs representing *CBP20* mini-genes and its mutated full versions. Mini-gene constructs containing 4th and 5th exons from *A. thaliana CBP20* gene and U12 introns derived from different plants and differing in length (from 134nt to 2733nt) were transfected to tabaccum mesophyll 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 the mini-gene construct containing a U2 intron in the U12 intron natural position revealed that 37% of mRNAs undergo alternative splicing. Additionally we prepared five constructs containing full *A. thaliana CBP20* gene in which i) the U12 intron was replaced by a U2 one, ii) the U12 intron was removed, iii) exons flanking the U12 intron were exchanged with each other, and iv-v) the 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* gene constructs (in which the U12 intron was moved to the other gene locations) generate additional isoforms containing up to 58% of alternatively 5′ spliced U2 intron, which was placed in U12 intron natural position. Simultaneously the U12 intron in different locations within *CBP20* gene body was correct and efficient spliced. Additionally we found that plants containing mutated *CBP20* gene in which the U12 intron was replaced with a U2 one show strong down-regulation of the *CBP20* mRNA and protein levels. All these data show that the U12 intron in the proper position in *A. thaliana CBP20* genes is necessary for correct pre-mRNA splicing and mRNA/protein level.

Poster 45

The first liverwort microtranscriptome: Liverworts share molecular traits exclusive for green algae and other land plants

P. Piszczalka[1], S. Alaba[2], H. Pietrykowska[1], P. Plewka[1], I. Sierocka[1], Kashmir Singh[3], W. Karlowski[2], Z. Szweykowska-Kulinska[1,2]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

[3] Department of Biotechnology, Panjab University, Chandigarh, India

MicroRNAs are key regulatory elements of eukaryotic gene expression. These short (18-24 nt) molecules act post-transcriptionally by sequence-specific guidance of RNA Induced Silencing Complex (RISC) to complementary mRNAs which results in slicing or translation inhibition of targeted mRNAs. Because of its importance in gene expression regulation, miRNAs are a field of intensive research and much is known for model plants or plants with great economic importance. Yet, until now there are no publicly available data on any liverwort microtranscriptome. Liverworts represent the most the basal group of land invaders and are the evolutionary oldest land plants present in our days. We applied t high-throughput sequencing technique (SOLEXA, Illumina) and sequenced small RNAs, transcriptome, and degradome from the dioecious liverwort *Pellia endiviifolia* species B. 311 miRNA families of conservative miRNA species that are identical to model moss *Physcomitrella patens* and/or other land plants miRNAs were identified. Surprisingly, our analysis reve1ed in *Pellia endiviifolia* the presence of 3 miRNA families identified exclusively in the green algae *C. reinhardtii.* We confirmed the presence of selected *C. reinhardtii* miRNAs by northern hybridization using RNA isolated from axenically grown *P. endiviifolia* plants. Also, the presence of selected conservative miRNAs with homologs in higher land plants identified in *P. endiviifolia* was confirmed by northern hybridization. With the use of bioinformatic approaches we studied also novel *Pellia endiviifolia* miRNA candidates which have not been previously described. Using northern hybridization we evidenced the presence of 41-21 nt long stable, small RNAs, which represent novel, unique liverwort miRNAs. Analysis of *P. endiviifolia* transcriptome re-

vealed the presence of at least twenty miRNA putative precursors. Ten of them were verified using experimental approaches like RACE and genome walking resulting in establishing the gene structure of the first known liverwort *MIR* genes and their primary transcripts. Four of identified *MIR* genes contain one or more introns. Interestingly, in all intron containing *MIR* genes the miRNA and its stem-and-loop sequence are located in last exon. Degradome sequencing revealed mRNA targets for 38 conservative miRNAs and 13 novel miRNAs. Targets for 3 miRNAs (miR160, miR166, and miR408) were evolutionary conserved while the remaining mRNAs represent new, previously unknown targets. Our results are the first to characterise liverwort microtranscriptome. This new data will supplement our knowledge and understanding of plant miRNA evolution and represent an interesting example of research case for other scientists.

Poster 46

Identification of molecular factors involved in biogenesis of tRNA-derived fragments (tRFs) in plants using next-generation sequencing

P. Plewka[1], M. Szymanski[2], A. Pacak[1], Z. Szweykowska-Kulinska[1], A. Jarmolowski[1], W. Karlowski[2]

[1] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Laboratory of Computational Genomics, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

The mechanism of negative regulation of gene expression by small non-coding RNAs (sRNAs) is among the most studied subjects in molecular biology. The sRNAs act by silencing of gene expression at both transcriptional and post-transcriptional levels, and they have been reported to play an essential role in plant growth and development as well as are recognized as important players in responses to environmental stresses. 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, non-coding RNAs, like rRNA, tRNA and snoRNA may, aside of their canonical functions, be a source of small regulatory RNAs. Current knowledge about biogenesis and function of these molecules in plants is poor, however studies on human and bacterial cells point out at their putative negative regulatory action at the gene expression level, mainly through translation inhibition. Considering a great regulatory potential of small RNAs, we decided to study the biogenesis of small RNAs derived from tRNAs, called tRFs from tRNA-derived fragments. They represent a newly discovered and interestingly the most conservative class of small RNAs (they have been identified in all three domains of life). The tRFs of 14-26 nucleotides in length have long been regarded as random byproducts of tRNA biogenesis or degradation process, but nowadays there are more and more evidence proving their stability and putative regulatory capabilities. Results of high-throughput sequencing experiments demonstrate that tRFs are not only generated through specific cleavage pattern that predominantly favors either the 3′ or 5′ end but also preferentially maintained in the cells. This asymmetric accumulation and defined length of tRFs molecules reflect characteristic features of microRNAs and siRNAs. What is more, experiments carried out on animal cells show that these molecules can be generated by the main endonuclease of miRNA maturation pathway – Dicer or alternatively, by RNase Z and RNase P which are responsible for maturation of the 3′ and 5′-end of tRNA molecules, respectively. However, there is no experimental data concerning the mechanism of generation of tRNA fragments in plants. In order to perform comprehensive, whole-genome studies on biogenesis of tRFs in *Arabidopsis thaliana*, we selected almost 50 mutants disrupted in biogenesis and degradation of microRNAs, siRNA and tRNA transcripts, assuming that sRNAs resulting from tRNA cleavages can represent byproducts of tRNA maturation and/or degradation machinery, or alternatively, they can be processed by the factors involved in siRNA/microRNA biogenesis. Until now, 22 representative *Arabidopsis* mutants were subjected to RNA

deep sequencing. Detailed bioinformatic analysis of obtained results has led to identification of almost 6300 unique sequences that map to 725 annotated genes encoding tRNA molecules and show differential expression in mutants in comparison to wild type plants. This work was supported by grant from the National Science Center 2011/03/B/NZ2/01416.

Poster 47

Analysis of *GASA* genes in germinating tomato seeds *Solanum lycopersicum* cv. Moneymaker

W.E. Pluskota, J. Perkowska, K. Glowacka, M.M. Jastrzebska, M. Pikulinski, P. Pupel, S.B. Okorska, R.J. Gorecki

Department of Plant Physiology, Genetics and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Gibberellin (GA) stimulated transcript (*GAST*) family genes, encoding a group of small cysteine rich proteins, has been reported in petunia (*Petunia hybrida*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), maize (*Zea mays*) and *Arabidopsis* (*Arabidopsis thaliana*). GAST proteins also known as GASA (*Arabidopsis*), GASR (rice) or SNAKIN (potato) proteins contain 18 - 29 amino acid residues, a highly divergent intermediate region with polar amino acid residues, and a conserved 60 amino acid C-terminal domain, named as GASA domain, containing 12 conserved cysteine residues. Those proteins are involved in plant development, plant responses to biotic and abiotic stress and hormone crosstalk and redox homeostasis. Microarray data indicates that two member of *SlGASA* gene family are expressed in the micropylar region of germinating tomato seeds. Nucleotide sequences of array probes show the highest similarities with *GAST1*, first GA-stimulated transcript cloned from tomato and *GASA4-like* gene from rose, respectively. Base on the literature some GASA proteins can promote and the others inhibit seed germination. Alignment of cloned cDNA and genomic nucleotide sequences reveal the structure of *SlGASA6*, the new member of *GASA* gene family from tomato. Predicted amino acids sequences of GASA6 was compared with amino acid sequences of GASA proteins available in GenBank. The temporal and spatial expressions of both *SlGASA* (*GAST1* and its new homolog *GASA6*) genes in germinating tomato seeds are presented. Changes in transcript level of mRNA *GAST1* and cloned *GASA6* as well as expressions of genes involved in biosynthesis of GA in dissected tomato seeds are shown. Moreover the 5′ upstream region of the *GASA6* gene was cloned and its activity was studied in transgenic *Arabidopsis thaliana* seeds using a green fluorescent protein and/or β-glucuronidase reporter gene. Additionally the potential *cis*-regulatory elements contained in 5′upstream region of *SlGASA6* are described.

Poster 48

Transcriptomic insights into SCL30a, an *Arabidopsis* SR protein involved in salt stress tolerance during seed germination

D.N. Richardson, P. Duque

Instituto Gulbenkian de Ciencia, Oeiras, Portugal

Despite the influx of new evidence in the animal world suggesting myriad roles for SR genes/proteins in diverse biological processes such as transcription, alternative splicing, gene and transposon silencing and response to biotic or abiotic stresses, little functional evidence exists for linking this key post-transcriptional regulatory mechanism

to plant stress tolerance. As SR genes typically have critically important roles in transcription and splicing, it is expected that their misregulation will have several downstream consequences on gene expression and alternative splicing, which in turn may affect several biological processes, including the response to environmental stress. To better understand the roles of SR genes in the abiotic stress response in plants, we have performed RNA-seq on an *Arabidopsis* SCL30a knockout mutant and overexpressor line during seed germination under high salt concentrations. At the phenotypic level, we observed in these distinct genotypes alterations in seed size and dormancy as well as in the response to salt stress during germination, which were dependent on an intact ABA pathway. Aside from these changes, we observed several differentially expressed genes (1667 in the SCL30a knockout and 434 in the overexpressor). Among these differentially expressed genes, 35 were associated to the term, "response to abscisic acid stimulus" in the knockout, whereas 13 were associated thusly in the overexpressor, of which 11 were common to the 35 in the knockout. Broad level gene ontology analysis using DAVID highlighted several biological processes correlated with the observed germination phenotypes, such as embryonic development, dormancy process, protein biogenesis, transport/localization and cell growth. Several differentially expressed genes were also associated with photosynthesis. Of the differentially expressed genes in the SCL30a knockout, the most up-regulated (22-fold) gene is a copia-like retrotransposon, leading us to suspect a role for SCL30a in transposon silencing. As SCL30a is a splicing regulator, our next step will be to analyze global changes in alternative splicing in the SCL30a misregulation lines using these RNA-seq data. Comparative analysis of alternative splicing with the list of differentially expressed genes should allow for a narrowing down of potential target genes for experimental validation to garner functional evidence for SCL30a's role in salt stress tolerance in *Arabidopsis*.

Poster 49

RNA-seq reveals global differences in gene expression between male and female gametophytes producing sex organs in dioecious liverwort *Pellia endiviifolia* sp B

I. Sierocka[1], S. Alaba[2], W. Karlowski[2], Z. Szweykowska-Kulinska[1,2]

[1]Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2]Laboratory of Computational Genomics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Regulation of gene expression plays a pivotal role in controlling all aspects of multicellular organisms development, including sexual reproduction. In flowering plants a number of genes has been identified which control the transition from vegetative to generative phase of life cycle. Among liverworts, the most basal lineage of bryophytes, there is almost no data about the genes and mechanisms controlling this transition. This fact puts liverworts in critical evolutionary position to investigate the genetic basis of key innovations which allowed them to survive in demanding terrestrial environment and to give fertile offspring. We have chosen *Pellia endiviifolia* species B, a dioecious liverwort from class *Jungermaniopsida* to profile the differences in transcripts level between different stages of the male and female thalli development. We applied the next generation sequencing technology to identify genes engaged in the antheridia and archegonia production in *P. endiviifolia*. RNA-seq was performed using four different developmental stages: the male thalli i) producing or ii) without antheridia, the female thalli iii) producing or iv) without archegonia. For each library over 40 mln reads were generated which were mapped to the reference *de novo* transcriptome sequencing data of *P. endiviifolia.* To select genes with the highest differences in expression between the male and female thalli producing/not producing sex organs bioinformatics analyses were performed with criterion log2_fold_change ≥ 10. As a result 72 Differentially expressed genes (DEGs) were selected. Out of 10 genes up-regulated in sperm-producing male thalli, 8 are also expressed in the vegetative phase of males thalli. In turn, out of 62

up-regulated genes in archegonia-producing female thalli, 46 are also expressed in the vegetative phase of females growth. To verify the differentially expressed genes selected from the RNA-seq, real-time PCR analysis was performed which validated 9 male and 47 female specifically expressed genes. All verified DEGs were analyzed by blast search to classify their gene function. The most enriched DEGs belong to RNA or DNA binding protein families, serine/threonine-protein phosphatases, LRR receptor-like kinases and ubiquitin protein ligases. 24 DEGs showed no similarity to known proteins or nucleotide sequences, and the lengths of these transcripts reach from ~250 to ~600 nt with no putative open reading frames. It cannot be excluded that these transcripts represent non-coding RNAs or represent partial sequences of untraslated regions of original mRNA molecules what needs to be further investigated. Our studies provide possibility to learn about the gene expression regulation within the representative of genus *Pellia*, which is recognized as the one of the most basal lineage of the simple thalloid liverworts. The obtained results are one of first steps to understand the processes that trigger the development of liverworts from vegetative to generative stage of life. The work was supported by the Foundation for Polish Science, grant number POMOST/2012-5/7.

Poster 50

Regulation of environmental stress memory in *Arabidopsis* through the *AGO1-miR156-SPL* module

A. Stief, S. Altmann, K. Hoffmann, B. Datt Pant, W.-R. Scheible, I. Baeurle

Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Max Planck Institute for Molecular Plant Physiology, Potsdam, Germany

Plants have developed coping strategies for reproductive success and survival under stressful conditions. The responses directly following abiotic stress such as high temperatures have been well characterized. In contrast, the mechanisms allowing adequate reaction to recurring environmental stress are poorly understood, despite their importance for organisms in their natural surroundings. We present molecular and physiological evidence for the involvement of *ARGONAUTE1* and the microRNA pathway in the adaptation to recurring heat stress, an active maintenance of acquired tolerance which we call heat stress memory. We show that besides *AGO1*, *DCL1* and *SUO* are required for heat stress memory. Different miRNAs are induced by high temperatures in *Arabidopsis* seedlings, and elevated expression is maintained for several days. The *miR156* family, and especially isoform *miR156h*, displays a particularly interesting expression profile of high induction and long maintenance, and we can show its functional requirement by manipulating the levels of mature *miR156*. Depletion of *miR156* leads to higher susceptibility towards recurring heat stress, while its overexpression improves memory. Using heat-inducible constructs we can show that *miR156* is only required after the heat stress. Interestingly, increased levels of *miR156h* clearly prolong the sustained expression of heat stress memory genes (such as *HSFA2*, *HSA32*, and other *HSPs*). We furthermore show that repression of *SPL* genes, prominent targets of the *miR156* family regulating the juvenile-to-adult phase transition, is required for heat stress memory. We demonstrate that *SPL* genes are post-transcriptionally downregulated after heat, and this repression is a direct effect of *miR156*, as *miR156*-resistant *SPL* transcripts are not downregulated equivalently. Seedlings expressing *rSPL* transcripts are more susceptible to recurring heat stress, showing a level of damage similar to that seen in *miR156* knockdown lines. Reduced heat stress memory in *rSPL* plants corresponds to reduced maintenance of heat stress memory genes, arguing for a role of SPL proteins as transcriptional repressors of these memory genes. Altogether, our analyses demonstrate a yet unknown, central role of the *miR156-SPL* module in the integration of development and environmental stress.

Poster 51

Different pattern of H2A.Z distribution in *Arabidopsis* genes

W. SURA[1], M. KUS[3], L. PAWELOSZEK[1], W. KARLOWSKI[2], J. SADOWSKI[1], P.A. ZIOLKOWSKI[1]

[1] Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland
[2] Laboratory of Computational Genomics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland
[3] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

The role of histone variant H2A.Z in the control of gene expression has been reported in a wide variety of eukaryotes. Moreover, its amino acid sequence is highly conserved and its presence is essential for viability in many species. Although it is known that H2A.Z-dependent regulation is based on transcription initiation and the variant occupies mainly gene transcription start sites (TSSs), its mode of action is still unclear. Possible models include modification of nucleosome stability or position, as well as recruitment of other factors to TSS. To investigate correlation between changes in presence of H2A.Z in chromatin and changes in gene transcription levels in *Arabidopsis thaliana* we studied its nucleosomal distribution at the whole-genome scale. We performed ChAP-seq (*chromatin-affinity purification* followed by high-throughput sequencing) and RNA-seq analyses of plants submitted to drought stress in comparison to control plants. Our results indicate that there are several different patterns of H2A.Z distribution along gene bodies. The most common pattern with distinctive 5′-end peak is typical for constitutive genes, while genes involved in stress response show more complex distribution of H2A.Z. It may suggest that this histone variant regulates expression of different genes in several ways. While we are still comparing H2A.Z distribution in genes which are either induced or repressed under drought stress, our initial analysis shows that the H2A.Z pattern is relatively constant and not subjected to alterations upon transcription. This may indicate that the main mode of H2A.Z action is based on attraction of additional regulation proteins, likely transcription factors, to regions enriched in H2A.Z-containing nucleosomes along gene bodies.

Poster 52

AtCCR4s degrade the poly(A) tail of *GBSS1* mRNA which is responsible for amylose synthesis

YUYA SUZUKI[1], P.J. GREEN[2], JUNJI YAMAGUCHI[1,3], YUKAKO CHIBA[1,3,4]

[1] Graduate School of Life Science, Hokkaido University, Sapporo, Japan
[2] Delaware Biotechnology Institute, University of Delaware, Delaware, USA
[3] Faculty of Science, Hokkaido University, Sapporo, Japan
[4] Japan Science and Technology Agency, Precursory Research for Embryonic Science and Technology, Kawaguchi, Japan

Gene expression is tightly regulated during plant growth and development as well as in response to internal or external environmental changes. Most studies regarding control of gene expression have largely focused on transcriptional regulations. However, steady-state levels of mRNA are in fact determined by the balance between mRNA synthesis and degradation. Removal of poly(A) tail, deadenylation, is the first and rate-limiting step of mRNA degradation and apparently an effective step not only for controlling mRNA stability, but also for translation in many eukaryotic transcripts. CARBON CATABOLITE REPRESSOR 4 (CCR4) has been identified as a major cytoplasmic deadenylase in yeast. The *Arabidopsis* homologs of yeast CCR4, AtCCR4a and AtCCR4b, have been identified by the sequence-based analysis; however their functions and physiological significance for plants remain to be elucidated. In this study,

we showed that FLAG-tagged AtCCR4a or AtCCR4b exhibited poly(A) specific degrading activity *in vitro*. Transient expression analysis using the GFP fusion of AtCCR4a or AtCCR4b indicated that both were localized in P-bodies, which are specific granules in the cytoplasm consisting of many enzymes involved in mRNA turnover. To understand the functional significance of AtCCR4a and AtCCR4b *in vivo*, we took an advantage of reverse genetics strategy. The double mutant of AtCCR4a and AtCCR4b showed the insensitivity to a high level of sucrose. Levels of sucrose in the seedlings of the double mutants were reduced, whereas no difference was observed in the glucose level. In addition, we revealed that the amylose fraction in the double mutants was slightly higher than in the control plants. Furthermore, the poly(A) length of the transcripts encoding GRANULE BOUND STARCH SYNTHASE 1 (GBSS1), which is the key enzyme for amylose synthesis, was longer in the double mutants. This result indicated that *GBSS1* transcript is the target of AtCCR4a and AtCCR4b. Our results presented here suggest the regulation of amylose contents via deadenylation of *GBSS1* transcripts by AtCCR4a and AtCCR4b.

Poster 53

miR172 is involved in control of somatic embryogenesis induced *in vitro* in *Arabidopsis*

A. SZCZYGIEL-SOMMER, M. GRZYB, K. SZYRAJEW, K. NOWAK, M.D. GAJ

Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

In plants, microRNAs were indicated to play a main role in regulation of many aspects of development and responses to the environment. Thus, the involvement of miRNAs in control of somatic embryogenesis (SE), a developmental process induced *in vitro* in somatic cells, can be assumed. In support, the *dcl1* mutant defective in DICER LIKE1 (DCL1) activity required for miRNAs biogenesis, was found totally unable for the SE-induction. Moreover, numerous *MIRNA* genes were found differentially expressed during SE-induction stage, including *MIR172c* and *MIR172d* to be highly up-regulated (100- and 31-folds, respectively) (Szyrajew, 2012a,b). To further verify the involvement of miR172 in SE the relation between *MIR172* expression level and explant capacity for SE was evaluated. To this end, the embryogenic potential of cultures derived from a transgenic line overexpressing *MIR172d* and three insertional mutants (*mir172b*, *mir172c* and *miR172d*) were analysed. It was found that all the analyzed genotypes were impaired in capacity for SE and displayed a significantly reduced number of responding explants and/or somatic embryos produced per explant. *In planta*, *MIR172* genes are involved in regulation of flowering time and floral organ identity in *Arabidopsis* and among their targets a small group of *APETALA2 (AP2)-like* transcription factor genes including *AP2, TOE1, TOE2, TOE3, SNZ* and *SMZ* were indicated (Aukerman and Sakai, 2003). The present results of qRT-PCR analysis revealed that two of miR172 targets, *AP2* and *SMZ*, are down-regulated during SE suggesting their role as the negative regulators of SE induction in *Arabidopsis*. In support, auxin treatment used to induce SE in cultured explants was found to significantly modulate expression of *AP2* and *SMZ*. In contrast to *AP2* and *SMZ*, expression profiles of *TOE* genes in embryogenic culture have not indicated their regulatory relation with miR172 during SE. However, a significant and auxin-dependent stimulation of *TOE1* and *TOE2* in SE together with a significantly decreased embryogenic response of *toe1* mutant suggest that these genes can positively control embryogenic transition in somatic cells of *Arabidopsis*. Further analysis are needed to reveal other genes of regulatory pathway operating during SE induction in which miR172 and its targets *in planta* (*AP2, SMZ, TOE1, TOE2*) seem to be involved.

Poster 54

At4g25290 has at least eight different splicing variants

O. Sztatelman, J. Labuz, A.K. Banas

Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

UVB is known to seriously harm living cells. Beside causing oxidative damage of proteins, lipids and nucleic acids it can also induce dimer formation between adjacent pyrimidines in a DNA strand. Such photoproducts may be both mutagenic and cytotoxic. They are removed via highly efficient light repair pathway by photolyases (photoreactivation repair) and/or relatively inefficient dark repair mechanisms (excision repair pathways). Up to date, three *Arabidopsis* genes have been shown to have photolyase activity: i) *At1G12370* (*PHR1* photolyase1, *UVR2* – UV resistance2); ii) *At3G15620* (*UVR3*); iii) *At5G24850* (*CRY3* cryptochrome3). Two others, *PHR2* and *At4g25290* have been classified as photolyases based on *in silico* analysis. The protein encoded by *At4g25290* has two domains, a photolyase at the N-terminus and a hydrolase at the C-terminus. When we used mRNA isolated from different plant organs as a template for the PCR reaction with *At4g25290* specific primers we had found eight splicing variants of this gene. The steady state mRNA levels of most of these forms were strongly up-regulated by light. Several of the splicing variants identified contained premature STOP codon and subsequently encoded a shorter protein having only a photolyase domain. Interestingly, due to ASIP database (http://www.plantgdb.org/tmp/ASIP/) one of the splicing forms can have alternative start of translation, located in the alternatively spliced area. Three other forms also contain long ORFs starting at different position in the alternatively spliced region. In all these cases proteins having only a hydrolase domain would be produced. Transcripts with premature translation-termination codons may undergo nonsense mediated mRNA decay (NMD). Thus, we have used *Arabidopsis* mutants of the NMD pathway (*upf1-5*, *upf3-1* and *upf3-2*) to address a possible role of alternative splicing in the regulation of *At4g25290* mRNA level. No differences in mRNA levels of one of the splicing variant as well as the pattern of its light up-regulation between wild type and the mutants have been observed. Acknowledgements: The study was supported by Polish National Science Centre, a grant no. UMO-2011/03/D/NZ3/00210

Poster 55

Whole genome computational analysis of small RNA fragments derived from tRNAs in response to abiotic stress

A. Thompson[1], M. Szymanski[1], A. Jarmolowski[2], W.M. Karlowski[1]

[1] Laboratory of Computational Genomics / Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

[2] Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Small RNA (sRNA) play an important role in regulation of gene expression, and as a consequence are essential for proper function of living cells. RNA silencing has been recognized for its critical role in development, stress response and housekeeping. Due to increasing amount of data from Next Generation Sequencing, the research community is continuously adding to the ever-expanding pool of newly identified sRNAs that potentially may contribute to the regulatory landscape of gene expression. One such recently discovered group are fragments derived from transfer RNA (tRNA) genes. Transfer RNAs are well-characterized, conserved components of the translational machinery

with lengths varying between 73 and 90 nucleotides and having a characteristic secondary and tertiary structure. Recent results demonstrate that their transcripts undergo processing to form fragments ranging between 19 and 26 nucleotides in length and are known to accumulate during stress conditions. In order to evaluate the regulatory potential of tRNA fragments we have selected a model plant *Arabidopsis thaliana*. We performed a genome-wide analysis and predicted approximately one hundred previously unclassified tRNA candidates for a total of seven hundred and twenty-five tRNA positions. The sequences of tRNA genes derived from the annotations provided by GtRNAdb and TAIR databases were validated with two criteria: i) the ability to fold into standard tRNA cloverleaf structure ii) the presence of promoter elements – boxA and boxB. As a means to investigate which factors are responsible for accumulation of tRNA fragments, we have collected close to seventy datasets from publically available Gene Expression Omnibus (GEO) database. The datasets consisted of wild type samples from different tissues and environmental conditions. We then analyzed small RNA sequencing data and created expression profiles for precursor tRNA transcripts. Our results demonstrated that tRNA fragments originate from both precursor and mature tRNAs. Our recent advances will be presented in the poster. This work was supported by grant from the National Science Center 2011/03/B/NZ2/01416.

Poster 56

Dicer-like proteins in *Medicago truncatula*, a model legume plant

A. Tworak[1], A. Urbanowicz[1], N. Koralewska[1], M. Pokornowska[1], A. Kurzynska-Kokorniak[1], J. Podkowinski[1], M. Figlerowicz[1,2]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

[2] Institute of Computing Science, Poznan University of Technology, Poznan, Poland

The biogenesis of both miRNAs and siRNAs in plants depends on a specific group of ribonucleases known as DICER-LIKE (DCL) proteins. Based on functional analysis of DCL proteins (DCL1-4) identified in *Arabidopsis thaliana*, four functional DCL types were distinguished in plants. DCL1-type ribonucleases mainly produce 21 nt long miRNAs. The products generated by DCL2-, DCL3-, and DCL4-type ribonucleases belong to various classes of siRNAs. DCL2-type enzymes are involved in the biogenesis of 22 nt long siRNAs from natural antisense transcripsts (nat-siRNA), DCL3-type ribonucleases produce an abundant class of 24 nt long heterochromatic small interfering RNAs (hc-siRNA) and DCL4-type proteins are mainly involved in 21 nt long trans-acting siRNAs (ta-siRNA) production. In addition, DCL2-, 3- and 4-type enzymes contribute to the plant defense against a diverse range of pathogens. In many plants duplications of the genes encoding DCL proteins have been observed. For example, the rice genome encodes two DCL2- and two DCL3-type proteins whereas in soybean two genes encoding DCL1-, DCL2- and DCL4-type ribonucleases were identified. *Medicago truncatula* is a model legume plant closely related to many economically important cultivable species. In order to increase our knowledge on miRNA and siRNA biogenesis in *Medicago* we have screened the current genome assembly available from *Medicago truncatula* Genome Project in search for DCL-coding genes. In addition to MtDCL1, 2 and 3 characterized in the previous studies we identified three other DCL genes: MtDCL4 and two new MtDCL2 homologs. We found that one of the newly identified MtDCL2 genes codes for a truncated version of DCL2 protein. Using droplet digital PCR (ddPCR) we confirmed the existence of all MtDCL transcripts in the total RNA fractions extracted from *Medicago* plants. Additionally, we identified an alternative splicing variant of MtDCL1 mRNA. Translation of the latter may result in the formation of the truncated DCL1 protein. Finally, we determined the expression profiles of the six MtDCL genes in different parts of the plant at various developmental stages. The mRNA abundance for all assayed DCL mRNAs was significantly increased in the

nodule, compared to root and other plant organs which may suggest the important role of DCL genes in nodule function. This work is co-financed by the European Regional Development Fund through the Operational Programme Innovative Economy, Innovation grants. This work was partially supported by the European Union Regional Development Fund within the PARENT-BRIDGE Programme of Foundation for Polish Science (Pomost/2011-3/5 to A.K.-K).

Poster 57

Changes in small RNA populations under herbicide stress in maize

A. Tyczewska[1], M. Zywicki[2], J. Gracz[1], L. Handschuh[1], K. Adamczewski[3], J. Adamczyk[3], M. Figlerowicz[1], T. Twardowski[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

[2] Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University in Poznan, Poland

[3] Plant Breeding and Acclimatization Institute, National Research Institute, Poznan, Poland

Maize, *Zea mays*, a plant that originally comes from South America, is currently cultivated in the world on a large scale. Its great potential is broadly used in food and fodder industry, textile industry but also in bioethanol production. Herbicides, commonly known as weedkillers, are compounds used to destroy or inhibit the growth of plants, especially weeds. The most popular weedkillers, widely used in the maize fields, are nonselective, which means that they affect not only weed populations but influence all plants that are growing in the sprayed area. To guarantee their survival under adverse environmental conditions plants have developed exquisite adjustments to stresses at all levels (anatomical, morphological, cellular, biochemical and molecular). It has been observed in the fields that there is a variety of phenotypic differences between maize lines in response to herbicide spraying. Therefore our goal is to identify molecular basis of plant's increased/decreased resistance to herbicides. First, we chose two maize lines that differ significantly in susceptibility to herbicide RoundUp, then using Next Generation Sequencing (NGS) we detected differences in small RNA populations. Bioinformatic analyses (miRDeep-P, miREvo and our own programs) were performed to profile the expression of known and identify new small RNA molecules that are involved in herbicide stress response, cDNA libraries were mapped to B73 maize genome (assembly AGPv2, March 2009). We identified over a hundred of small RNA molecules with changed expression profiles in tested lines (treated or not with the herbicide), nearly 50 of them have never been described before in the literature. Funding acknowledgement: the work is supported by a grant no. N N310 769040 from Ministry of Science and Higher Education, Poland.

Poster 58

Bioenergetic cross-talk between RNA silencing and photosynthesis in plants

F.G. Verret[1], N.E. Ioannidis[2], X. Kotakis[2], K. Kotzabasis[2], K. Kalantidis[1,2]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece

[2]Department of Biology, University of Crete, Heraklion, Greece

RNA silencing is the regulatory control of gene and genome expressions by small (21-25 nt) non-coding RNAs including short interfering RNAs (siRNAs) and microRNAs (miRNAs). In plants RNA silencing is involved during

development, genome stability, defence against virus, and responses to abiotic stressors. Conversely RNA silencing efficiency is affected by environmental factors such as temperature and photoperiod. Using *Nicotiana benthamiana* transgenic line exhibiting spontaneous RNA silencing of a GFP transgene we have recently shown that photoadaptation to high light condition increases both the frequency of RNA silencing and the transcript levels of key components of the RNA silencing machinery. Here analysis of the composition and activity of the photosynthetic apparatus during ongoing PTGS is reported. *In vivo* photosynthetic measurements using chlorophyll fluorescence based approaches and qPCR analysis of key photosynthetic genes have been carried out in *A. thaliana* and *N. benthamiana* transgenic lines exhibiting PTGS against a GUS and a GFP transgene respectively. Our project aims to assess the presence of a bioenergetic cross-talk between RNA silencing and photosynthesis, identify its molecular players and provide a better understanding of the place of the RNA silencing machinery within the plant physiology.

Poster 59

Auxin inhibits flowering of *Ipomoea nil* through stimulation of *JASMONIC ACID CARBOXYL METHYLTRANSFERASE* expression

E. WILMOWICZ, K. FRANKOWSKI, A. KUCKO, K. MARCINIAK, J. KOPCEWICZ

[1] Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Torun, Poland

[2] Centre for modern interdisciplinary technologies, Nicolaus Copernicus University, Torun, Poland

Studies indicate the great significance of interaction between indole-3-acetic acid (IAA) and jasmonates (JAs) in the regulation of various physiological processes in plants, e.g. stem cell growth, abscission, secondary abscission zone formation, tendril coiling and wounding, but still little is known about their interaction in the transition from vegetative to reproductive phase. In *A. thaliana* flowers, auxin signalling requires AUXIN RESPONSE FACTOR6 (ARF6) and ARF8, both of which induce the expression of JA biosynthesis genes in filaments. We investigated the interaction between IAA and JAs in photoperiodic flower induction of short-day plant *Ipomoea nil* using Real-Time PCR and GC-MS methods. Also we investigated the effect of IAA on the endogenous jasmonates (JA, MeJA) level in cotyledons of *Ipomoea nil* during 16-h long inductive night and the expression of JAs biosynthesis (*InLOX2* – LIPOXYGENASE, *InAOS* – ALLENE OXIDE SYNTHASE, *InOPR3* – 12-OXOPHYTODIENOATE REDUCTASE) and metabolism genes (*InJMT* – JA CARBOXYL METHYLTRANSFERASE, *InJAR* – JASMONATE RESISTANT). We have shown that IAA applied to the cotyledons before the inductive night inhibits flower induction. The level of endogenous JAMe in cotyledons of *I. nil* during inductive night was low. Application of IAA just before the inductive night increased the level of endogenous MeJA in the cotyledons and also stimulated the expression of *InJMT*. Our results demonstrate that IAA inhibits flower induction through the stimulation of *InJMT* and as a consequence enhancement MeJA content, which is considered as another flowering inhibitor in *I. nil*.

Poster 60

Identification and preliminary expression analysis of *LlFPA* gene from *Lupinus luteus*

W. Wojciechowski, M. Banach, J. Kesy, P. Glazinska, E. Wilmowicz, A. Kucko, K. Marciniak, J. Kopcewicz, A. Tretyn

Chair of Plant Physiology and Biotechnology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Torun, Poland

The induction of flowering is one of the most important stages of the development of higher plants. A lot of data indicates the necessity of cooperation of many factors both exogenous and endogenous to provide an optimum time of flowering. At least 4 pathways controlling this process (photoperiodic, vernalization, autonomous, and hormonal) were distinguished with the model long day plant *Arabidopsis thaliana.* All pathways control of flowering cooperate regulation of key flowering genes which name integrator genes. The autonomous pathway includes at least 7 of flowering induction genes. The key task of the proteins coded by them is to stop the transcript activity of the FLC (*FLOWERING LOCUS C*) gene flowering inhibitor. The mutants of AP (autonomous pathway) genes showed an increased level of mRNA *FLC*. The *FLC* inactivation occurs on the road of two mechanisms of remodeling chromatin and mRNA processing of this gene. In these study we identify homologue of *FPA* in vegetative organs from *Lupinus luteus*. Known full-length cDNA sequence shows high identity to the known gene cDNAs described in other legume species (*Glycine max*, *Medicago truncatula*). Into the predicted amino acid sequence LlFPA evolutionarily conserved RRM (RNA recognition motifs) domain was found. This suggests that in the lupine as in others species this protein takes part in alternative cleavage and polyadenylation of RNAs. Examined the transcriptional activity of the gene was determined in vegetative organs (leaves, petioles roots) of lupine. No significant differences in the expression of *LlFPA* were detected. These results may indicate that the protein encoded by this gene is involved in many physiological processes. Acknowledgements: 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

Poster 61

miR393 controls somatic embryogenesis in *Arabidopsis* through regulation of auxin signaling components (TIR1 and AFB2)

A.M. Wojcik, M.D. Gaj

Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

MicroRNAs (miRNAs) play essential role in regulation of gene expression and their involvement in control of developmental processes in plants and animals was indicated. In plants, a key regulatory role of miRNAs in morphogenic processes, including zygotic embryogenesis and hormone signaling was documented. Beside development *in planta*, miRNAs are also believed to control morphogenic processes induced in somatic cells cultured *in vitro*, including somatic embryogenesis (SE). In support for this hypothesis, we found the mutant in *DICER-LIKE1 (DCL1),* a gene required for miRNAs biogenesis pathway, incapable of SE induced in culture of *Arabidopsis* explants. Moreover, *MIR393A* and *MIR393B* genes involved in auxin signaling, a regulatory pathway expected to be essential for SE induction, were found up-regulated in SE induction and the respective mutants (*miR393a, miR393b* and *miR393ab*) showed impaired embryogenic potential. In the present study, the involvement of *MIR393A* and *MIR393B* genes in

SE was further confirmed and overexpression of these genes was found to negatively affect culture capacity for SE. To extend the knowledge on miR393-controlled mechanism of SE induction, four candidate miR393-target genes involved in auxin perception were analyzed, including: *AFB1 (AUXIN SIGNALING F-BOX1), AFB2, AFB3* and *TIR1 (TRANSPORT INHIBITOR RESPONSE1).* The candidate targets are members of the TIR1/AFB2 clade of auxin receptors (TAARs) in the AFB family of plant F-box proteins. We found insertional mutants in the candidate targets (*tir1-1, afb1-3, afb2-3* and *afb3-4*) significantly defected in embryogenic response induced *in vitro*. In addition, expression analysis with the use of real time RT-PCR indicated that two of the candidate genes, *TIR1* and *AFB2,* are negatively regulated by miR393 molecules in SE. In contrast to SE-stimulated *MIR393* expression, the transcription of *TIR1* and *AFB2* was found down-regulated during this process. Expression profiles of *AFB1* and *AFB3* did not suggest their miR393-controlled involvement in SE. In addition, a spatiotemporal expression pattern of the candidate miR393-target genes was analyzed in explants undergoing SE induction with the use of GFP reporter lines (*TIR1pro:GFP, AFB1pro:GFP, AFB2pro:GFP* and *AFB3pro:GFP*). GFP analysis indicated that *TIR1* and *AFB2* are highly expressed in explant areas involved in embryogenic transition (cotyledons, proximity of SAM) while expression of *AFB1* and *AFB3* is localized mainly in explant parts not undergoing SE induction (root, epidermis). Collectively, the results indicated that miR393 molecules control SE induction in *Arabidopsis* through regulation of *TIR1* and *AFB2* genes encoding auxin receptors.

Poster 62

Post-transcriptional gene silencing of putative *HvGSK* leads to enhanced salt stress tolerance of barley seedlings

Y. YANUSHEVSKA[1], A. NADOLSKA-ORCZYK[2], W. ORCZYK[1]

[1] Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

[2] Department of Functional Genomics, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Blonie, Poland

Brassinosteroids (BRs) are a class of naturally occurring steroidal plant hormones involved in diverse biological processes. BRs play a key role in plant growth and development and function as crucial regulators of plant tolerance for biotic and environmental stresses. The goal was to verify that clone AK251287, showing high similarity to *OsGSK1* (Koh i in., 2007), was a putative ortolog of this gene and, similar to *OsGSK1*, functioned as a negative regulator on BR-dependent signalling pathway and was involved in salt stress tolerance. Transcript assigned as AK251287 was amplified using barley cv. Golden Promise cDNA as a template, cloned and sequenced. Nucleotide and encoded amino acid sequence confirmed high similarity to *OsGSK1* and *BIN2/AtSK21* – a gene earlier recognized as negative regulator of BR signaling. Fragments of ORF, 5′- and 3′-UTRs were cloned into pBRACT207 silencing cassette and used for barley transformation, via *Agrobacterium*. The 11 primary transgenic plants (T_0) were confirmed for the presence of T-DNA. Relative expression of the analyzed gene, a putative *HvGSK* in T1 plants ranged between 0,09 and 0,22 of the expression determined in the control plants. Transgenic T_1 plants with silenced expression of analyzed gene showed better seedling development under salt stress (25 mM NaCl germination and 200 mM NaCl growth) as well as normal conditions compared with control non-transgenic plants. Biomass of randomly selected 14 days old T_1 seedlings compared with control was between 71% and 117% for seedlings grown in normal conditions and between 67% and 145% for salt stress conditions. Analyzed seedlings represented T1 generation and the observed range of results might reflect segregation of the silencing cassette in the selected transgenic plants. In the summary we can state that silencing of the analyzed gene *HvGSK* resulted in better growth of seedlings in both – normal and salt stress conditions. Similarity of the nucleotide sequence of the genes (*HvGSK* and *OsGSK1*) as well as the similar phenotypes of *OsGSK1*-knock-out rice and *HvGSK*-silenced barley indicate that identified barley gene

is a homolog of *OsGSK1*. Due to the expected bigger number of barley genes in *GSK3* gene family, further study is required to establish detailed phylogenetic and functional characteristics of the gene. The work was financed from National Science Centre grants nr. 718/N-COST/2010/0 and UMO-2011/01/B/NZ9/02387.

Poster 63

Transcriptome surveillance by the nuclear 5′-3′ exonuclease XRN3 in *Arabidopsis*

M. Zakrzewska-Placzek[1], M. Krzyszton[1], G. Barton[2], N. Schurch[2], A. Sherstnev[2], G. Simpson[3], J. Kufel[1]

[1] Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland

[2] Division of Computational Biology, University of Dundee, Dundee, United Kingdom

[3] Division of Plant Sciences, College of Life Sciences, University of Dundee, Scotland, United Kingdom and Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Scotland, United Kingdom

Two nuclear 5′-3′ exonucleases AtXRN2/3 in *Arabidopsis thaliana* are homologs of the yeast Xrn2/Rat1, which is involved in degradation and processing of several classes of nuclear RNAs and in transcription termination of RNA polymerases I and II. We have recently shown that AtXRN2/3 contribute to polyadenylation-mediated nuclear quality control of rRNA precursors and excised spacer fragments. Here, we report that several mRNAs and pri-miRNAs are significantly upregulated in *xrn3* mutants, and this is accompanied by a strong accumulation of non-coding RNAs that originate from these loci. As chromatin immunoprecipitation confirmed increased PolII occupancy in these regions, these species most likely represent readthrough transcripts that result from termination defects. In addition, our high-throughput data reveal a prevailing accumulation of lncRNAs corresponding to intergenic regions in *xrn3* mutants. These transcripts, named XATs (XRN3-associated transcripts), are produced by PolII and often contain fragments of coding regions and poly(A) tails, but mostly lack the 5′ cap structure. Considering that plant XRN2/3 act as endogenous silencing suppressors, these aberrant RNAs could in principle activate RNAi, however, we were not able to detect changes in DNA methylation or histone modifications in XAT-generating regions. Although XAT transcripts have an impact on the expression of neighbouring genes, the mechanism of their action is still unclear. We propose two models for XATs biogenesis. One of them assumes that XATs are readthrough transcripts resulting from XRN3 involvement in transcription termination. The alternative mechanism considers XATs a result of pervasive transcription of the genome and implicates their rapid removal by AtXRN3. The two pathways are not mutually exclusive and AtXRN3 may be involved in both regulation of transcriptional termination and surveillance of genome-wide transcription.

Poster 64

Reference Gene Selection for Quantitative Real-time PCR Normalization in *Quercus robur*

M. Zapalska, K. Dudziak, M. Nowak, K. Kowalczyk

Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Lublin, Poland

Gene expression can vary across tissues, developmental stages as well as under different experimental conditions. The relative quantification of gene expression by reverse transcription quantitative PCR (RT-qPCR) requires reliable internal controls in order to avoid misinterpretation of experimental results. Recent studies confirm that

an universal reference gene does not exist, therefore a selection of most suitable RG for each species or condition is essential. *Quercus robur* is a deciduous tree of great ecological and industrial importance. Due to its significance and scarce knowledge of gene expression in this species, an attempt of RG selection for pedunculate oak was carried out. In this study, several candidate reference genes were evaluated to determine the most stable internal reference for quantitative PCR normalization in *Quercus robur*. Among tested genes were *ACT* (*ACTIN*), *EF-1α* (*ELONGATION-FACTOR-1ALPHA*), *GAPDH* (*GLYCERALDEHYDE-3-PHOPHATE DEHYDROGENASE*), *β-TUB* (*β-TUBULIN*), *UBQ* (*UBIQUITIN*) and *18S rRNA*. The transcript abundance of these genes was analyzed in leaves tissue of 1-year old *Quercus robur* seedlings subjected to three different $CdCl_2$ concentrations (0 μM, 10 μM and 50 μM). Total RNA extraction was carried out according to Le Provost et al., (2007) protocol. RNase contamination from glass and plastic surfaces was removed with the use of RNase*Zap*® RNase Decontamination Solution (Ambion, cat.# AM9780). cDNA was synthesized from 1 μg of total RNA using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). The Quantitative Real-time PCR analysis was performed with the SYBR Select Master Mix (Applied Biosystems) chemistry, using gene-specific primers. Specificity of amplification of the transcripts was checked by the analysis of melting curves and by gel electrophoresis, showing a single PCR amplification product with the expected size for each gene. The gene expression stability was assessed with the NormFinder statistical approach. From all candidate genes tested, *GAPDH* and *β-TUB* were identified as the most stable, while *ACT* showed the lowest expression stability. In this study, we have identified and validated reference genes in pedunculate oak that can be used for quantification of target gene expression in leaves tissues under heavy metal treatment and will be useful as a starting point for further gene expression studies in this species.

Poster 65

The microRNA transcriptome change during tuberization in potato

RUNXUAN ZHANG, M. LINEY, D. MARSHALL, G.J. BRYAN, C. HORNYIK

The James Hutton Institute, Invergowrie, Dundee, United Kingdom

Potato is a major global food crop, and is cultivated for its underground storage stems (tubers), rich in starch and nutrients. Potato is unique among the major crops in tuber formation. It is extremely important to understand the as yet poorly known molecular events playing roles in development or responses upon biotic or abiotic stresses, ultimately impacting on breeding of cultivars with improved tuber characteristics (tuber initiation, yield, size distribution, shape etc.). Our aim is to explore the potato microRNA (miRNA) transcriptome to investigate the role of these small non-coding RNAs in one of the most important potato developmental processes, tuberization. Using this knowledge we can identify the genes which play a role in the switch which lead to stolon and tuber intiation and tuber development. Previously, we used small RNA next generation sequencing approach to identify small RNAs and the recently published potato genome for the prediction of microRNAs (miRNAs). We have developed a pipeline for the analysis of miRNAs in potato. The prediction of candidate miRNAs is based mainly on plant miRNA characteristics, such as minimum folding energy of candidate precursors, read distributions and matching patterns of the mature miRNA and 'star' sequences. Conserved and potato specific miRNAs were identified and non-conserved miRNAs were validated experimentally. Additionally, we predicted targets for all of the miRNAs applying different levels of stringency using the potato transcriptome data. In our study we used a photoperiod inducible tuberization system (*Solanum tuberosum* group *Andigena*). This primitive cultivar can tuberize under short days (SD) but do not make tubers under long day (LD) conditions. During a time course experiment we sampled plants under SD and LD conditions from the induction of tuberization (early time points) until SD plants had small tubers. Using high-throughput

sequencing approach we identified small RNAs in different samples. Both leaf and stolon tissues were used in biological replicates to compare the miRNA transcriptome between SD and LD plant at different time points. Conserved and potato specific miRNAs were predicted with their targets and miRNA expression was calculated at the different time points between SD and LD plants. Data will be presented on how the expression levels of miRNAs change during this unique developmental transition. Our findings will ultimately facilitate the molecular biology in potato through the knowledge of miRNAs and their predicted targets.

Poster 66

Distribution of AGO1, HYL1 and AGO4 in *Hyacinthus orientalis* L. male gametophytes

K. Niedojadlo[1], M. Kupiecka[1], A. Kolowerzo[1,2], R. Lenartowski[3], J. Niedojadlo[1]

[1] Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

[2] Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Torun, Poland

[3] Laboratory of Isotope and Instrumental Analysis of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland

Small non-coding RNAs (sRNAs) have emerged as key guide molecules in the regulation of various biological processes in plants, including developmental transition and patterning, responses to the environment, maintaining genome stability and defense against viruses and bacteria. Our knowledge of small RNAs biogenesis and their mechanisms of action has dynamically expanded over the past decade, but their functions in the regulation of plant sexual reproduction are still not fully understood. The present study uses immunofluorescence techniques to examine the spatial and temporal distribution of the molecules involved in the biogenesis of small non-coding RNAs, i.e. AGO1 (ARGONAUTE 1), HYL1 (HYPONASTIC LEAVES 1) and AGO4 (ARGONAUTE 4), in mature pollen grain and *in vitro* growing pollen tubes of *Hyacinthus orientalis*. AGO1 and HYL1 are specifically associated with the miRNA pathway. AGO4 is involved in siRNA gene silencing and in RNA-directed DNA methylation. The results have demonstrated a different localization of these antigens in mature pollen grain. Also, changes in the pattern of the labeling were visible during pollen tube growth in the vegetative and generative nuclei and after division generative cell were visible in sperm cells in the pollen tube after 8-12 h of growth in medium. Our preliminary study indicates that in *H. orientalis* processes including sRNAs take place and play an important role in the regulation of gene expression in these cells during male gametophyte development and fertilization. The research was supported by the National Science Centre (NCN) grant 2011/03/D/NZ3/00603