Session 7: Plant molecular biology

Lectures

L7.1

Double-strand break induced genome engineering in plants

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Sequence-specific nucleases can be used to induce site-specific double-strand breaks (DSBs) in plant genomes. In the past we could show that thus gene targeting (GT) by homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Moreover, by inducing several DSBs sequences can be deleted out of the genome and chromosome arms exchanged. In the last years the CRISPR/Cas system became the major tool for targeted mutagenesis in plants. We were able to demonstrate *Streptococcus pyogenes* (Spy)Cas9 nuclease induced, NHEJ mediated, heritable targeted mutagenesis in *Arabidopsis thaliana* as well as homology dependent in planta GT. A major concern for biotechnological applications is the specificity of the Cas9 nuclease. Off-target effects might be avoided using two adjacent sgRNA target sequences to guide a Cas9 protein that was transformed from a nuclease to a nickase to each of the two DNA strands, resulting in the formation of adjacent single strand breaks (SSBs). We could show that this Cas9 paired nickase strategy has a mutagenic potential at the target site comparable to that of the nuclease. Interestingly; sequence duplications are a prominent outcome of this approach, hinting to the possibility that in general the repair of adjacent SSBs is a major cause of sequence duplications during genome evolution of plants. Recently we applied the Cas9 orthologues from *Streptococcus thermophilus* (Sth1Cas9) and *Staphylococcus aureus* (SauCas9) for error-prone non-homologous end-joining (NHEJ)-mediated targeted mutagenesis in *A. thaliana*. We obtained efficiencies at least comparable to those of SpyCas9. Stable inheritance of the induced targeted mutations was demonstrated for both nucleases at high frequencies. We were also able to show that the SauCas9 and SpyCas9 proteins only work in the presence of their species-specific single guide (sg) RNAs. These proteins are not prone to inter-species interference with heterologous sgRNA expression constructs. Thus, the Cas9 proteins of *S. pyogenes* and *S. aureus* should be appropriate for simultaneously addressing different sequence motifs with different enzyme activities in the same plant cell. The simultaneous use of different Cas9 orthologues will offer the opportunity to control genetic information of plant cells on more complex levels than before and will lay the basis for future synthetic approaches in plant biology.

L7.2

Pausing transcription to pause development. Molecular biology of seed dormancy establishment

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Seed dormancy is one of the earliest developmental transitions in plant life cycle. Therefore plants have evolved elaborate mechanism of its regulation including DOG1, a dedicated seed dormancy regulator. DOG1 controls seed dormancy in natural Arabidopsis populations, and has been shown to contribute to seed dormancy in agronomicaly important plants. This is reflected by the evolutionary conservation of the functional short alternatively polyadenylated form of the DOG1 mRNA. I will present our characterization of short DOG1 protein partners and the biochemical activity of DOG1 protein. Notably, the 3' region of DOG1 gene, including the last exon that is not included in short functional transcript isoform, shows a high level of conservation at the DNA level, but the encoded polypeptide is poorly conserved. I will show our data that this 3' tail of DOG1 gene is a crucial regulator of seed dormancy establishment and propose a molecular mechanism behind.

L7.3

Identification and functional analysis of the *HvD14* gene involved in strigolactone signalling in barley

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In the presented studies, the barley *HvD14* gene encoding α/β hydrolase, which is involved in strigolactone (SL) signalling, was identified. Bioinformatics analysis revealed that the identified gene is an orthologue of the *D14*, *AtD14* and *PhDAD2* genes that have been described in *Oryza sativa*, *Arabidopsis thaliana* and *Petunia hybrida*, respectively. Using TILLING strategy, a *hvd14.d* mutant that carried the G725A transition, located in the second exon, was identified. This mutation led to the substitution of a highly conserved glycine-193 to glutamic acid in the conserved domain for the α/β hydrolase family of HvD14 protein. The plants that carry the *hvd14.d* allele were semi-dwarf and produced a higher number of tillers in comparison to the wild-type (WT) parent cultivar. Additionally, the root architecture of mutant plants was affected: the total length of the seminal roots was significantly reduced, and the density of the lateral roots was higher than in the WT. Plants with the *hvd14.d* allele were insensitive to treatment with GR24, which is the synthetic analogue of SL. Analysis of the IAA concentration in the lateral buds showed no differences between the WT and mutant plants. By contrast, the WT seedlings treated with GR24 developed a lower number of tillers, longer primary roots with a reduced number of lateral roots and had an increased concentration of IAA in lateral buds. This studies describe the first barley strigolactone mutant and show the potential functions of strigolactones in barley growth and development.

Posters

P7.1

Construction of innovative platform based on Arabidopsis, human cell lines, mice and chemoinformatics modelling for identification of drugs targeting metabolome-related human diseases

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Some human diseases, such as clear cell renal cell carcinoma (ccRCC) are related to metabolome disorders, featured by the TOR (Target of rapamycin) serine-threonine kinase hyperactivation and correlate with aberrant activity of SWI/SNF ATP-dependent chromatin remodeling complex. Given a recent increase in the number of ccRCC cases, there is an urgent need to address and fill the current gaps in our knowledge on the etiology of this disease, and to develop innovative evidence-based treatments.

The TOR kinase is responsible for integrating signals connected to environmental stress, cell energy status and availability of amino acids. Homologues of TOR kinases were among others identified in plants. TOR kinase appears in cytoplasm and nucleus, but its nuclear function remained thus far largely unknown. Given that the TOR kinase pathway regulates transcription, it is highly probable that there is a functional interdependence between the TOR pathway and machineries responsible for chromatin remodeling enabling precise control of gene expression.

Our findings indicate the existence of functional interdependences between TOR and SWI/SNF chromatin remodeling complexes in Arabidopsis and human. We showed that functional SWI/SNF is necessary for proper function of TOR pathway as well as for metabolism control. Subsequently, we constructed a unique innovative platform enabling both the study of evolutionary conserved processes behind development of ccRCC and identification of potential drugs targeting the TOR kinase pathways. This Arabidopsis, human, mice and chemoinformatics based platform provides an attractive and cost effective alternative to other currently used approaches.

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P7.2

AtHB8 gene and the mechanical stress response

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The visualization of the *GUS/GFP* gene activity under the control of the *AtHB8* promoter is a widely used method to identify the early stages of vascular development. *AtHB8* (*Arabidopsis thaliana HOMEOBOX GENE 8*), a member of a family of the HD-ZIP III genes, has no obvious phenotype of the respective mutant and its function is still not really resolved. *AtHB8* gene is known to be expressed in preprocambial and procambial cells during vascular tissues formation, and is possibly required for the determination of the differentiation of vascular cells. Our experiments with the *pAtHB8:GUS* transgenic plants have revealed a potential new function of this gene and thus the results of our research will be discussed in the context of the *AtBH8* role in the mechanical stress response.

P7.3

What triggers SAM arrest in the *ftsh4* mutants during growth in mildly elevated temperature

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Continuous growth and development of a plant is enabled by the maintenance of the Shoot Apical Meristem (SAM). We aimed to analyze the impact of mitochondria impairment, caused by a loss of an important member of *A. thaliana* mitochondrial protein quality control system, the protease AtFTSH4, on the maintenance of the SAM. The *ftsh4* mutant plants, in suboptimal growth conditions (continuous mildly elevated temperature of 30°C), displayed a striking phenotype of the precocious termination of the inflorescence meristem. In our study, we have shown that *AtFTSH4* gene is predominantly expressed just prior to and during the reproductive phase of growth, especially in intensively dividing tissues, and that the premature meristem termination is associated with H_2O_2 accumulation and loss of the mitochondria functionality directly in the SAM tissues. Importantly, within the SAM, the most affected were the crucial stem cells, which normally must be maintained throughout the plant's life. All the observed defects concomitantly caused a drop in the stem cells activity. We have concluded that the SAM termination, observed in *ftsh4* mutants, is caused both by internal oxidative stress, which accumulates with time/age, and by the tissue specific role of AtFTSH4 around the flowering transition. The results of our research will be discussed in the context of the role of *AtFTSH4* gene for the SAM maintenance at the cellular and molecular level.

Key words: *Arabidopsis, AtFTSH4* gene, SAM, oxidative stress, mitochondria impairment

P7.4

The importance of a proteolytically active serine and PDZ domains for chaperone activity of chloroplast protease AtDeg2

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AtDeg2 is a ATP-independent, serine-type chloroplast protease, peripherally attached to the stromal side of thylakoid membrane, containing a protease domain and two PDZ domains (PDZ1 and PDZ2), which play an essential role in oligomerization of the the enzyme, substrate recognition and activation of the protease domain. The results of recent studies allowed to conclude that besides being a protease AtDeg2 exhibits a chaperone activity consisting in an ability to suppress the aggregation of lysozyme in the presence of DTT. It prompted us to perform studies regarding a localization of chaperone activity in linear structure of AtDeg2 molecule. Therefore a chaperone activity of several AtDeg2 mutants was tested, such as *S268G* (proteolytically active serine substituted by glycine) and those in which PDZ domains have been deleted – ΔPDZ1, ΔPDZ2 and Δ(PDZ1 + PDZ2).

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P7.5

Cadmium effect on the expression of *AHA* genes in *Arabidopsis thaliana* brassinosteroid mutants.

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Despite increasing knowledge about plant responses to cadmium (Cd), many elements of plant mechanism adaptation to Cd stress remain unclear,s including hormone crosstalk. Transcriptome analysis revealed significant similarity between Cd and brassinosteroids (BR) induced responses at the gene expression level, suggesting that the reaction of plants to Cd may activate signaling pathways dependent on BR. Our previous study indicated that plasma membrane (PM) H^+-ATPase, is the key enzyme in adaptation of plants to various stress conditions, including cadmium stress. Moreover we have earlier demonstrated a stimulating effect of exogenous BR on the activity of PM H^+-ATPase. In the present study we have examined the role of BR in plant adaptation to cadmium stress mediated through PM H^+-ATPase activity. PM H^+-ATPase is encoded by a multigene family and modification of PM H^+-ATPase activity may include transcriptional level. To explain the role of BR in modification of PM H^+-ATPase activity under Cd stress, the expression of *AHA* genes in *Arabidopsis thaliana* mutants with dysfunctional BR biosynthesis (*dwarf*4) or signaling (*bri*1, *bak*1) was performed. The six-week old plants was treated with 10 μM $CdCl_2$ for 6 days. Some of the plants after 3 days of 10 μM $CdCl_2$ exposure were transferred to a nutrient medium without heavy metals for another 3 days (post-stressed plants). Treatment of wild type plants with cadmium increased transcription of *AHA2* gene, which correlated with an increase of H^+-ATPase activity in the same conditions. However in *dwarf*4, *bri*1 and *bak*1 mutants treated with cadmium, significant decrease of *AHA1, AHA2, AHA3 and AHA4* transcript level was observed. Based on this results, we suggest that BRs play an important role in the adaptation of plants to cadmium stress by affecting the transcription of *AHA* genes encoding of PM H^+-ATPase in *Arabidopsis thaliana.* Taken together our results show some elements of the brassinosteroid signaling pathway activated under cadmium stress, thus significantly contribute to the advancement of knowledge on adaptation mechanisms and hormone signal transduction pathways in plants subjected to abiotic stresses.

P7.6

Transcriptional analysis of Rubisco assembly chaperones in *Arabidopis thaliana* plants with silenced *rbcX* or *raf1* genes

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Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) is an enzyme responsible for incorporation of carbon dioxide into ribulose-1,5-bisphosphate and therefore for the first step of biomass generation in the biosphere. For its proper biogenesis Rubisco requires a set of auxiliary factors like chaperonins (i.e. Cpn60/Cpn20/Cpn10) or assembly chaperones. Recently function of two representatives of the latter group – RbcX and RAF1 - was characterized. RbcX is crucial for cyanobacterial Rubisco assembly. Although its plant orthologs have been found, their participation in eukaryotic enzyme biosynthesis has yet to be confirmed. RAF1 was firstly identified in maize cells. Its disruption in these C_4 plants is lethal. Cyanobacterial homologs of RAF1 act post-folding *in vitro* but their physiological function have not been determined up to date.
Arabidopsis thaliana carries two genes encoding RbcX (*AtRbcX1*, *AtRbcX2*) and two encoding RAF1 (*AtRAF1.1*, *AtRAF1.2*) homologs respectively. To elucidate the function of each of these assembly chaperones separately we generated *A. thaliana* mutants with decreased transcription level of each chaperone-encoding gene using siRNA approach. Although, silencing led to over 95% decrease of specific transcript abundance in all cases, modified plants lines do not exhibit any obvious phenotypes under normal growth condition. However, silencing of each single chaperone-encoding gene caused decrease of the transcript abundance of at least a part of the remaining ones. Moreover, this effect was additionally counterbalanced by significant increase of transcription level of RbcS encoding genes. Presented data suggest that the control of Rubisco assembly process is strictly organized on previously unreported transcription level.

P7.7

Manipulation of vanillin synthesis in genetically modified flax

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Flax (*Linum usitatissimum* L.) is an annual dicotyledonous plant used by a human for about 10 thousand years. It serves as a source of: seeds from which is produced oil and the fibers used widely in textile and paper industry. Also, flax fibers are rich in phenolic acids and flavonoids, which possess antiallergenic, antiviral and antiinflammantory properties. These properties are mainly due to the presence of secondary metabolites in flax.
Plant secondary metabolites exhibit crucial functions for plant, such as cell growth modulation, plant elongation, photoprotection and regulation of permeability and fluency of plasma membranes. Main groups of secondary metabolites in plants consist of terpenes, alkaloids and phenolics. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of such metabolites (phenylopropanoids) which is crucial in food and cosmetic industry. Vanillin also has valuable antioxidant and anti-inflammatory properties. It plays an important protective role against oxidation of proteins and lipid peroxidation. It is also involved in removing the excess of hydrogen peroxide and free radicals. In addition, it decreases the expression of proinflammatory cytokines such as interleukin (IL) -1β, IL-6, interferon-γ, and tumor necrosisfactor-alpha (TNF-α). Because of these properties, flax overproduction of vanillin could be used in the pharmaceutical industry and in medicine. In flax, vanillin is synthesized in the benzoic pathway, which is part of phenylopropanoid pathway. Exploration of the pathway will allow for creation of plants with increased amount of vanillin. It was analyzed how introduced changes to phenylopropanoids pathway affected vanillin synthesis.
In our study we created plants with overexpression of vanillin synthase gene in *in vitro* culture. The agro-transformation introduced two genes from *Pseudomonas sp*. The genes known as *fcs* and *ech* are responsible for encoding feruloyl-CoA synthetase and enoyl-CoA hydratase/aldolase respectively - enzymes involved in bioconversion of ferulic acid to vanillin in this bacteria. These results are the basis for the introduction of a field crop and to analyze the expression of level of genes and level of metabolites related of the phenylpropanoid pathway in the next generation of transgenic plants.

P7.8

Selection of reliable reference genes for expression analyzes in yellow lupin subjected to *Fusarium* spp.

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Soil-borne fungal diseases caused by *Fusarium* spp. is one of the major factor causing severe losses in lupins yield. Yellow lupin (*Lupinus luteus* L.) is characterized with the highest level of resistance against *Fusarium* among lupin crops although no specific genes related to resistance have been identified. To address this issue RNA sequencing of susceptible and resistant genotypes was performed towards identification of differentially expressed genes potentially involved in the resistance process. Candidate genes will be further validated in the expression analyses by qPCR. Accurate normalization of qPCR data through availability of stably expressed reference genes is crucial to ensure that the expression of gene of interest is unaffected by experimental conditions.

In this study we aimed at identifying reference genes suitable for the quantification of gene expression level in yellow lupin leaf tissue. In an attempt to identify the best internal control, 7 putative reference genes were tested in 3 susceptible and 3 resistant genotypes growing in a field under both standard condition and subjected to *Fusarium* infection. Gene expression stability was calculated using three softwares: geNorm, NormFinder, and Bestkeeper. Actine and ATP-synthase genes were found as the most appropriate genes for qPCR normalization by both NormFinder and geNorm programs, while the most stably expressed genes according to BestKeeper algorithm was alcohol dehydrogenase (ADH3) and ATP-synthase.

The results of our study provide guidelines for reference genes selection to be used in gene expression studies of *Fusarium* spp. infection in yellow lupin.

P7.9

Identification of functional interdependence between SWI/SNF dependent chromatin remodeling and pre-mRNA splicing in Arabidopsis

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SWI/SNF type chromatin remodeling complexes (CRCs) are evolutionarily conserved multiprotein machineries controlling DNA accessibility by regulating chromatin structure. The core of Arabidopsis SWI/SNF complex contains one of the four ATPases (BRM, SYD, CHR12, CHR23), two of the four SWI3 type proteins (SWI3A, SWI3B, SWI3C, SWI3D) and one SNF5 type protein (BSH). SWI/SNF CRCs play an important role in the regulation of transcription, cell cycle and DNA replication. Moreover, recent data indicate that the SWI/SNF complex is likely involved in the regulation of pre-mRNA splicing in humans, *Drosophila* and yeast. In eukaryotes, intron sequences from pre-mRNAs transcribed by RNA polymerase II are removed by the spliceosome to produce mature mRNAs. The spliceosome is composed of small nuclear snRNAs and associated proteins. Catalytic activation of the spliceosome is critically dependent on its association with the evolutionary conserved NineTeen Complex (NTC), which among others carries the core PRP19A/B, CDC5 and SPF27 subunits. The aim of our study is to identify a regulatory link between chromatin remodeling and pre-mRNA splicing in Arabidopsis. According to the STRING protein-protein interaction database, several subunits of the SWI/SNF complex interact with components of the NTC. To confirm the conservation of these protein interactions in Arabidopsis, we performed yeast two-hybrid (YTH) interaction studies. In addition, using combinations of existing T-DNA insertion mutations, we identified epistatic genetic interactions between SWI/SNF and NTC, which provide evidence for functional interdependence between chromatin remodeling and pre-mRNA splicing mechanisms in Arabidopsis.

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P7.10

Insights into mechanism of herbicide resistance by comparative analysis of the transcriptomes derived from two maize lines with differential susceptibility to glyphosate

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As a non-selective, broad-spectrum herbicide, glyphosate become one of the most widely used herbicide in the world. One of the reasons of its popularity was an introduction of glyphosate-resistant genetically-modified crops which allow for selective removal of the weeds in the filed. However, it is also possible to obtain non-GMO plants with similar levels of tolerance by selective breeding. Moreover, with the long-term use of herbicides, the increasing number of cases of spontaneous acquisition of glyphosate resistance in weeds is reported. Thus, it is necessary to understand the mechanism of gaining natural resistance to herbicide.

Here, we address this question by *de novo* assembly and comparative analysis of two *Zea mays* transcriptomes derived from lines with a varying resistance to glyphosate. The samples of both types of plants were taken right after glyphosate was applied, 1 day and 7 days after treatment. Using RNA-seq data from next generation sequencing we were able to analyze not only dynamic changes in gene expression, but also in alternative splicing of mRNAs. The experimental setup allowed us to investigate the baseline differences between the lines prior to the treatment and the differences in the response of to the glyphosate treatment. The results reveal significant differences of both, gene expression and splicing patterns between the glyphosate-resistant and sensitive plants.

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P7.11

Flavonoid C-glucosides from flax straw extracts reduce human breast cancer cell growth *in vitro*

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Flax straw of flax varieties which are grown for oil production, are leftovers, and contribute to the high biomass production. Therefore their putative application for human use is of high interest. Our study revealed that flax straw is rich in flavonoid C-glucosides, including vitexin, orientin and isoorientin. The objective of this study was to evaluate the cytotoxicity and proapoptotic effect of flax straw derived C-glucosides of flavonoids in the human breast adenocarcinoma cell line (MCF-7). The effects of flax straw derived flavonoid C-glucosides on cell proliferation of MCF-7 cells were evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl-tetrazolium bromide (MTT) and sulforhodamine B (SRB) assays. The expressions of apoptosis-related genes were assessed by RT-PCR. Our data revealed that flax C-glusosides as well as pure compounds are cytotoxic towards MCF-7 cells and inhibit their proliferation. Moreover the induction of apoptosis was correlated with the changes in the mRNA level of pro-apoptotic genes. The increased expressions of bax, caspase-7, -8, and -9 and the decreased mRNA expressions of bcl-2 was observed, whereas the mRNA levels of p53 and mdm2 were not altered. These results clearly demonstrated that flax straw metabolites effectively induced growth inhibition and apoptosis in human breast adenocarcinoma cells.

P7.12

Cucumber MTP5 and MTP12 proteins form a functional heterodimeric complex involved in the import of zinc into the Golgi compartment

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Members of cation diffusion facilitator (CDF) family catalyze the H^+-coupled efflux of heavy metal ions from the cytoplasm. The so far characterized plant CDF proteins (designated as metal tolerance proteins MTP) contribute to the maintaining of Zn^{2+} or Mn^{2+} homeostasis and tolerance through the export of metals out of the cells or through the intercellular compartmentalization of metals excess within the vacuoles (zinc and manganese transporters) or the Golgi/secretory pathway vesicles (manganese transporters). Though phylogenetically related MTP proteins appear to have similar substrate specificity, experimental confirmation of metal substrates for some of the plant MTPs is still lacking. Here we describe a novel metal tolerance protein CsMTP5 from cucumber that localizes to Golgi compartment and is equally distributed in cucumber tissues and organs. CsMTP5 is predicted to have 400 amino acids and 6 transmembrane helices (TMH) with TMDII and TMDV containing the two conservative motifs typical for zinc CDFs. *Split-ubiquitin* two-hybrid assay, as well as *coimmunoprecipitation* experiments revealed that CsMTP5 forms a heteromeric complex with a high molecular mass metal tolerance protein CsMTP12. Heterologous expression in yeast deficient in endogeneous ER zinc importer revealed that heteromerization of CsMTP5 with CsMTP12 is necessary to complement the low-zinc sensitive phenotype of the yeast mutant. The cytosolic labile zinc as well as the total cellular zinc have been markedly reduced in yeast expressing CsMTP5-CsMTP12 complex, indicating the function of the heterodimer in zinc loading into the ER lumen and the secretory pathway of yeast cells. The level of CsMTP5 protein was markedly increased in cucumber roots upon Zn excess suggesting that in cucumber cells, the CsMTP5-CsMTP12 complex may contribute to the loading of Zn excess to the secretory pathway vesicles to remove metal out of the cell via exocytosis. These results are in line with the generally postulated role of plant MTP proteins in detoxification of plant cells from heavy metals excess.

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P7.13

ABA transport in Medicago-between drought stress response and changes in root morphogenesis

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There is growing evidence that ABA movement within plants can determine proper ABA perception and subsequently trigger adequate responses under unfavorable conditions. The studies conducted on Arabidopsis showed that ABC transporters belonging to the G subfamily participate in ABA translocation. Little is known about the ABA transport and ABA transporters in legumes. Different effects of ABA on the lateral root formation in Arabidopsis and *Medicago truncatula* indicate species-specific ABA action. Moreover it was reported that in *M. truncatula*, ABA affects the nodulation process as a negative regulator to reduce costly establishment of nodules under stressful conditions. Based on phylogenetic tree of the half-size ABCG transporters, we have selected four potential Medicago ABC proteins involved in ABA transport. We have shown that their expression is strongly upregulated upon drought stress. A spatial expression pattern analyses with GUS reporter gene as well as NLS:GFP revealed that *MtABCG20* and *MtABCG26* promoters are active mostly within vascular bundles where ABA is predominantly biosynthesized. Two other, namely *MtABCG27* and *MtABCG29*, are expressed in meristems and cortex root elongation zone, respectively, where ABA stimulates lateral root growth. The use of transgenic Medicago carrying a dominant-negative allele of *abi1* revealed that the ABA-inducible genes acts independently of the ABA core signaling pathway. The corresponding proteins are located in the plasma membrane. The Bimolecular Fluorescence Complementation (BiFC) assays confirmed that MtABCG20 and MtABCG26 have an ability to make homodimers and heterodimers. Finally, transport experiments indicated MtABCG20 contribution to ABA export from cell.

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P7.14

Calcium-Dependent Protein Kinase family members in potato (*Solanum tuberosum*)

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Calcium-Dependent Protein Kinases (CDPKs), unique for plants, are both important sensors and effectors of Ca^{2+} flux in plants. They are involved in growth and developmental processes as well as in defence strategy against different environmental stresses. CDPKs are encoded by multi-gene families. Plant are known to express a large number of *CDPK*s genes, mostly from 20 to 40 genes. Despite extensive studies of *CDPK*s in many species, knowledge concerning the specific expression patterns and evolutionary history of the CDPK family in potato (*Solanum tuberosum*) remains very limited.
Therefore, the aim of the study was determination of the phylogenetic relationships and expression profiles of the *CDPK* genes identified in the potato genome sequenced by Potato Genome Sequencing Consortium.
To identify CDPK family members in potato, bioinformatics methods were used to gather information on this family. CDPKs were divided into four subfamilies based on a phylogenetic tree and gene structures. Finally, detailed QPCR expression analysis were carried out for the *CDPK* genes in different organs of potato such as young and mature leaves, stems, shoots, roots, stolons, swollen stolons, flowers and mature tubers. The expression of all *CDPKs* was observed in all potato organs analysed, although the level of their expression varied greatly.
To our knowledge, this is the first genome-wide study of the potato CDPKs gene superfamily. Because of that, obtained results provide a perspective on the evolutionary history and general biological roles of the potato CDPK family.

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P7.15

Generation of nitric oxide in cucumber roots in response to low temperature – involvement of NR and/or NOS dependent pathways

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Nitric oxide is widely recognized signaling molecule generated during different abiotic stresses. Two major enzymatic NO sources are present in plants, one associated with nitrate reductase (NR) and the other with NO synthase (NOS) like activity. Cytoplasm localized NR is a key enzyme in nitrate assimilation pathway, responsible for reduction of nitrate to nitrite. Additionally, it can reduce nitrite directly to NO under stress conditions. In plants, NR activity associated with plasma membrane (PM-NR) was also recognized. PM-NR produces NO_2^- as a substrate for NO generation by nitrite/NO reductase (Ni-NOR) in apoplast. Whereas NO synthase catalyzes oxidation of arginine to citruline and NO. Although plant NOS protein has not been yet identified, arginine-dependent NOS-like activity was well documented.
In the present study, involvement of both NR and NOS-like activities in cucumber roots subjected to low temperature (LT, 10ºC) was analyzed. All experiments were conducted on cucumber seedlings stressed with 10ºC for a short (1d) and long (6d) time. Some of the plants after 3 day exposure to LT were transferred to control conditions for another 3 days (post-stressed plants, 3d+3d).
The amount of NO, detected using fluorescent dye DAF-2D, was higher in LT stressed plants than in control. Increase of the NO level was correlated with higher NR and PM-NR activities. The NR stimulation was due to post-translational modifications, especially of cytosolic NR, and a rise of *CsNR* gene expression. Beside, decrease of nitrite reductase (NiR) activity after 1d LT treatment and increase of NO_2^- level after 6d LT and 3d+3d treatment was observed in roots.These conditions favorNR -mediated NO production. Moreover, NOS-like activity was also changed in roots of plants under LT stress. Involvement of NR and NOS-like in NO generation has been confirmed in experiments with commonly used inhibitors: sodium tungstate for NR and L-NNA and AET for NOS-like activity. Application of inhibitorsreduces LT-induced increase of NO level in cucumber roots.
Summarizing, we suggest that NO biosynthesis occurring in LT stressed and post-stressed plants is mediated by NR, PM-NR and NOS-like enzymes.

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P7.16

Structural studies of the full-length *Arabidopsis thaliana* transcription factor WRKY50

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The WRKY proteins are a large superfamily of transcription factors with 72 representatives in *Arabidopsis thaliana*,induced upon pathogen infection and during certain stages of plant development. In spite of the strong conservation of the DNA binding domain which contains an invariant WRKYGQK sequence and zinc binding motif, the overall structures of individual representatives are highly divergent.
In present study we developed an efficient method for expression and purification of recombinant AtWRKY50 protein retaining the biological activity of the DNA binding.
The CD spectrum and bioinformatics sequence analyses allowed to deduce that AtWRKY50 lack of well-defined secondary structure and is intrinsically disordered protein (IDP).
Crystallization attempts proved impossible and therefore we employed a set of biophysical techniques: dynamic/static light scattering (DLS/SLS), small angle X-ray scattering (SAXS), circular dichroism (CD), infrared spectroscopy (FTIR) and complementary bioinformatics sequence analyses to characterize secondary structure of protein of interests.Compilation of these data, analysis of radius of gyration, hydrodynamic radius and volume-parameters were used for bioinformatics modeling of AtWRKY 3D-structure and let us demonstrate the AtWRKY50 elongated shape, with N-terminal flexible tail (residues 1-107) and globular well-ordered C-terminal containing WRKY domain.
Using Isothermal Titration Calorimetry (ITC), AtWRKY50 was demonstrated to bind dsDNA containing one W-box sequence motif with K_d of 237 nM and 1:1 stoichiometry. As expected, AtWRKY50 protein binds one DNA molecule because this protein possess only one region responsible for DNA binding defined as the N-terminal WRKY domain. This result were in good agreement with those obtained for WRKY4-C domain where surface plasmon resonance was applied to determine the K_d. The calculated value was 260nM[1].DNA bindingn properties of AtWRKY50 were also proved using electromobility shift assay (EMSA).

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P7.17

Identification of genes involved in the response to a long-term salt stress in sugar beet and its halophytic ancestor, *Beta vulgaris ssp. maritima*

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A halophytic subspecies of beet, *Beta vulgaris ssp. maritima* is considered as an ancestor of domesticated beet varieties, such as sugar beet. Sugar beets display several traits of salt-tolerance inherited from the halophytic ancestor. However, *B. vulgaris ssp. maritima* is characterized by elevated salt stress-resistance, when compared to sugar beet. In this study a transcriptomic response to a long-term treatments with salinity was assessed in leaves of young plants representing *B. vulgaris ssp. maritima* and sugar beet variety *Huzar*. For the salt treatment, potted plants representing *Beta vulgaris ssp. maritima* or *Beta vulgaris* cv. *Huzar*were subjected to either moderate or severe salt stress during 32-day-long culture period. Following the treatments, Illumina paired-end sequencing technology was used to study gene expression profiles in leaves. Simultaneously, selected biochemical parameters such as relative water content, chlorophyll, proline and abscisic acid concentration were tested. The study revealed that more genes were altered in their expression level in sugar beet than in halophytic beet, in response to salt treatment. Whereas, 249 genes were up-regulated, and 669 were down-regulated in cv. Huzar, the number of 95 up-regulated and 192 down-regulated genes was identified in leaves of *B. vulgaris ssp. maritima*. Gene ontology (GO) analysis revealed that most represented GO categories among salt-responsive genes in both, *B. vulgaris ssp. maritima* and *Beta vulgaris* cv. *Huzar* were "oxidation-reduction process", "membrane", and "protein binding" which emphasises the importance of the antioxidant protection, regulation of membrane transport and stability and protein chaperoning, under long-term treatment with salinity. More GO categories representing salt-responsive genes were found un sugar beet than in halophytic beet. The finding suggests that the adaptation to salinity in sugar beet variety requires more profound reorganization of leaf transcriptom, than in its halophyte relative. Significant number of genes of unknown function were differentially expressed under salt administration in plants representing both genotypes. These genes might provide a link to so far uncharacterised mechanisms of salinity tolerance.

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P7.18

An antisense long non-coding RNA serves as a sensor for environmental signals during development and stress responses in Arabidopsis

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Long non-coding RNAs represent diverse classes of transcripts longer than 200 nucleotides. Recent genome-wide transcriptome analysis, such as tiling arrays and next generation sequencing, has revealed a large number of stress-responsive ncRNAs in various species. Several studies have recently characterized a varieties of long-noncoding RNAs as an important gene regulators involved in stress responses, disease regulation and adaptation upon changeable environmental conditions.

DOG1 (*Delay of germination 1*) is a master regulator of seeds dormancy in Arabidopsis and has important contribution to seed dormancy regulation in other plants. We have previously shown the presence of an antisense transcript produced in opposite orientation to the *DOG1* gene and characterized it function in seed dormancy regulation.

Here we present detailed bioinformatics analysis of 3`untranslated *DOG1* genomic region including last intron, 3rd exon, 3`UTR and part of downstream genomic sequences that co-localize with *DOG1* antisense transcription start site (TSS). This revealed substantial enrichment of classical and specific hormone-responsive *"cis"*- regulatory elements (TATA-boxes, ABRE-element etc.). In addition, we find high H3K4me3 levels at this sequence suggesting that this genomic region can serve as a promoter sequence controlling *DOG1* antisense mRNA expression. Cloning of putative *DOG1* antisense promoter in frame to IRES-luciferase *(LUC)* marker gene fusion showed tissue-specific expression that partially overlapped with *DOG1* sense promoter`s activity. We will describe our efforts to understand an interaction between *DOG1* sense- and antisense-oriented transcriptional machineries. Including classical approaches for promoters study such as gene's promoter activity as well as mutagenesis, deletion studies and *"cis"* or *"trans"* interactions.

Our results demonstrated a novel regulatory mechanism of *DOG1* gene regulation that highlights unexpected function of *DOG1* during vegetative grow that involves environmental and hormonal signal sensing by a long antisense non-coding RNA.