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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

iPlanta

iPLANTA COST Action CA15223

2nd iPLANTA Conference

RNAi: THE FUTURE OF CROSS TALK

Focus on RNAi Technology (WG1) and Applications (WG2)

ABSTRACT BOOK

14-16 february 2018
Poznań, Poland

Faculty of Biology
Adam Mickiewicz University

Adam Mickiewicz University Poznań
KNOW
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iPLANTA COST *Action* CA15223

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Focus on RNAi Technology (WG1) and Applications (WG2)

Faculty of Biology, Adam Mickiewicz University
Poznań, Poland

http://iplanta2018.home.amu.edu.pl/

FEBRUARY, 14-16, 2018
BOOK OF ABSTRACT

Grosshans H, ***Filipowicz W****.Nature.* ***2008***

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2nd iPLANTA Conference AIMS AND THEMES

The 2nd iPLANTA Conference will focus on the unique aspects of Double-stranded RNAi technologies to induce resistance to pests and pathogens. The small RNAs (sRNAs) that mediate cross-kingdom RNAi in plant hosts represent a novel class of pathogen effectors that inhibit host immunity for successful infection. e.g. sRNA trafficking from fungal pathogens to host plants. Plant transgene-derived artificial sRNAs can induce gene silencing in certain interacting pests and pathogens, a phenomenon called host-induced gene silencing (HIGS). To improve the use of RNAi technology, bidirectional cross-kingdom RNAi and two-way sRNA trafficking between pests/pathogens and hosts need to be better understood. Furthermore, information is needed on the specificity of silencing and whether the produced dsRNA may also trigger non-specific (off-target) effects that affect plant physiology or non-targets. Information is also required on the efficiency and specificity of RNAi systems so that they can be designed to maximize the desired gene silencing and minimize off-target effects. The main focus of the 2nd iPlanta conference will be addressed to understanding RNAi mechanisms and specificity (WG1) and the novel pest and disease control applications (WG2) that are unlikely to be developed by other new breeding technologies (NBT), such as gene editing.

The conference will also consider the issues discussed at iPlanta Ghent workshop on RNAi biosafety (WG3), which relate to data requirements for environmental risk assessment of RNAi systems for pest and disease control and to the likelihood of off target and non-target effects. The conference will consider what form future risk assessments should take so that they are proportionate to potential risks and proportionate for organisations developing new systems.

The WG4 plan for creating a framework of existing knowledge on the socio-economic impacts of new technologies on the food production, processing and marketing, will be presented. In addition, a study of the contribution of RNAi technologies to this production and marketing chain is being considered. A proposal for a survey of EU scientist's attitude and perceptions of RNAi and NBT technologies, will be also presented and discussed.

The WG5 communication plan to stakeholders and consumers will be presented and discussed, including the identification of a specific message to be communicated with a podcast based on a "story telling approach" and an event to be organized at the EU parliament.

Additionally, it will be organized a Satellite meeting "RNAi research in Poland", an event that aims to attract and disseminate the iPlanta COST action in Poland.

COMMITTEES

Scientific Committee:

- Prof. **Bruno Mezzetti** – Chair of the iPLANTA Cost Action
- Dr. **Jeremy Street** – vice-Chair of the iPLANTA Cost Action
- Prof. **Guy Smagghe** – WG1 leader
- Prof. **Huw Jones** -WG2 leader
- Prof. **Salvatore ARPAIA**, WG3 leader
- Prof **Justus WESSELER**, WG4 leader
- Prof **Hilde-Gunn OPSAHL-SORTEBERG**, WG5 leader
- Prof. **Michel Ravelonandro**, Vice-leader WG1
- Prof. **Hely Häggman**, Vice-leader WG2
- Prof. **Antje Dietz-Pfeilstetter**, Vice-leader WG3
- Prof. **Konstantinos Karantininis**, Vice-leader WG4
- Prof. **Matina Tsalavouta**, Vice-leader WG5
- Prof. **Attila Molnar**
- Prof. **Gijs Kleter**
- Prof. **Dario Frisio**
- Prof. **Vera Ventura**
- Prof. **Joost Dessein**
- Prof. **Mirco Montefiori**
- Prof. **Lorenzo Burgos**
- Prof. **Kit Greenop**
- Dr. **Jorge Paiva**, Coordinator STSM

Organizing Committee:

- **Jorge Paiva** – Polish delegate to MC, STSM coordinator, IGR PAN
- **Zofia Szweykowska-Kulińska** – UAM Poznan
- **Iwona Kanonik-Jędrzejak** – UAM Poznan
- **Tomasz Bielecki** – UAM Poznan
- **Andrzej Pacak** – UAM Poznan
- **Mortaza Khodaeiaminjan** – IGR PAN
- **Carolina Gomes** – IGR PAN
- **Aleksandra Smoczyńska** – UAM Poznan
- **Aleksandra Grabowska** – UAM Poznan

Program

DAY 1 – WEDNESDAY FEBRUARY 14TH, 2018

8:00 –Registration

9:00 Welcome – Satellite meeting: *RNAi Research in Poland*

9:15 Satellite meeting: RNAi Research in Poland

Chairman: Andrzej Pacak

AUTHORS	ORAL Presentation	
SZYMON SWIEŻEWSKI	MAKING SENSE OF ANTISENSE	15 min
ROBERT MALINOWSKI	CHEMICALLY INDUCIBLE GENE SILENCING FOR PRECISE UNDERSTANDING OF PLANT DEVELOPMENT	15 min
MATEUSZ BAJCZYK	A ROLE OF CROSSTALK BETWEEN THE NEXT COMPLEX AND SERRATE IN DEGRADATION OF MIRNA PRECURSOR FRAGMENTS	15 min
SUSHEEL SAGAR BHAT	*ARABIDOPSIS THALIANA* M^6A METHYL TRANSFERASE (MTA) AS A PUTATIVE PLAYER IN miRNA BIOGENESIS REGULATORY PATHWAY**.**	15 min

10:30 – *Coffee Break and Poster Session*

11:00 Satellite meeting: RNAi Research in Poland

Chairman: Andrzej Pacak

AUTHORS	ORAL Presentation	
PAWEŁ SEGA	GLOBAL ANALYSIS OF SMALL RNAS LEVEL CHANGES IN BARLEY ROOTS AND SHOOTS DURING PHOSPHATE STARVATION	15 min
A SMOCZYŃSKA	CHARACTERIZATION OF NOVEL MIRNAS IN BARLEY	15 min
A GRABOWSKA	ARE BARLEY microRNA PRECURSORS FROM mIR444 FAMILY ASSOCIATED WITH RIBOSOMES?	15 min
IWONA MORKUNAS,	THE EFFECT OF LEAD AND A. PISUM ON EXPRESSION LEVELS OF PHENYLALANINE AMMONIALYASE AND CHALCONE SYNTHASE GENES IN PEA SEEDLINGS	15 min

11:30 Registration 2nd iPLANTA Conference

12:30 *Lunch*

13:30 Opening 2nd iPLANTA Conference

14:00 International Invited Lecture

Chairman: Zofia Szweykowska-Kulińska

ANDRZEJ WIERZBICKI	MECHANISMS OF RNA-DIRECTED DNA METHYLATION	45 min

14:45 Open session 1 – RNAi Technology

Chairman: Michel Ravelonandro

AUTHORS	ORAL PRESENTATION	
Y.ZHAO, J.SUN, **L.SWEVERS**	VIRAL-LIKE PARTICLES BASED ON CYTOPLASMIC POLYHEDROSIS VIRUS FOR DELIVERY OF DSRNA IN INSECTS	15 min
U. MANSKE, A. DIETZ-PFEILSTETTER	COMPARISON OF DIFFERENT METHODS FOR THE ESTABLISHMENT OF RNA SILENCING IN PLANTS	15 min
W.ORCZYK, Y.YANUSHEVSKA, J. GROSZYK, W.M. KARŁOWSKI, A.ZIELEZIŃSKI, A. NADOLSKA-ORCZYK	THE RNAi APPROACH TO STUDY BRASSINOSTEROID REGULATOR ENCODING GENES IN BARLEY	15 min
A.STEPIEN, T.GULANICZ, M.BAJCZYK, D. SMOLINSKI, Z.SZWEYKOWSKA-KULINSKA, A.JARMOLOWSKI	THE ROLE OF THE U1 SNRNP PROTEIN, PRP40, IN PLANT MIRNA BIOGENESIS	15 min

***15:45*-16:45** *Coffee break*

16:15 Open session 2 – RNAi Technology

Chairman: Guy Smagghe

AUTHORS	ORAL PRESENTATION	
A. KOCH, L. HOEFLE, E. SECIC, S. ZANINI, **K.H. KOGEL**	SMALL RNA EFFECTORS IN HIGS AND SIGS APPROACHES	30 min
A. NADOLSKA-ORCZYK, S. GASPARIS, W. ORCZYK	EFFECTIVNESS OF THE ARTIFICIAL MICRORNA- AND SIRNA-BASED SPECIFIC GENE SILENCING OF AGRONOMICALLY IMPORTANT GENES, AND SI-RNA-MEDIATED IMPROVEMENT OF PRODUCTIVITY IN CEREALS	15 min
M.RAVELONANDRO, P. BRIARD, R. SCORZA	THE Mi AND Mi-SiRNA APPROACH TO CONTROL PLUM POX VIRUS GENOME IN PLUMS	15 min
M. PETEK, K. Gruden	TESTING THE ACTIVITY OF DOUBLE-STRANDED RNAS AGAINST COLORADO POTATO BEETLE	15 min
JR PARREIRA, P FEVEREIRO, **SS ARAÚJO**	MODULATING SEED DEVELOPMENT IN COMMON BEAN (PHASEOLUS VULGARIS): AN APPROACH TO ENHANCE SEED QUALITY?	15 min

17:45 *General Discussion*

18:00 Poster Session + *Cheese, wine and pierogi party*

DAY 2 – THURSDAY FEBRUARY 15TH, 2018

9:00 Open session 3 – RNAi Technology

Chairman: Wacław Orczyk

AUTHORS	ORAL PRESENTATION	
Y. FEI, T. NYIKO, **A. MOLNAR**	DECIPHERING THE MOLECULAR POSTCODES IN EPIGENETIC GENE SILENCING	30 min
A.SULKOWSKA, A.AUBER, D.SILHAVY, J.KUFEL, I.WAWER	NEW PROTEIN FACTORS INVOLVED IN PLANT NMD	15 min
INES WYRSCH, THOMAS BOLLER, MANFRED HEINLEIN, **ANNETTE NIEHL**	PLAYERS AND MECHANISMS IN ANTIVIRAL PATTERN-TRIGGERED IMMUNITY IN PLANTS	15 min
F. NEGRINI, M. CHATTERJEE, T. ZHANG, M.F. MAD ATARI, K. O'GRADY, B. MEZZETTI, **E.BARALDI** AND K.FOLTA	ASSESSING SILENCING EFFECTIVENESS AND SPECIFICITY AMONG RAPID ALKALINIZATION FACTOR (RALF) FAMILY GENES FOR Fragaria vesca RNAi PLANTS	15 min
E. KAFKAS, M. ZARIFIKHOSROSHAHI, H. TOPCU, M. KHODAEIAMINJAN, M.A.GUNDESLİ, N. ÇOBAN, **M.GUNEY**, H. PAIZILA, H.KARCI, S.KEFAYATI, S. KAFKAS	EXPRESSION OF CANDIDATE GENES RESPONSIBLE FOR BUD ABSCISSION IN PISTACHIO	15 min

10:30-11:00 *Coffee break*

11:00 Open session – 4 RNAi Applications issues

Chairman: Huw Jones

AUTHORS	ORAL PRESENTATION	
H.D. JONES, J. MARTINEZ-FORTUN, D.W. PHILLIPS	SPEAKING UP FOR SILENCED GENES: CAUGHT IN THE CROSS TALK BETWEEN RNAI AND EDITING	30 min
A. ALBURQUERQUE, C. PETRI, L. FAIZE, **L. BURGOS**	SILENCING OF AGROBACTERIUM TUMEFACIENS ONCOGENES WITH A SHORT-LENGTH SINGLE CHIMERICAL TRANSGENE INDUCES RESISTANCE TO CROWN GALL DISEASE IN PLUM BUT NOT IN APRICOT	15 min

P.P. SOSOI, **M.C. ICHIM**	USE OF RNAI TOOLS TO INCREASE BARLEY'S RESISTANCE TO BIOTIC STRESS	15 min
G. RAKLEOVA, L. PETROVA, A. ATANASSOV, **I. PANTCHEV**	RNA INTERFERENCE AND GENE EDITING – COMPLEMENTING OR MERGING IN ACTION	15 min

12:30 *Discussion*

Conference group photo

13:00 *Lunch*

14:00 International Invited Lecture

Chairman: Huw Jones

QIANG CAI, LULU QIAO, MING WANG, BAOYE HE, ARNE WEIBERG, **HAILING JIN** (UNIVERSITY CALIFORNIA, RIVERSIDE)	CROSS KINGDOM RNAI AND SPRAY-INDUCED GENE SILENCING FOR CROP PROTECTION	45 min

14:45 Open session 5 – RNAi Applications

Chairman: Huw Jones

AUTHORS	ORAL PRESENTATION	
P. WIECZOREK, M. TAUBE, Z. SZWEYKOWSKA-KULIŃSKA, A. JARMOŁOWSKI, M. KOZAK	SMALL-ANGLE X-RAY SCATTERING STUDY OF THE OLIGOMERIC STATE AND DIMER STRUCTURE OF THE SERRATE194-579 PROTEIN IN SOLUTION – A KEY PLAYER IN THE PROCESS OF miRNA BIOGENESIS IN ARABIDOPSIS	15 min
JANA SCHUCK, SELMA GAGO-ZACHERT, MARIE KNOBLICH, TORSTEN GURSINSKY AND **SVEN-ERIK BEHRENS**	IDENTIFICATION OF HIGHLY EFFECTIVE SMALL RNAs FOR ANTIVIRAL PLANT PROTECTION	15 min
LIDIA JIMÉNEZ, DELPHINE M. POTT, ELSA MARTÍNEZ-FERRI, ALISDAIR FERNIE, JOSE G. VALLARINO, **SONIA OSORIO**	THE BALANCE BETWEEN FUMARATE AND MALATE PLAYS AN IMPORTANT ROLE IN PLANT DEVELOPMENT AND POSTHARVEST QUALITY IN TOMATO FRUIT.	15 min

15:30 -16:00 *Coffee break*

16:00 Open session 6 – Biosafety issues

Chairman: Antje Dietz-Pfeilstetter

AUTHORS	ORAL PRESENTATION	
W.SCHENKEL	THE CURRENT REGULATORY ENVIRONMENT FOR RNAI PLANTS IN THE EU	30 min
A. GATHMANN	CHALLENGES FOR THE AUTHORISATION OF SPRAYABLE RNAI BASED PLANT PROTECTION PRODUCTS	15 min
A. DIETZ-PFEILSTETTER, S. ARPAIA	BIOSAFETY ASSESSMENT OF CURRENT RNAi APPLICATIONS: CASE STUDY OF INSECT RESISTANT MON 87411 MAIZE	15 min
O. CHRISTIAENS, S. ARPAIA, I. URRU, K. KOSTOV, T. DJAMBAZOVA, M. REDDY JOGA, G. SMAGGHE AND J. SWEET	LITERATURE REVIEW OF BASELINE INFORMATION ON RNAi THAT COULD SUPPORT THE ENVIRONMENTAL RISK ASSESSMENT OF RNAi-BASED GM PLANTS	15 min
HARRY A. KUIPER, **GIJS A. KLETER** AND ESTHER J. KOK	DESIGNING FOOD SAFETY ASSESSMENT APPROACHES FOR RNAI –MODIFIED PLANTS AND DERIVED PRODUCTS	15 min

17:45 Open session 7 STMS

Chairman: Jorge Paiva

AUTHORS	ORAL PRESENTATION	
TUMBAS ŠAPONJAC V., PETEK M., PAIVA J.A.P.	ONE YEAR OF SHORT TERM SCIENTIFIC MISSION IN THE FRAME OF THE COST ACTION 15223: DEVELOPING SYNERGIES, COMPETENCES AND SKILLS OM RNAI	15 min

18:00*General Discussion and Closure*

18:30 Social Dinner

DAY 3- FRIDAY FEBRUARY 16TH, 2018

8:30 Open session 7 – SOCIO ECONOMIC IMPACT AND COMMUNICATION

Chairman: Dario Frisio and Hilde-Gunn Opsahl Sorteber

AUTHORS	ORAL PRESENTATION	
MATINA TSALAVOUTA	STRATEGIC COMMUNICATIONS FOR RESEARCH AND IMPACT: WHY AND HOW	30 min
K. GREENOP, E. FERRI	AN EVENT TO UNITE EUROPEAN POLICY MAKERS ON THE VALUE OF RNA SILENCING	15 min
JOOST DESSEIN	'THE GM-DEBATE IS NOT A GM-DEBATE. REFLECTIONS ON THE GM-DEBATE IN EUROPE FROM A SOCIAL SCIENCES PERSPECTIVE.	15 min
D.G. FRISIO, V. VENTURA	EXPLORING THE FUTURE DEVELOPMENT OF RNAi/NBTs APPLICATIONS IN PLANT THROUGH FIELD TRIALS DATA	15 min
L.CASELLA, P.CASTAGNO, E.A.MUSA, A.ATANASSI, C.MINOIA	DESPITE NUMEROUS EFFORTS DONE IN THE LAST DECADES TO CONTROL RICE BLAST, THIS FUNGUS REMAINS THE BIGGEST ISSUE FOR EUROPEAN RICE CULTIVATION. COULD RNAI TECHNOLOGY BE A HOPE?	15 min
A.PASCALE	FROM PINOCCHIO TO MASTERSCHEF. FROM HUNGER TO ABUNDANCE. THE HISTORY OF AGRICULTURE IN A FEW FAMILY PHOTOS	15 min

10:15 *Discussion*

10:45 *Coffee Break*

11:15 CLOSED SESSION- iPlanta MC meeting

iPlanta MC meeting (only iPlanta MC members) to discuss future program and Communication strategy (WG leaders to present plans) including workshops, training schools, conferences. Discussion of STSMs.

14:00 **Closure**

Oral Presentations

DAY 1 - WEDNESDAY FEBRUARY 14TH, 2018

9:00 Satellite meeting: RNAi Research in Poland

MAKING SENSE OF ANTISENSE

SZYMON SWIEZEWSKI

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warszawa, Poland

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. Our efforts to understand DOG1 regulation lead to description of an antisense transcript, that resulted in discovery of an antisense dependent mode of gene expression regulation by a conserved chromatin-remodeling complex. I will present our recent efforts to understand the regulation of this antisense transcript as well as the mechanism of DOG1 silencing by this non-protein coding transcript.

CHEMICALLY INDUCIBLE GENE SILENCING FOR PRECISE UNDERSTANDING OF PLANT DEVELOPMENT

M.OLSZAK[1], A. FLEMING[2], R.MALINOWSKI[1]

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[1]Department of Integrative Plant Sciences, Institute of Plant Genetics of the Polish Academy of Sciences, Poznań, PL

[2]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

Expression systems, cell cycle, plant development

Plant development is governed by complex regulatory networks, therefore its proper understanding needs precise tools allowing spatial and temporal modification of gene activity. Here we would like to present usefulness of dexamethasone inducible gene expression method for precise modulation of transcript accumulation in Arabidopsis. In particular we will describe our attempts to link the DEX inducible method with AmiRNA or PTGS. We were using these approaches to understand leaf development or plant – pathogen biotic interactions.

We will also present effects of engineering miRNA recognition sites in gene of interest for exploring endogenous developmental regulatory patterns of plants.

We propose that inducible systems can be a good alternative to classical studies based on knock-outs or 35S-driven PTGS lines.

A ROLE OF CROSSTALK BETWEEN THE NEXT COMPLEX AND SERRATE IN DEGRADATION OF MIRNA PRECURSOR FRAGMENTS

MATEUSZ BAJCZYK[1], ŁUKASZ SZEWC[1], DAWID BIELEWICZ[1], AGATA STEPIEN[1], ZOFIA SZWEYKOWSKA-KULINSKA[1], ARTUR JARMOLOWSKI[1]

mateusz.bajczyk@amu.edu.pl

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

SERRATE, miRNA, NEXT, EXOSOME, DEGRADATION

The *Arabidopsis thaliana* SERRATE protein (SE), which is homologue of human ARS2 protein, is involved in two important pathways of RNA metabolism in plant: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA 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 accuracy of pri-miRNA cleavages catalyzed by DCL1. The Arabidopsis *se* null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the nuclear cap-binding protein complex (CBC), have been also ascribed to splicing of pre-mRNA. We have shown that SE interacts with CBC and both have influence of alternative splicing. In order to understand this dual role of SE and CBC in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the *se-1* mutant genetic background. The SE-bound proteins were identified by mass spectrometry, and the putative protein interactions were confirmed by the yeast two hybrid system and pull-down experiments. Additionally we confirmed this interaction in living cells using FLIM FRET techniq in *Arabidopsis* protoplasts. Our results have clearly demonstrated that SE as like ARS2 in humans contacts directly the Nuclear Exosome Targeting complex (NEXT). Our study has characterized accurate interaction place between SE and NEXT complex. Moreover we have shown that the NEXT complex is necessary for proper degradation of 5' pri-miRNA fragments after excision of miRNA by DCL1. We suggest that molecular interactions between CBC, SE and the NEXT complex is important for the quality control of miRNA precursors and degradation by the nuclear exosome 5' pri-miRNA fragments produced during miRNA biogenesis in the plant cell nucleus.

This work was supported by grants from the National Science Center
UMO-2014/13/N/NZ1/00049

ARABIDOPSIS THALIANA M^{6}A METHYL TRANSFERASE (MTA) AS A PUTATIVE PLAYER IN miRNA BIOGENESIS REGULATORY PATHWAY.

SUSHEEL SAGAR BHAT[1], DAWID BIELEWICZ[1], TOMASZ GULANICZ[1], DARIUSZSMOLINSKI[2], ARTUR JARMOLOWSKI[1] AND ZOFIA SZWEYKOWSKA-KULIŃSKA[1]

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[1]Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland; [2]Department of Cell Biology, Nicolaus Copernicus Univeristy, Torun, Poland

Among various mRNA modifications, methylation of adenosine at N6 position is the most abundant mRNA modification. It is present near the 3'UTR's and the stop codons within a specific motif –RRACH (R= G/A; H= A/C/U), and is present in almost 3-5 sites per mRNA molecule in plants as well as mammals. Methyl Transferase Like 3 (METTL3) is the key enzyme introducing m^{6}A mark to mammalian RNAs. METTL3 is a part of Methyl Transferase complex containing in addition Methyl Transferase Like 14 (METTL14) and Wilms Tumor 1-Associated Protein (WTAP) proteins. The *Arabidopsis* homologues of the Methyl Transferase complex proteins have been identified as m^{6}A Methyl Transferase, MTA (METTL3), FKBP12-interacting protein of 37 kDa, FIP37 (WTAP) and MTB (METTL14).

m^{6}A methylation has also been shown to be a mark for further processing of animal pri-miRNA. HNRNPA2B1 protein has been shown to read the m6A mark and recruit the micro-processor machinery facilitating the processing of pri-miRNAs.

In this study we aim to identify the role of MTA and m6A methylation in plant miRNA biogenesis. Interaction of MTA with other proteins involved in miRNA biogenesis was checked using Yeast Two Hybrid system. These experiments showed interactions of MTA with proteins involved in very early stages of miRNA biogenesis, namely Cycling DOF factor2 (CDF2), Negative on TATA less 2b (NOT2b) and its isoform, and TOUGH (TGH). Using fluorescent microscopy, co-localization of MTA with CDF2, NOT2b and TGH was shown. Interactions between MTA and TGH were confirmed using FLIM-FRET. Co-immunolocalization also showed MTA to co-localize with RNA pol II. Using high throughput sequencing data from mutants with very low level of MTA protein I found 37 miRNAs whose levels were changed in the mutant, 33 of which were downregulated. I also found that 30 pri-miRNAs were upregulated in the mutant and 14 of them formed pairs with downregulated miRNA. The results indicate that m^{6}A methylation and MTA have some influence on miRNA biogenesis in plants. To study this role further, experiments like ChIP, RIP and Co-IP will be done to understand the influence of MTA and m^{6}A modification on plant miRNA biogenesis.

GLOBAL ANALYSIS OF SMALL RNAs LEVEL CHANGES IN BARLEY ROOTS AND SHOOTS DURING PHOSPHATE STARVATION

P. SEGA, K. KRUSZKA, W. KARLOWSKI, Z. SZWEYKOWSKA-KULINSKA, A. PACAK1

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

Barley, small RNAs, microRNA, degradome, gene silencing

Inorganic phosphate (Pi) is an important factor for plant growth and development as an available form of phosphorus (P) - the fundamental macronutrient for the structural and metabolic needs of plants. In plants, Pi level is controlled and phosphate homeostasis is maintained by the regulatory network of Pi signaling pathways. Small RNAs: microRNA399 and microRNA827 play an important role in Pi level regulation. These microRNAs target barley mRNAs encoding PHOSPHATE2 (ubiquitin-conjugating E2 enzyme) in the case of miR399 and NLA (Nitrogen Limitation Adaptation, E3 enzyme in Arabidopsis) and SPX-MFS (implicated in Pi sensing or transport) in the case of miR827. We analyzed changes in small RNAs expression profiles in barley roots and shoots during Pi starvation. We performed degradome analysis to find a correlation between small RNAs level changes and sequences that could be recognized by small RNAs and next cut by Ago proteins. Firstly we selected small RNAs which level was significantly changed during Pi starvation (EDGE test: Bonferroni and FDR P-value correction). Then small RNA sequences were mapped to miRbase and to barley sequences deposited in Ensembl Plants database (release 37). We found that most of changes were detected for reads derived from non-translating RNAs and rRNA sequences in roots and tRNAs in shoots. We identified new sites within 5'UTR of *PHO2* mRNA which are recognized by microRNA399 as well as novel targets for microRNA399 and microRNA827. To identify the role of small RNAs in abiotic stress response we plan to introduce a small RNA sequence into the barley microRNA166 or microRNA167 precursor backbone sequence and transform barley using the construct via Agrobacterium transformation. Herein, we present the barley Pi-response network composed of small RNAs, their targets and protein products.

CHARACTERIZATION OF NOVEL MIRNAS IN BARLEY

A.SMOCZYŃSKA, A.PACAK, Z.SZWEYKOWSKA-KULIŃSKA

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

Hordeum vulgare, miRNA, drought response, degradome

MiRNAs are small ribonucleic acid molecules usually 21 nucleotides in length that takes crucial part in transcriptional and post-transcriptional regulation of gene expression when incorporated into multiprotein complex- RISC (RNA-induced silencing complex) in all biological processes occuring in plants.

Currently there are only 71 barley mature miRNAs annotated in MirBase, much less than in other crop species such as wheat (119 miRNAs), rice (713 miRNAs) or maize (321 miRNAs). That suggests a large portion of miRNA molecules still undetected therefore vast part of regulatory mechanisms unexplored. We decided to overcome these differences, identify and validate novel miRNAs in barley.

In order to complete this task we prepared and sequenced small RNA libraries from five barley developmental stages and extracted a ranking list of potentially new miRNAs in barley, which was then subjected to verification.

Using Northern blot we proved the presence of 9 new miRNAs, for which precursor structures have been identified. With 5' and 3' RACE we identified structure of miRNA- coding genes and using degradome data we determined their target mRNAs. Our data show the accumulation of miRNAs in 68th day of development (spike stage) and interesting changes in expression in particular flower organs such as stamen, which suggests involvement in the development and function of this organ.

We also analysed expression level of novel miRNAs in seven different stresses and found that 7 miRNAs are down-regulated upon drougth conditions which correlated with up-regulation of their targets.

Overall our data provide important information about the molecular mechanisms underlying the development of barley and its response to environmental stress expanding the current state of knowledge.

ARE BARLEY microRNA PRECURSORS FROM mIR444 FAMILY ASSOCIATED WITH RIBOSOMES?

A. GRABOWSKA, A. PACAK, Z. SZWEYKOWSKA-KULIŃSKA

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, *Adam Mickiewicz University in, Poznań, Poland*

Barley, microRNA, pri-miRNA, splicing

MicroRNAs from the *MIR444* family have been found exclusively in monocots. The exceptionally feature of the MIR444 gene structure is the presence of an intron that separates two halves of pre- miR444 structure and encodes one half containing miR444 in one exon while the another half containing miR444* is encoded in the neighbour exon. Based on data from NGS Illumina small RNA sequencing we deduced that there are three *MIR444* genes in barley genome. RACE and RT-PCR experiments show that all three pri-miRNAs undergo extensive alternative splicing generating multiple pri-miRNA444 isoforms which can be functional or non-functional (microRNA cannot be generated).

Bioinformatics analysis revealed the presence of short potential open reading frames (ORFs) in almost all of the pri-miRNAs444. Others previous studies revealed that plant pri-miRNAs encode regulatory peptides [Lauressergues et al., 2015]. That prompted us to analyse cytoplasmic fraction for the presence of pri-miRNA. We carried out polysome profiling and using RT-PCR we identified that both, non-functional and functional pri-miRNAs444 are associated with ribosomes. As a negative control we used U3 snoRNA and we did not find it to be associated with the ribosomes. As a positive control we used spliced CBP20 mRNA that is dominant in the cytoplasmic fraction. Our results show that we indetified splice isoforms from all *MIR444* family genes are exported from the nucleus to the cytoplasm and play yet unknown role in plant metabolism.

THE EFFECT OF LEAD AND *A. PISUM* ON EXPRESSION LEVELS OF PHENYLALANINE AMMONIALYASE AND CHALCONE SYNTHASE GENES IN PEA SEEDLINGS

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Lead, Acyrthosiphon pisum, flavonoid biosynthesis enzymes; defense responses, Pisum sativum

The aim of this study was to determine the effect of an abiotic factor, i.e., lead at various concentrations (low causing a hormesis effect and high causing the toxic effect), on the expression levels of genes encoding enzymes of the flavonoid biosynthesis pathway (phenylalanine ammonialyase and chalcone synthase in pea (*Pisum sativum* L. cv. Cysterski) seedlings and then during infestation by the pea aphid (*Acyrthosiphon pisum* Harris). Phenylalanine ammonialyase (PAL) is an enzyme initiating phenylpropanoid metabolism, and chalcone synthase (CHS) catalyses the first committed step in the flavonoid biosynthetic pathway. Semi-quantitative RT-PCR analysis revealed that pea aphid feeding alone (+aphids), lead administration at the high and low concentrations in the medium (0.5 mM Pb^{2+} and 0.075 mM Pb^{2+}) as well as the cross-talk of lead and *A. pisum* infestation (0.5mMPb^{2+}+aphids and 0.075mMPb^{2+}+ aphids) upregulated mRNA levels for PAL and CHS in relation to the control. However, these stress factors much more strongly upregulated CHS than PAL. Very high upregulation of mRNA for the CHS genes were observed as a result of the impact of lead or the cross-talk of lead and *A. pisum*, especially at the toxic concentration of lead. The obtained resultes indicate involment of these enzymes in regulation of flavonoids syntesis and in the defence strategy of pea against stresses.

13:30 Opening 2nd iPLANTA Conference

14:00 International Invited Lecture

MECHANISMS OF RNA-DIRECTED DNA METHYLATION

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RNA-directed DNA methylation is a conserved process where small RNAs target transposons and other sequences for silencing by establishing repressive chromatin modifications. A central element of this process is long noncoding RNA (lncRNA), which has been proposed to serve as a binding scaffold for small RNAs and associated proteins. In *Arabidopsis thaliana*, this lncRNA is produced by a specialized RNA polymerase known as Pol V. Pol V transcripts serve as a binding scaffold for several RNA-binding proteins, which mediate the recruitment of chromatin modifying enzymes. These enzymes contribute to the repression of transposon activity and help determine the boundaries between heterochromatic transposons and their euchromatic environment. RNA-directed DNA methylation has also been implicated in controlling organization of chromosomes, which may explain how it affects expression of distant genes. Overall, the existing knowledge about the mechanisms of RNA-directed DNA methylation provide strong support for non-coding transcription serving as a key factor controlling the structure of chromatin.

15:00 Open session 1 - RNAi Technology

VIRAL-LIKE PARTICLES BASED ON CYTOPLASMIC POLYHEDROSIS VIRUS FOR DELIVERY OF DSRNA IN INSECTS

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CPV, Silkworm, MultiBac, viral-like particles, dsRNA

Cytoplasmic polyhedrosis virus (CPV; *Cypovirus*, Reoviridae) provides an interesting platform for the engineering of viral-like particles capable to deliver dsRNA cargo in target cells. Detailed cryo-electron microscopy (cryo-EM) studies have provided great insights in the structure of the virions that provide the basis for the construction of viral-like particles (VLPs). Because CPV is characterized by a segmented dsRNA genome, CPV-based VLPs can be considered as attractive candidates for delivery of dsRNA fragments. For construction of VLPs, following structural proteins can be considered important: (1) capsid shell protein (CSP) that can spontaneously assemble into single-shelled VLPs; (2) turret protein (TP) that houses the mRNA capping complex; (3) A-spike, involved in cell attachment and penetration; and (4) large protruding protein (LPP) that functions as a clamp to stabilize the virion shell structure. The MultiBac baculovirus/insect cell expression system that was specifically designed to express large multiprotein complexes will be harnessed to generate VLPs of different structural protein composition. Characterization of VLPs will include assessment of stability to environmental conditions, capacity of cell entry and recognition by the innate immune system of the insect. A major challenge constitutes the loading of VLPs with dsRNA that will need further engineering of the MultiBac expression system. Engineering of CPV-based VLPs is part of our research efforts to develop new methods for delivery of RNAi triggers in insects with increased specificity and efficiency.

COMPARISON OF DIFFERENT METHODS FOR THE ESTABLISHMENT OF RNA SILENCING IN PLANTS

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Hairpin RNA, Agroinfiltration, Transient gene silencing

RNA interference (RNAi) has proven to be one of the most powerful tools for studying plant gene function and for the specific silencing of gene expression. It has been widely used for the generation of crops with improved pathogen or pest resistance and for metabolic modifications. For preliminary testing of multiple silencing constructs, silencing inducing dsRNA is often delivered transiently by means of agroinfiltration, a rapid method which is independent of plant regeneration. In order to get more insight into the applicability of transient gene silencing, we compared different transient and stable approaches to induce silencing of the ß-glucuronidase (*gus*) transgene by the expression of *gus* hairpin (*hpGus*) transcripts. While stable expression of a *gus* hairpin always resulted in specific siRNA mediated gene silencing, transiently expressed *hpGus* transcripts could only efficiently trigger silencing when the hairpin construct was introduced simultaneously with or prior to the target gene. This time dependence of expression of the silencing inducer was only partly explained by the stability of the GUS protein. Our work demonstrates the limitations of transient gene silencing approaches and is relevant for elucidating requirements for siRNA mediated gene silencing.

THE RNAi APPROACH TO STUDY BRASSINOSTEROID REGULATOR ENCODING GENES IN BARLEY

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Barley, *GSK3*, Shaggy-like kinase, gene annotation, *stress tolerance*

GLYCOGEN SYNTHASE KINASE3 (GSK3) is a highly conserved kinase present in all eukaryotes and functioning as a key regulator of a wide range of processes. The kinase, known in land plants as GSK3/SHAGGY-like kinase (GSK) is a key player in brassinosteroid (BR) signaling. The GSKs are encoded by multigene families and function as key regulators of diverse developmental processes and modulate responses for environmental stresses. Based on 10 Arabidopsis *GSK* genes and encoded proteins we identified 7 transcriptionally active *GSK* genes in barley, assigned them to four groups based on their evolutionary relationships and analyzed their expression profiles. For 2 genes we re-evaluated *GSK* annotation in the current release of barley genome. 5 of the identified genes *HvGSK1.1*, *HvGSK1.2*, *HvGSK2.1*, *HvGSK3.1* and *HvGSK4.1* representing the four phylogenic groups were selected for functional analysis. Using RNAi-based strategy, we obtained barley plants with a significantly lowered level of the target gene transcripts. Silenced expression of *HvSK1.1* was additionally associated with altered expression of four other barley *GSK* paralogs. The most pronounced phenotype in lines with lowered *HvGSK1.1* expression showed better growth of seedlings in normal and in salt stress conditions compared with the non-transgenic control. The trait was correlated with the silencing rate of the analyzed gene. Additional phenotypic changes observed in plants included wider leaf blades, shorter internodes, bigger kernels, longer spikes and altered leaf inclination angles and are in agreement with the traits known to be regulated by BRs. The experiments with the remaining four genes are in progress and the presentation will include the first results. The siRNA-induced silencing is a feasible strategy to get the knowledge about biological function of barley *GSK* genes and it also provides the basis for future strategies of crop improvement.

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THE ROLE OF THE U1 SNRNP PROTEIN, PRP40, IN PLANT MIRNA BIOGENESIS

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microRNA biogenesis, PRP40, SERRATE

MicroRNAs (miRNAs) are small noncoding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. Plant miRNA biogenesis takes place in the nucleus, and DCL1 (Dicer Like 1), HYL1 (HYPONASTIC LEAVES 1) and SE (SERRATE) are key factors responsible for miRNA production. Interestingly, SE is also involved in pre-mRNA splicing. Many miRNA genes (*MIR*s) contain introns that have to be spliced from primary miRNA precursors (pri-miRNAs) by the spliceosome. We have already shown that splicing of intron containing pri-miRNAs influences the expression levels of mature miRNAs and that in the communication between the spliceosome and the microprocessor the interaction between SE and U1 snRNP is involved. We identified four binding partners of SE among U1 snRNP auxiliary proteins: PRP39b, PRP40a, PRP40b and LUC7rl. The interplay between SE and PRP40 has been found to be particularly important for the plant development since triple (*se*/*prp40a*/*prp40b*) knockout Arabidopsis plants are embryo-lethal. The goal of the study was to understand the role of PRP40 in miRNA biogenesis and Arabidopsis development.

We have found downregulation of 50% of all Arabidopsis pri-miRNAs in the *prp40ab* mutant, suggesting a role of PRP40 in *MIR* transcription regulation. Interestingly we have also observed that SE is localized in RNAPII containing nuclear foci and forms a complex with PRP40b and CTD of RNAPII. It raises a question about the co-transcriptional character of pri-miRNA processing in plants, and a special role of SE/PRP40 interaction in this crosstalk. The molecular mechanism of the interplay between U1 snRNP and the microprocessor in plants and its role in miRNA biogenesis will be discussed.

This work was supported by the Foundation for Polish Science (grant no. START 2017 to A.S.) and KNOW RNA Research Centre in Poznan (No. 01/KNOW2/2014).

16:15 Open session 2 - RNAi Technology

SMALL RNA EFFECTORS IN HIGS AND SIGS APPROACHES

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Fusarium, HIGS, SIGS, CYP51, azole

Small RNA effectors play a crucial role in the outcome of host-pathogen interactions and have shown great potential for controlling pest and diseases in crops (Koch and Kogel 2014). Previously we have demonstrated that the 791 nt CYP3-dsRNA, which targets the two *sterol 14α-demethylase* genes *FgCYP51A* and *FgCYP51B* and the fungal virulence factor *FgCYP51C,* inhibits growth of the ascomycete *Fusarium graminearum in vitro* and *in planta* upon delivery through transgene expression (host-induced gene silencing, HIGS) or spray application (spray-induced gene silencing, SIGS) (Koch et al. 2013; 2016). Here we compare the efficiencies of different dsRNA delivery strategies to assess the activity of novel dsRNA species that were designed to target one or two *FgCYP51* genes. Using barley as a cereal model, we found that dsRNA constructs targeting two *FgCYP51* genes inhibited fungal growth more efficient than single constructs, although both types of dsRNAs decreased fungal infections. While inhibitory activity of single dsRNA constructs is unexpected and contrasts to earlier experiments showing that single *FgCYP51* deletions do not affect fungal development, our findings are attributable to co-silencing effects of CYP51-targeting dsRNAs on respective non-target *FgCYP51* genes. We also show that fungal sensitivity to dsRNAs in *in vitro* cultures is a good proxy for their activities in HIGS and SIGS setups.

Koch, A. & Kogel, K.-H. New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing. *Plant biotechnology journal* **12,** 821–831 (2014).

Koch, A. *et al.* Host-induced gene silencing of cytochrome P450 lanosterol C14alpha-demethylase-encoding genes confers strong resistance to Fusarium species. *Proceedings of the National Academy of Sciences of the United States of America* **110,** 19324–19329 (2013).

Koch, A. *et al.* An RNAi-Based Control of Fusarium graminearum Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal Silencing Machinery. *PLoS pathogens* **12,** e1005901 (2016).

EFFECTIVNESS OF THE ARTIFICIAL MICRORNA- AND SIRNA-BASED SPECIFIC GENE SILENCING OF AGRONOMICALLY IMPORTANT GENES, AND SI-RNA-MEDIATED IMPROVEMENT OF PRODUCTIVITY IN CEREALS

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Cereals, amiRNA, siRNA, gene silencing, agronomic improvement, wheat, barley

Gene silencing by RNA interference is a particularly important tool in the study of gene function of agronomically important polyploid (wheat, triticale) and diploid (barley) cereal species for which the collections of natural or induced mutants are very limited. In polyploid cereals we have studied effectiveness of two RNAi-based silencing pathways, namely siRNA (siR) and amiRNA (amiR), in investigations of grain hardness genes. For the purpose two amiR and two hpRNA cassettes were designed to silence *Puroindoline a* (*Pina*) and *Puroindoline b* (*Pinb*) hardness genes in wheat and their orthologues *Secaloindoline a* (*Sina*) and *Secaloindoline b* (*Sinb*) genes in triticale. Each of the two amiR cassettes contained 21 nt microR precursor derived from conserved regions of *Pina*/*Sina* or *Pinb*/*Sinb* genes, respectively. Silencing of the *Pin* genes in wheat and the *Sin* genes in triticale was highly efficient in the T_1 generation. The transcript level of both genes, independent on the pathways of silencing was reduced up to 92% in wheat and up to 98% (by amiR) in triticale. Moreover, the RNA-mediated silencing of one of the *Pin*/*Sin* genes simultaneously decreased the expression of the other. High down regulation of both *Pin* and *Sin* genes in T_1 plants of wheat and triticale was associated with strong expression of *Pinb*-derived amiR. Silencing of the target genes by siR and amiR correlated with increased grain hardness in both species. Although, the *Pinb*-derived amiR cassette was stably inherited in the T_2 generation of wheat and triticale the silencing effect (decreased expression of silenced genes, phenotype, other) completely in opposition to siR, was not transmitted.

In diploid barley and polyploid wheat silencing of *HvCKX* and *TaCKX* genes by siRNA was applied to study their function and exploring the possibility of improving the productivity.

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THE Mi AND Mi-SiRNA APPROACH TO CONTROL PLUM POX VIRUS GENOME IN PLUMS

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Plum-tree, silencing, RNAi, resistance

Fight against plum pox virus (PPV) disease is now well-known through genetic engineering of *Prunus domestica* (plum-tree). As documented in the literature, the HoneySweet plum is among the first clone well-characterized (Ravelonandro et al. 1997, Scorza et al. 2016). The use of new gene constructs built for tackling either PPV genome translation via miRNA or PPV genome replication via siRNA was reported here. Following to the introduction of these constructs into plum genome, we showed the results of 24 clones. These plants were respectively produced via the pHellsgate-miCPRNA and pHellsgate-misiCPRNA plant transformation vectors. Following to the preliminary characterization, 15 clones accumulated miRNA and 9 transcribing mi-and siRNAs. Plants were vegetatively propagated onto the *Prunus marianna* GF8-1 rootstocks prior to challenging to graft-infection with PPV. Utilizing the dormancy cycle as the seasonal changes that led buds to become functional after bud-breaking, virus movement were followed with shoot elongation. Moving through the vascular system from roots to shoots, PPV was monitored through symptom observations, serological and molecular analyses. Any uninfected scions were re-infected. Over four dormancy cycles, the majority of miCPRNA plums were infected and some recovered. When referred to misiCPRNA clones, 2/9 recovered. Two clones misiCP-RNA-3 and -4 could show PPV infection then rapidly recovered. Interestingly, when compared to HoneySweet plum, the scions of misiCPRNA-7 plum remained uninfected. This is an unexpected observation because PPV is widely spread in the rootstocks. At this stage, the potential application of the mi-siCPRNA-7 plum enabled us to claim the robust silencing deployed by this clone. Among the future studies to assess was the relationship between plant responses to virus infection and RNAi distribution. On the basis of these interactions, we will asses how mi-siRNA interact with PPV genome (timing, dose-effect…)?

Ravelonandro,M., et al. 1997. Resistance of Prunus domestica L. to plum pox virus infection. Plant Disease 81, 1231-1235.

Scorza R., et al. 2016. HoneySweet (C5) the first genetically engineered plum pox virus-resistant plum (Prunus domestica L.) cultivar. Hortscience, 51 (5) 601-603.

TESTING THE ACTIVITY OF DOUBLE-STRANDED RNAS AGAINST COLORADO POTATO BEETLE

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Leptinotarsa decemlineata, double-stranded RNAs (dsRNAs), target genes, gene expression

Colorado potato beetle (*Leptinotarsa decemlineata*; CPB) is a serious pest of Solanaceous crops such as potato, tomato and eggplant. The possibility to effectively silence CPB genes by feeding larvae with long dsRNAs makes this beetle a convenient candidate for the study of RNA interference mechanisms and development of RNAi-based pest management treatments.

In order to identify new CPB pest management targets, we designed dsRNAs against three genes expressed in its gut and tested their insecticidal activity in feeding trials. For these trials, *in-vitro* synthetized dsRNAs were sprayed onto potato leaves and used to feed the beetles. We followed beetle survival, weight gain and development and measured target gene knockdown by qPCR. We show that the efficiency of target knockdown differs between the targets and how this correlates with dsRNA's insecticidal activity. Additionally, we are aiming at profiling the non-sequence specific transcriptional response to dsRNAs in CPB using RNA sequencing.

MODULATING SEED DEVELOPMENT IN COMMON BEAN (PHASEOLUS VULGARIS): AN APPROACH TO ENHANCE SEED QUALITY?

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candidate genes, common bean, functional validation, seed development, seed quality

Common bean (*Phaseolus vulgaris*) is an important staple food worldwide, being the most consumed grain legume in Portugal. Despite the agronomic and economic importance of this pulse, the knowledge on common bean seed development (SD) is scarce but needed to improve traits related to nutritional value and seed yield. During the last years, we have been investigating the molecular mechanisms underlying SD in common bean by using an integrative Omics approach. Proteomic and coding transcriptomics profiling studies were conducted on developing seeds at 10, 20, 30 and 40 days after anthesis, spanning from late embryogenesis until seed dehydration. These combined approaches unveiled a set of metabolic pathways differentially activated during the timeframe of SD. As an example, transcriptomics unveiled that DNA damage sensing and repair are crucial during early SD stages, when cell division and differentiation occur. On the other hand, maintenance of chromatin structure during dehydration seems to be essential to maintain the viability of the desiccated seed. The proteomics study demonstrated an accumulation of proteins associated with storage, signaling, starch synthesis and cell wall was observed mid SD, when seed filling and maturation occurs. Candidate genes with a potential role in driving these biological processes were retrieved and can be used to design legume seeds with beneficial quality and yield traits. Our challenge now is to validate their function *in planta* using, as example, RNA silencing approaches. Future directions on this challenge will be discussed.

DAY 2 - THURSDAY FEBRUARY 15TH, 2018

9:00 Open session 3 - RNAi Technology

DECIPHERING THE MOLECULAR POSTCODES IN EPIGENETIC GENE SILENCING

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Short non-coding RNA molecules (sRNAs) play a fundamental role in gene regulation and development in higher organisms. sRNAs act as molecular postcodes and guide proteins to target specific RNA and DNA molecules. In plants, sRNA-targeted mRNAs are destroyed, reducing gene expression. In contrast, sRNA-targeted DNA sequences undergo a specific modification, involving addition of methyl groups to cytosine bases in DNA. Cytosine methylation can be inherited but does not change the sequence of the DNA, therefore it is referred to as an epigenetic modification. However, cytosine methylation can suppress transcription, thus sRNAs are potent regulators of gene expression. Recent experiments further demonstrated that sRNAs are mobile and can induce epigenetic modifications in distant parts of plants, including the cells from which flowers and subsequently seeds are produced. Therefore mobile sRNAs can initiate epigenetic changes through the plant that might influence development, stress-response and immunity and that can be passed on to subsequent generations. Knowing how sRNAs function is therefore fundamental to our understanding how plant gene expression is regulated. It is particularly relevant to development, which is known to involve sRNAs, and to hybrid vigour, in which sRNAs have also been implicated. It would also open up the possibility of using sRNAs to change gene expression heritably without changing DNA sequences. I will discuss how sRNAs can find their target DNA to induce epigenetic gene silencing.

NEW PROTEIN FACTORS INVOLVED IN PLANT NMD

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NMD, UPF1, new NMD interactors, RNA quality control

Nonsense-mediated mRNA decay (NMD) is a conserved mRNA surveillance mechanism that prevents the production of potentially harmful proteins by eliminating aberrant mRNAs carrying premature translation termination codons (PTC) that are often generated by alternative splicing. The key NMD effectors, ATP-dependent RNA helicase UPF1 together with UPF2 and UPF3 form a core of the NMD complex in all organisms. In addition, SMG1, SMG5-9, ribosome, the exon-exon junction complex (EJC) and eukaryotic release factors ERF1 and ERF3A were shown to be involved in degradation of nonsense mRNA in vertebrates. Extensive studies in yeast, *C.elegans*, flies and mammals established a whole set of additional and auxiliary NMD components. Most of these proteins, including RNA helicases, subunits of eukaryotic initiation factor 3 (eIF3), transcription-export (TREX) complex and nucleus-associated RNA-binding proteins, are conserved and essential for growth. In contrast, only a few major players, including UPF1-3 proteins, SMG7 and EJC components, were identified in plants. To identify new plant NMD factors we have analysed UPF1-interacting proteins by affinity purification using a transgenic Arabidopsis line expressing tagged UPF1. Besides UPF2 and UPF3 we have identified several proteins including ribosomal and RNA-binding proteins, splicing factors, RNA helicases, subunits of eukaryotic initiation factor 3 and 4, and proteins involved in nuclear transport and proteolysis. To investigate the NMD-related function of the best and most interesting candidates we have applied the VIGS approach (Virus-Induced Gene Silencing agroinfiltration transient NMD assay), which allows for quick and efficient testing of many potential NMD factors using transient transfection of *N. benthamiana* leaves. I will present the results of these proteomic and functional analyses.

PLAYERS AND MECHANISMS IN ANTIVIRAL PATTERN-TRIGGERED IMMUNITY IN PLANTS

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double-stranded RNA, pathogen-associated molecular pattern (PAMP), co-receptor kinase, signaling network, Arabidopsis thaliana

In search of factors influencing resistance and susceptibility to viruses we recently showed that virus infection in plants is restricted by pattern-triggered immunity (PTI), a defense response, which relies on the perception of conserved pathogen associated molecular patterns (PAMPs). Furthermore, we found that double-stranded (ds)RNAs act as elicitors to induce PTI responses in the model plant *Arabidopsis thaliana* dependent on the co-receptor kinase SERK1 and that treatment with the synthetic dsRNA analogue poly(I:C) restricts virus infection in plants. This sequence-unspecific plant defense response appears to be independent of RNA silencing and may act in addition to this sequence-specific antiviral defense mechanism. First dissection of the signaling cascade induced upon dsRNA treatment revealed that dsRNA-mediated PTI involves the typical, PTI-related mitogen activated protein kinases MPK6 and MPK3, the induction of ethylene production, induction of typical PTI-related gene expression, but also high-amplitude induction of expression of salicylic acid- and jasmonic acid-dependent defense genes (e.g. EDS5 and LOX3). However, dsRNA-recognition does not seem to induce the production of significant levels of hydrogen peroxide. dsRNA sensitivity varies among Arabidopsis ecotypes, and evidence exists that dsRNA sensitivity is conserved in different plant species, as we obtained typical PTI responses upon treatment of *N. benthamiana* plants with dsRNA or the synthetic dsRNA analogue poly(I:C). By further elucidating the molecular mechanisms underlying dsRNA-mediated antiviral PTI and its suppression by viral effectors, we expect to gain valuable insight into this newly identified antiviral immune response. Moreover, application of dsRNA to crops for the induction of broad-spectrum antiviral resistance may present an attractive addition to conventional crop protection strategies.

References:

Kørner, C. J., et al. (2013). "The immunity regulator BAK1 contributes to resistance against diverse RNA viruses." Molecular Plant Microbe Interactions **26**(11): 1271-1280.

Niehl, A., et al. (2016). "Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants."New Phytologist **211**(3): 1008-1019.

ASSESSING SILENCING EFFECTIVENESS AND SPECIFICITY AMONG RAPID ALKALINIZATION FACTOR (RALF) FAMILY GENES FOR *Fragaria vesca* RNAi PLANTS

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Strawberry, genetic transformation, RALF, gene silencing,

Rapid alkalinization factor (RALF) genes code for ubiquitous small peptides that are involved in multiple aspects of plant physiology. *Arabidopsis thaliana* has thirty seven RALF genes, while in the diploid woodland strawberry (*Fragaria vesca*) only nine genes were found. Generally, they are synthesized as propeptide and subsequently activated by protease cleavage. The resulting mature peptides share a highly similar sequence. RALF, binding to its receptor FERONIA, increases apoplast alkalinization, affecting cell growth and the fertilization process. Furthermore, RALF peptides may act as a negative regulator of the immune plant response, inhibiting the formation of the signal receptor complex for immune activation. Recently RALF homologues were identified in different fungal pathogen genomes, where they play a role in host infection ability. Furthermore, it was observed, in *Arabidopsis* and in tomato, an overexpression of some RALF genes upon pathogen infection. Overall, RALF genes appear to be crucial during plant infection process. In order to study plant-pathogen interactions and discover novel susceptibility genes, three lines of RNAi transgenic plants were developed for silencing of one RALF gene in *Fragaria vesca* genotype Hawaii 4. The aim of this study is to assess the effectiveness of RNAi silencing in a gene family with members sharing highly similar sequence, and to evaluate the impact and the consequences of the off-target effects. This knowledge is necessary to possibly use RALF gene silencing mechanisms to decrease strawberry susceptibility to fruit diseases.

EXPRESSION OF CANDIDATE GENES RESPONSIBLE FOR BUD ABSCISSION IN PISTACHIO

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Pistachio, alternate bearing, bud abscission

Alternate bearing is a very widespread phenomenon occurring in both deciduous and evergreen trees. Alternate bearing konwn also irregular bearing is the phenomenon by which trees bear an irregular crop year after year, usually heavy yields ("ON" year) which are followed by light ("OFF") ones. Generally, the presence of fruit is the key factor controlling flower initiation. Bud abscission caused by alternate bearing phenomena is one of the most important problem in pistachio also. In Turkey, due to the abscission of inflorescence buds alternate bearing occur especially commercially grown "Uzun" pistachio variety severely. In "Uzun" pistachio variety fruit production fluctuates between an 'on' year of high yields and an 'off' year of low yields. As far as we know there are very limited papers previously published on histological analysis on pistachio related to flower bud abscission. Therefore, in this study it was aimed to detect begining of the flower bud abscission time on "Uzun" pistachio by scanning electron microscope (SEM). Based on SEM results, RNA-seq was performed in the buds from ON and OFF year trees at 10 days intervals. Candidate genes responsible for bud abscission in pistachio were determined and their expression analysis were performed during two concecutive years at different organs such as leaf, nut, kernel, bud and shoot. The results will be given in the meeting.

11:00 Open session - 4 RNAi Applications issues

SPEAKING UP FOR SILENCED GENES: CAUGHT IN THE CROSS TALK BETWEEN RNAI AND EDITING

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RNAi, gene silencing, CRISPR Cas-9, genome editing, phenocopy, biotechnology, GMO

There are many challenges to maintain a secure, affordable, and nutritious food supply over the next fifty years and plant breeding will surely play a part in the solution. The development of new crop varieties with higher yields, improved nutritional or functional qualities and better resistance to pests and diseases has been a key part of agriculture for centuries. However, plant breeding is currently experiencing a revolution driven by the availability of new genomic data and the ability to genetically transform, edit and culture plant cells *in vitro*.
Conventional *in planta* RNAi silencing approaches that target the expression of specific plant genes are ubiquitous in molecular genetic research and have already been applied in many commercial GM crops currently on the market. For example, Arctic Apples developed by Okanagan depend on RNAi silencing to lower the levels of polyphenol oxidase (the enzyme that cause browning when cut). The soy varieties, Vistive Gold (Monsanto) and Plenish (DuPont-Pioneer) use a gene silencing effect to make high oleic and lower linolenic oil that the companies claim result in better heat stability for frying, longer fry life and improved flavour of fried products. Innate™ potatoes made by the J.R. Simplot Company have reduced free asparagine, a lower content of reducing sugars and a non-browning phenotype due to RNAi targeting the expression of four genes; asparagine synthetase-1, polyphenol oxidase-5, potato phosphorylase, and the starch-associated R1 gene.
However, these advantageous phenotypes can also be readily achieved using the newer technologies of genome editing which have the significant advantage of not being captured by GMO legislations in many regions of the world. Alternatively there are other applications of RNAi that genome editing cannot readily replace. I will discuss this technology crosstalk and identify the applications where the future RNAi is likely to be limited, and others where it is set to make major breakthroughs in future agriculture.

SILENCING OF AGROBACTERIUM TUMEFACIENS ONCOGENES WITH A SHORT-LENGTH SINGLE CHIMERICAL TRANSGENE INDUCES RESISTANCE TO CROWN GALL DISEASE IN PLUM BUT NOT IN APRICOT

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Crown gall, iaaM, ipt, siRNA, 6b

A short chimeric transgene with self-complementary sequences from conserved oncogenes fragments was tested transforming tobacco. The silencing response was triggered in most T0 transgenic tobacco lines and T1 seedlings, evaluated *in vitro* or in the greenhouse, which had intermediate or very low susceptibility to *A. tumefaciens* as compared with the wild type plants. Low levels of transgene hairpin RNA (hpRNA) coupled with small interfering RNA (siRNA) accumulation correlated with oncogene silencing and, therefore, resistance to crown gall. Also, much lower levels of the oncogenes' mRNA were found in resistant lines than in wild type plants after infection. However, resistant plants infected with the oncogenic A281 strain only showed a slight reduction in symptoms. This was related to a slightly lower homology of the *ipt* gene of A281 with the consensus fragment and to a specific distribution of mismatches. Therefore, a specific fragment with high homology to A281 *ipt* was later added to the transgene. Then, transgenic plum and apricot lines were produced and evaluated *in vitro* to identify susceptible lines and to reduce the number of lines to be evaluated in the greenhouse. Five transgenic plum lines, expressing transgene-derived small interfering RNA (siRNA) and low levels of transgene hairpin RNA (hpRNA), showed a significant reduction in the development of the disease after infection with *Agrobacterium* strains C58 and A281 under greenhouse conditions. However, unexpectedly, all transgenic apricot lines were susceptible. The infection of apricot plants with a binary vector containing only the 6b oncogene demonstrated that the expression of this gene is involved in the induction of tumors in the apricot species. RNAi-mediated gene silencing can be used for inducing crown gall resistance in plum rootstocks. These could be used to graft non-genetically modified fruit cultivars reducing, or eliminating, the disease symptoms.

USE OF RNAI TOOLS TO INCREASE BARLEY'S RESISTANCE TO BIOTIC STRESS

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Hordeum vulgare, biotic stress, disease resistance, hdRNAi, gene silencing

RNA interference (RNAi) is a sequence-specific, double stranded RNA (dsRNA) induced gene-silencing mechanism that operates at transcriptional or post-transcriptional levels by inducing degeneration in the chain sequence of particular target messenger RNA in the cytoplasm. Since the RNAi mechanism was first demonstrated in *Caenorhabditis elegans* in 1998, RNAi has emerged as a potent gene-silencing tool for loss-of-function analyses in a wide range of organisms.

Three RNAi based concepts have potential applications in plant functional genomics and agriculture. These concepts are tissue specific silencing, inducible silencing and host delivered RNAi (hdRNAi) during plant-pest interaction. Tissue specific promoters driving RNAi constructs can induce gene silencing in a particular organelle or tissue. Also, RNAi constructs with stress or chemical inducible promoters can be used to induce gene. In the hdRNAi, dsRNA generated in an RNAi transgenic plant is delivered to interacting target organism (pest), activating gene silencing in the target organism. RNAi as a reverse genetics approach has been successfully applied to identify function of genes involved in biotic stresses in *Medicago*, *Arabidopsis*, maize and other crop species.

In the light of the information already available from other species, we will use the RNAi tools on *Hordeum vulgare* (barley) to exploit plant disease resistance by knock-down the susceptible genes which cause the expression of novel genes for stress resistance. We propose to test several RNAi constructs with one or two promoters: (i) constructs with one promoter regulating a sense and antisense sequence of the target gene separated by a spacer/intron region, and (ii) constructs with 2 promoters in inverse orientation flanking a DNA sequence of the target gene which showed very good results in plants. The cloned vector will be transferred into *H. vulgare* embryos via *Agrobacterium* transfection, followed by *in vitro* culture. The obtained plantlets will be tested under controlled environmental conditions in our laboratory and growth chamber.

The expected result will be to develop transgenic barley lines that display biotic and/or abiotic resistance under particular agro-ecological conditions relevant for Eastern Europe, and Romania especially.

RNA INTERFERENCE AND GENE EDITING – COMPLEMENTING OR MERGING IN ACTION

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RNA interference, gene editing, transformation, cloning

Employment of the intrinsic cell system of RNA interference opened a new chapter in functional genetics research with immense impact on current understanding of gene functions and related properties. RNA interference mechanisms offered not only targeted RNA inactivation but also targeted modulation of gene expression through epigenetic regulation.

On the other hand, several approaches for gene editing (mainly CRISPR/Cas but not limited to) now offer similar capabilities in terms of gene regulation through chromatin remodeling.

Both systems share similar experimental designs but also have a number of differences: i.e. while RNAi depends on intrinsic cellular elements, CRISPR/Cas requires external protein for its function. Despite these key differences, there might be an opportunity for RNAi system to adopt some features of CRISPR/Cas.

In this work we are making an attempt to outline the possibilities to expand the area of applicability for RNAi.

14:00 International Invited Lecture

CROSS KINGDOM RNAI AND SPRAY-INDUCED GENE SILENCING FOR CROP PROTECTION

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Cross-kingdom RNAi, Spray induced gene silencing

Small RNAs (sRNAs) play a critical role in both host innate immunity and pathogen virulence. We have demonstrated that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, the fungal pathogen that causes grey mold disease on more than 1000 plant species, are translocated into host plant cells and suppress host immunity genes for successful infection. These sRNAs act as a novel class of pathogen effectors that translocate into host cells to suppress host immunity.

Conversely, we also found that some plant-derived sRNAs, including both exogenous and endogenous sRNAs, are transferred into interacting fungal cells. Transgenic plants expressing hairpin RNAs that targeting *Botrytis* Dicer 1 and Dicer 2 genes could effectively silence fungal DCL genes, and inhibit the generation of fungal sRNA effectors to suppress grey mold disease. This strategy can be easily designed to control multiple fungal diseases simultaneously. We also identified a set of host endogenous sRNAs that are delivered into fungal cells, mainly through extracellular vesicles, to suppress fungal genes that are involved in pathogenicity. Strikingly, we discovered that some fungal pathogens, such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*. could take up double-stranded RNAs and sRNAs from the environment. Applying sRNAs or dsRNAs that target fungal *Dicers* and pathogenicity-related genes on the surface of fruits, vegetables and flowers significantly inhibits fungal diseases. Such pathogen gene-targeting RNAs represent a new generation of effective and eco-friendly fungicides.

14:45 Open session 5 - RNAi Applications

SMALL-ANGLE X-RAY SCATTERING STUDY OF THE OLIGOMERIC STATE AND DIMER STRUCTURE OF THE SERRATE$_{194-579}$ PROTEIN IN SOLUTION – A KEY PLAYER IN THE PROCESS OF miRNA BIOGENESIS IN *ARABIDOPSIS*

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MicroRNA, SAXS, protein, structure, Arabidopsis

The SERRATE (SE) protein is a crucial component of the molecular machinery processing miRNA precursors in *Arabidopsis thaliana*. It was found to significantly improve the accuracy of miRNA excision from its precursor. The fragment consisting of amino acid residues 194-579 of the protein ($SE_{194-579}$) was shown to be sufficient to restore the wild type-like levels of miRNAs in SE-deficient *Arabidopsis* plants and to bind the precursor of miRNA-164c (pre-miRNA). Crystallographic structure of the shorter fragment ($SE_{194-543}$) adopts a walking man-like shape, with the C-terminal zinc finger and N-terminal domains standing for the „legs" attached to the middle domain. We examined the structure of recombinant $SE_{194-579}$ protein in solution by the small-angle X-ray scattering (SAXS). Based on the SAXS data, a molecular mass of $SE_{194-579}$ protein was estimated, pointing that it existed mainly in a dimeric state. On the basis of SAXS data, a modelling of the structure was performed. The crystal structure of $SE_{194-543}$ was used as a constraint. First, the loops and the C-terminal tail, missing from the crystal structure, were modelled by the Modeller software. Next, low-energy structures were generated by a molecular dynamics simulation using the AllosMod tool. Each of the structures was separately provided to the FoXSDock tool. FoXSDock models the dimer structure, simultaneously fitting it to the experimental SAXS data, taking into account the contribution from the monomer. The dimer with the best score and the corresponding monomer were subjected to the OLIGOMER algorithm, assessing fraction of the dimer in a mixture at about 64%. The best dimer model has an asymmetric structure. The main monomer-monomer contacts are provided by the N-terminal domain of one monomer and the first loop of the middle domain of the other.

IDENTIFICATION OF HIGHLY EFFECTIVE SMALL RNAS FOR ANTIVIRAL PLANT PROTECTION

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Infections with plant viruses cause major, worldwide crop losses. Recently, the situation has considerably aggravated due to the increasing global trade and the climate change, which accelerate the emergence of novel types of viruses and the insect-mediated virus spread. Agrochemicals that are currently applied for insect control may result in environmental degradation and human hazards, and traditional as well as genetic engineering-driven breeding procedures to generate virus-resistant plants are time consuming and do not guarantee protection. Towards the development of sustainable alternatives, we present a new procedure that is expected to substantially improve the use of RNA-based antiviral crop protection. It considers earlier findings that from a multitude of siRNAs, which are generated from viral RNAs during the plant's RNA silencing immune response, only a few are functional (1). Accordingly, we developed a screen that enables a reliable identification of *highly effective antiviral siRNAs* (*hesiRNAs*), *i.e.*, virus-derived siRNAs that act effectively antivirally. The identification of *hesiRNAs* essentially relies on an *in vitro* system of cytoplasmic extract that is prepared from cultured plant cells (*Nicotiana tabacum* BY2). These 'BY2-lysates, BYL' reproduce the replication of various (+)-strand RNA viruses. Moreover, the system recapitulates the plant's primary RNA silencing response. As in a natural infection, BYL-endogenous Dicer like proteins (DCLs) process double-stranded (ds) RNA elements into siRNAs. If the BYL is further supplemented with a defined Argonaute (AGO) protein, this AGO incorporates siRNA-guide strands and, as part of RNA-induced silencing complexes (RISC), may inhibit viral replication by endonucleolytic slicing (1). Notably, the BYL-system enables the testing of a whole pool of endogenously generated siRNAs but also of exogenously added, synthetic siRNAs. By applying a *hesiRNA* screen to the (+)-strand RNA virus *Tomato bushy stunt virus* (*TBSV*), a concise number of virus derived siRNAs was identified that support AGO-catalyzed degradation of the *TBSV* target RNA at excellent efficacy. Most importantly, in a *proof of concept* study, *in vitro* identified *hesiRNAs* were applied to plants in vaccination approaches and showed an effective and long lasting protection against challenge with the cognate virus. Current aims involve to establish procedures that enable the mass production of *hesiRNAs* and a non-transgenic application of these RNAs to plants.

(1) Schuck J, Gursinsky T, Pantaleo V, Burgyán J, Behrens SE. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 2013 May;41(9):5090-103.

THE BALANCE BETWEEN FUMARATE AND MALATE PLAYS AN IMPORTANT ROLE IN PLANT DEVELOPMENT AND POSTHARVEST QUALITY IN TOMATO FRUIT.

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Organic acids, produced as intermediates of the tricarboxylic cycle, play a crucial role in the plant primary metabolism and are considered as being ones of the most important quality traits in edible fruits. Even if they are key metabolites in a multitude of cellular functions, little is known about their physiological relevance and regulation. Transgenic tomato (*Solanum lycopersicum*) plants expressing constitutively a bacterial maleate isomerase, which converts reversibly maleate to fumarate, were generated in order to improve our knowledge about the role of organic acids in the crop and fruit metabolism. Growth and reproduction were affected by the unbalance of tricarboxylic cycle intermediates, as a dwarf phenotype and a flowering delay were observed in the transgenic plants. In addition, a delay in chlorophyll synthesis, a decrease in the numbers of stomata and significant changes in some photosynthetic parameters indicated alterations in central primary metabolism. Postharvest was also impaired, as transgenic fruits showed increased water lost and deterioration, indicating a possible role of the organic acids in cell wall metabolism. Finally, preliminary metabolomics analysis pointed out important changes during fruit ripening in flavor-related metabolites, such as acids and sugars, revealing the importance of organic acids in fruit metabolism. Taken together, these data indicate a pivotal role of tricarboxylic cycle intermediates, such as malate or fumarate, as regulatory metabolites. Besides their role in quality fruit characteristics, they are involved in a multitude of functions including growth and photosynthesis.

15:45 Open session 6 - Biosafety issues

THE CURRENT REGULATORY ENVIRONMENT FOR RNAI PLANTS IN THE EU

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RNAi, new breeding techniques, authorisation process

Discussion is ongoing throughout the EU as to whether organisms developed with the help of so-called "new plant breeding technologies" fall under the scope of the current regulation of GMO and products thereof. Various methods of applying RNAi technology in plants are also discussed in this context.

Although the stable expression of dsRNA generally requires the use of recombinant DNA techniques, resulting in organisms that fall within current EU-legislation for GMO and products thereof, there are applications conceivable were this assumption may be challenged. Besides classical genetic modification, cisgenic or intragenic plants, grafting on GM-rootstocks or intermediate GMOs could be considered to utilize RNAi effects.

The decision for an organism to be regarded as GMO or a product to be regarded as produced from GMO requires detailed knowledge of the process used for its production and the molecular genetics of the final product.

Equally important is a careful interpretation of the legal basis, Directive 2001/18/EC. In this context the wording of the definition of GMO needs to be scrutinized. In addition, the intentions of the law need to be identified, as the technologies under discussion were not known or anticipated at the time of law-making.

For this reason it is important to consider legal aspects from the very beginning when developing RNAi plants. An overview over decisive points within the ongoing discussion will be presented.

CHALLENGES FOR THE AUTHORISATION OF SPRAYABLE RNAI BASED PLANT PROTECTION PRODUCTS

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RNAi, sprayable plant protection products, authorisation process

RNA interference (RNAi) is a means of reducing or switching-off the expression of individual genes, often described as 'gene silencing'. RNAi is a natural process with important defence and regulatory functions in animals, plants and fungi. RNA technology is widely used in GM plants. Prominent examples are virus resistances, e.g. in squash, papaya or plum, quality traits, e.g. in potato and apple, oil composition of soybeans, and pest regulation of the western corn root worm.

Additionally, sprayable RNAi based plant protection products are in the pipeline aiming at different targets such as, flea beetles in oil seed rape, or fusarium diseases in barley or weed control to overcome resistant weeds.

RNAi is a new mode of action in "conventional plant protection products". This might challenge the risk assessment and risk management. For some aspects/areas, the characteristics of RNAi as active ingredient needs adaptions of existing or the development of new risk assessment tools. For other parts, it might on the other hand ease the risk assessment. Additionally, new formulations to resist degradation of RNAi such as liquid encapsulation, conjunction with polymers or nanoparticles might challenge risk assessments.

The presentation will introduce the new challenges, identify similarities and differences in risk assessment of biotechnical and classical plant protection products, and discuss how these challenges might be considered in the authorisation process of sprayable RNAi plant protection products.

BIOSAFETY ASSESSMENT OF CURRENT RNAi APPLICATIONS: CASE STUDY OF INSECT RESISTANT MON 87411 MAIZE

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Risk assessment, insect resistance, biosafety, regulation, dsRNA

RNA interference (RNAi) is a powerful technique to specifically shut-down gene expression, which has been used for the generation of genetically modified (GM) crop plants with improved metabolic traits as well as for pest and pathogen resistance. Several RNAi based developments have already been approved for cultivation and food/feed use in the US and other non-EU countries. One of these RNAi crops, maize MON 87411 with increased corn rootworm resistance, is currently under risk assessment by EFSA. Data delivered by the applicant for safety evaluation by the US Federal Drug Administration (FDA) and for determination of Non-regulated status of MON 87411 maize by USDA-APHIS will be considered in relation to EFSA requirements for GM plants to be placed on the market. The presentation will focus on the question whether there are specific biosafety issues connected to RNAi plants which might not be captured by the current EFSA risk assessment standards.

LITERATURE REVIEW OF BASELINE INFORMATION ON RNAI THAT COULD SUPPORT THE ENVIRONMENTAL RISK ASSESSMENT OF RNAI-BASED GM PLANTS

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In the context of an EFSA tender on the novel RNAi-based pest control technologies, a systematic literature search was executed to collect all available peer-reviewed publications reporting on RNAi in invertebrate species belonging to Nematoda, Arthropoda, Mollusca and Annelida. Our search resulted in a total of 5,075 publications after elimination of duplicate studies, non-peer reviewed studies and studies deemed irrelevant based on the inclusion and exclusion criteria. Based on this database of studies, an overview was distilled of all studies involving oral delivery of sRNAs to these invertebrate studies. This overview includes information on tested species, life stage, sRNA molecule type, target gene, concentrations used, outcomes, etc. Furthermore, we have written several narrative reviews on different topics such as environmental and cellular uptake of sRNAs, RNAi efficiency and factors involved in sensitivity, possible exposure routes of (non-)target organisms to GM-produced sRNAs, potential unintended effects by sRNAs on invertebrate species in the agroecosystem and also on the availability and use of genomic data in risk assessment of RNAi-based GM crops. Here, we present an overview of the literature search and the conclusions of the narrative reviews.

DESIGNING FOOD SAFETY ASSESSMENT APPROACHES FOR RNAI –MODIFIED PLANTS AND DERIVED PRODUCTS

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RNAi-mediated food crops, food safety and nutritional assessment strategies, targeted compositional analysis, genomic profiling, transient gene expression, phyto- nutritional improvement of food crops, spraying of RNAi containing nanoparticles as insecticidal use, adaptation of current GM safety /nutritional guidance.

RNAi Interference (RNAi) is a post-transcriptional process in eukaryotes that leads to specific *gene silencing* through degradation of target mRNA. It may also provide broad spectrum resistance against plant pathogens. This contribution is focussed on the design of strategies for the safety and nutritional assessment of RNAi-plants and derived foods and feed.

Risk assessment strategies for foods derived from modern biotechnology have been designed by various international organizations like WHO/FAO, OECD and EFSA. The risk assessment is *comparative* and based on characterization of the properties of the genetically modified (GM) food crop and appropriate non-modified counterpart(s). Focus of the assessment is on the characterization of newly inserted protein(s) and other identified differences with respect to their safety and nutritional impact. Specific attention is paid to possibly occurring *unintended* alterations in the GM food crop.

The safety assessment strategy designed for GM plants, is also applicable to RNAi-mediated plants and derived products. While no new protein(s) are expressed, particular attention should be paid to the possible occurrence of off-target alterations in the composition of the RNAi modified plants, which might negatively impact the safety/nutritional profile for humans/animals . To this end new genomic profiling technologies will be applied..

Several new RNAi applications with food crops will be discussed like (i) (transient) genomic introduction of RNAi's for improvement of the phyto-nutritional content of crops, (ii) control of mycotoxin contamination in RNAi modified plants, and (iii) the use of RNAi-nanoparticles for spraying of plants against plant pathogens. Furthermore possible adaptations of current safety/nutritional assessment strategies for RNAi modified plants will be proposed.

17:45 Open session 7 - STMS

ONE YEAR OF SHORT TERM SCIENTIFIC MISSION IN THE FRAME OF THE COST ACTION 15223: DEVELOPING SYNERGIES, COMPETENCES AND SKILLS OM RNAI

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Mobility, STSM

Short Term Scientific Missions (STSM) are exchange visits aimed to strengthen the scientific objectives of the Cost Action, by supporting inter-lab exchange visits of young scientists in/between COST countries. STSM aims at consolidating the existing networks and to create new network between institutions or laboratories of Action members in other COST countries (e.g. undertake research, to draft proposals and articles, etc.), facilitate the transfer of knowledge, training in new techniques, the use of important new equipment, and experimentation in the lab and field., and finally to foster personal connections and provide opportunities for future career development of the Early Career Investigators researchers (ESR). During the 2nd Grant Period, three calls for STSM grants were opened. Eleven applications from 8 countries were granted during this Grant Period - Poland (2), Italy (2); Greece (2), Spain (1), Serbia (1), Romania (1), Finland (1), Belgium (1). The participants had the opportunity to develop their skills and competences in different laboratories located in France Denmark, UK, Belgium, Italy and Israel. For the 3rd Grant Period of the Cost Action 15233, two new calls are expected to be opened. For rules and procedures how to apply for STSM grants, please consult the iPlanta website, at http://iplanta.univpm.it/sites/iplanta.univpm.it/files/STSM/STSM%20rules%20x%20web.pdf.

DAY 3- FRIDAY FEBRUARY 16TH, 2018

8:30 Session 7 – SOCIO ECONOMIC IMPACT AND COMMUNICATION

STRATEGIC COMMUNICATIONS FOR RESEARCH AND IMPACT: WHY AND HOW

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Strategic communications, engagement, communications plans

Engagement in dialogue about scientific innovation has a strong potential to enable the delivery of impact of the research activity and support the development of solutions for the relevant end users and society as whole. Often among stakeholders there are different views, perceptions and understanding of the value of new technologies such as genetic modification (GM) and what it can deliver for farmers and consumers. Defining and implementing an effective communication strategy is key to ensuring public and stakeholder perceptions about the impact of novel plant breeding technologies are addressed in a transparent and coherent manner. The development of a communications strategy in order to be effective needs to be aligned and support the overall project objectives in any given situation and the stakeholders need to be identified and prioritised so that both the communications and overall project/business objectives can be achieved. Thus, the identification and definition of communications objectives as well the stakeholder mapping requires input and discussion of all involved in a project and dialogue during the strategy development stage. Once these key elements of the strategy have been developed then an implementation plan can follow that essentially outlines key actions and communications deliverables with targeted and nuanced messages for each stakeholder group. Having a strategy and a communications plan enables the effective, efficient and proactive engagement with all concerned in order to achieve the project objectives, impact through the research activity and benefit users and consumers in society of technological advances. Examples of how such strategies have been developed and what have delivered at institutional and project level will be presented.

AN EVENT TO UNITE EUROPEAN POLICY MAKERS ON THE VALUE OF RNA SILENCING

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With every great scientific breakthrough that changes the landscape comes the challenge of preparing a policy environment that recognizes the policy challenges, accepts the added value of the breakthrough and reaches a consensus with the scientific community on policy solutions. With a strong competence to regulate the agriculture and nutrition market, the EU has been at the forefront of discussions on GMO's but the discussions about the value of scientific breakthroughs have largely surrounded safety concerns. The disconnect between public opinion and scientific fact has led to a confused policy environment. The future acceptance of RNA silencing will depend on the effective communication to policy makers around independent international research dealing with innovation and sustainable agriculture and the benefits this highlights for the environment and nutrition.

As such, high level engagement with EU policy makers throughout the actions of COST iPlanta is essential to prepare acceptance of the value of RNA silencing and to develop consensus of required policy solutions amongst EU stakeholders. This could take the form of a high-level policy event in Brussels, bringing together policy makers from the European Parliament and the Commission as well as stakeholders from the food and agricultural sectors to effectively transfer new evidence into better policy making. This event could be held under the auspices of several politicians in the European Parliament, creating lasting partnerships to ensure that the added value of RNA silencing is introduced in a positive, science based, environment.

‘THE GM-DEBATE IS NOT A GM-DEBATE’. REFLECTIONS ON THE GM-DEBATE IN EUROPE FROM A SOCIAL SCIENCES PERSPECTIVE.

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GM-debate, Europe, mediation theory

Large parts of the GM-debate about GM crop applications within EU can be understood as an attempt to debate how we have organised EU society today, rather than a mere critique on GM-technology as such. To substantiate this argument, this contribution will refer to Verbeeks mediation theory (Verbeek 2011, Inghelbrecht et al. 2017) and illustrate how ethical concerns about agricultural practices have co-evolved with the technological development of GM crops. It will discuss how technology and human beings relate. Is technology merely instrumental (the externalist view)? Or does technology actively shape human behavior and interpretation? And does the mediation between technology and human beings unveil the real meaning and significance of technology, in real practices?

From this perspective, the essence of the public debate on GM crops in the EU goes beyond the portrayed dichotomous Yes/No framing (in which both proponents and opponents are trapped), and is an attempt to discuss how EU agriculture is, could and should be organised. It shifts the question from a technocratic assessment of the (economic) gains and losses of a technology, towards the fundamentally political question of how technology applications help to shape society. A perspective of technological mediation can therefore contribute to re-politicising technology and technological design.

The presentation will use illustrations from GM-controversies at Member State level (Belgium) and at EU level.

References

Inghelbrecht, L., Goeminne, G., Van Huylenbroeck, G. and J. Dessein. (2016) When technology is more than instrumental: How ethical concerns in EU agriculture co-evolve with the development of GM crops. Agriculture and Human Values 3 (33),

Verbeek, P.P. 2011. Moralizing technology: Understanding and designing the morality of things. Chicago/London: University of Chicago Press

EXPLORING THE FUTURE DEVELOPMENT OF RNAi/NBTs APPLICATIONS IN PLANT THROUGH FIELD TRIALS DATA

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RNAi, NBTs, field trials, R&D, innovations

In the scenario of the global challenges that modern agriculture needs to deal with, new breeding technologies represents one of the most promising tools for the implementation of new plant varieties able to improve field productivity and agricultural sustainability. Hence, the evaluation of their future impact on the agricultural sector is of paramount importance, due to their potential in creating innovative plants/traits combinations.
Nevertheless, as a consequence of their recent development, data on commercial authorization and cultivation are still lacking. Thus, the economic analysis of this specific breeding sector require to be otherwise performed focusing on previous innovation steps prior to commercial phase. Therefore, this work focus on R&D data about field trials authorizations of plant varieties developed through modern breeding technologies, with a specific focus on RNAi technique. Data are collected from the database USDA-APHIS service, which regulates the introduction (importation, interstate movement, or environmental release) of biotech plants in the United States. Expected results shall cover the current state of play about field trials authorization, the patterns of plants and traits introduced and the main biotech players involved, in order to perform a global estimation of the future application of modern breeding techniques in agriculture.

DESPITE NUMEROUS EFFORTS DONE IN THE LAST DECADES TO CONTROL RICE BLAST, THIS FUNGUS REMAINS THE BIGGEST ISSUE FOR EUROPEAN RICE CULTIVATION. COULD RNAI TECHNOLOGY BE A HOPE?

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Rice, blast

SA.PI.SE. Coop. Agr. was founded in 1978 by 11 Italian rice seed growers with the aim of producing and selling certified rice seed. The SA.PI.SE. headquarter was located in Vercelli, the center of the largest European rice growing area. In 1989 SA.PI.SE. created an R&D Unit and started a rice breeding program for the development of new rice varieties. In the last 20 years SA.PI.SE. has released almost 30 rice varieties. Now SA.PI.SE. produces, selects and sells more than 16500 tons of certified seeds marketed in many Mediterranean countries (Spain, France, Portugal, Greece, Bulgary, Romania, Turkey and Morocco).

SA.PI.SE. main breeding activities focus on improving rice grain quality and on resistance to abiotic and biotic stresses. The most relevant biotic stress is known as rice blast and it is caused by the fungus *Magnaporthe oryzae*. Rice blast was described for the first time in Italy by Cavara in 1981 and it can affect all above ground parts of a rice plant: leaf, collar, node, neck, parts of panicle, and sometimes leaf sheath. A leaf blast infection can kill seedlings or plants up to the tillering stage. At later growth stages, a severe neck blast infection can seriously compromise grain yield.

Despite many efforts throughout the world to try to control this disease, blast remains one of the most destructive diseases of rice. Although more than 80 blast resistance genes have been discovered, it seems very difficult to achieve broad-spectrum and durable resistance.

The result is that most of the European rice varieties are susceptible to the disease, so farmers routinely spray fungicide to prevent the disease (1-3 treatments/season).

We are wondering if RNAi technology could be an appropriate strategy to control this significant disease of rice.

FROM PINOCCHIO TO MASTERSCHEF. FROM HUNGER TO ABUNDANCE. THE HISTORY OF AGRICULTURE IN A FEW FAMILY PHOTOS

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This short speech has two main aims, first: to tell the long and fascinating history of agriculture in the world through three family photos, my grandfather Antonio, my father Luigi e and I myself. Basically, from Pinocchio (the story of hungry) to Masterchef (The story of the land of plenty) As we did? Second: to face complex agricultural issues in a clear. What are the benefits of green revolution? What about the real cost of green revolution? The point is: agriculture is a field that everybody's talking about, but so few have the expertise to do this. To use a metaphor: it's like people they talk about football but they don't know the playing field. So, for to improve communication, i guess the question we should ask ourselves is what do we talk about when we talk about agriculture? I mean, story telling first.

Poster Sessions

P1 - -A ROLE OF CROSSTALK BETWEEN THE NEXT COMPLEX AND SERRATE IN DEGRADATION OF MIRNA PRECURSOR FRAGMENTS

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SERRATE, miRNA, NEXT, EXOSOME, DEGRADATION

The *Arabidopsis thaliana* SERRATE protein (SE), which is homologue of human ARS2 protein, is involved in two important pathways of RNA metabolism in plant: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA 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 accuracy of pri-miRNA cleavages catalyzed by DCL1. The Arabidopsis *se* null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the nuclear cap-binding protein complex (CBC), have been also ascribed to splicing of pre-mRNA. We have shown that SE interacts with CBC and both have influence of alternative splicing. In order to understand this dual role of SE and CBC in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the *se-1* mutant genetic background. The SE-bound proteins were identified by mass spectrometry, and the putative protein interactions were confirmed by the yeast two hybrid system and pull-down experiments. Additionally we confirmed this interaction in living cells using FLIM FRET techniq in *Arabidopsis* protoplasts. Our results have clearly demonstrated that SE as like ARS2 in humans contacts directly the Nuclear Exosome Targeting complex (NEXT). Our study has characterized accurate interaction place between SE and NEXT complex. Moreover we have shown that the NEXT complex is necessary for proper degradation of 5' pri-miRNA fragments after excision of miRNA by DCL1. We suggest that molecular interactions between CBC, SE and the NEXT complex is important for the quality control of miRNA precursors and degradation by the nuclear exosome 5' pri-miRNA fragments produced during miRNA biogenesis in the plant cell nucleus.

This work was supported by grants from the National Science Center
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P2 - -MicroRNAs EXPRESSION IN BARLEY DURING DROUGHT AND REHYDRATION STRESS

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Barley, drought stress, rehydration, microRNAs, SnRK2 kinase

MicroRNAs (miRNAs) are RNA molecules, mostly of 21 nucleotides in length. They are gene expression regulators, as they target mRNAs cleavage or repress its translation. In this study we analyzed miRNAs expression in barley response to drought and rehydration stress. The analysis was proceeded with NGS technology and confirmed with northern. One of the drought-regulated molecules is miRNA172b-5p. It is downregulated at the level of miRNA, nevertheless the pri-miRNA172b-5p level rises. Rehydration brings back the miRNA172b-5p level, while its precursor expression is constantly elevated. These suggest that the maturation of miRNA172b-5p is drought stress inhibited. With Parallel Analysis of RNA Ends method we identified serine-threonine kinase (SnRK2) as a target of miRNA172b-5p. The SnRK2 mRNA level is upregulated in drought and drops down after rehydration. We showed that the SnRK2 is drought stress induced and ABA-regulated kinase. To sum up, our data describe changes in the expression profile of miRNAs and their pri-miRNAs during drought and after rehydration. Drought stress regulates the level of barley miRNAs at the level of transcription and at the step of miRNAs maturation (posttranscriptional regulation). Also, we provide first evidence of SnRK2 level regulation during drought and rehydration by microRNA in barley. Work sponsored by POLAPGEN-BD UDA.POIG.01.03.01-00-101/08, subject 20: "The role of micro RNA in regulation of mechanisms leading to drought adaptation in plants"; Innovative Economy Programme 2007-2013, subject „Biological progress in agriculture and environment protection” ”; and by the KNOW RNA Research Centre in Poznan 01/KNOW2/2014.

P3 - INVESTIGATING THE RNA SILENCING SUPPRESSOR ATRLI2 OF *ARABIDOPSIS THALIANA*

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Arabidopsis thaliana, *AtRLI2*, *RNA silencing suppressor*

ATP-binding cassette sub-family E member 1 (ABCE1) is a highly conserved protein among eukaryotes and archaea. First identified as RNase L inhibitor, ABCE1 is currently recognized as an essential translation factor involved in several stages of eukaryotic translation and ribosome biogenesis. We have demonstrated that AtRLI2, the homolog of ABCE1 in *Arabidopsis thaliana*, is an endogenous suppressor of RNA silencing. Mutational analysis of AtRLI2 shows that the N-terminal domain with two iron-sulfur clusters is important for the suppression function. Also nucleotide binding domains seem to be related to the suppressor function.

Interestingly, ABCE1 is crucial for the viability of several organisms (knockouts in yeast, *C. elegans* and *Trypanosoma brucei* are lethal). We have determined that *AtRLI2* is ubiquitously expressed, showing an increased expression in flowers and siliques. At the moment we are characterizing one *Arabidopsis thaliana* T-DNA insertional line of *AtRLI2* and have not been able to obtain homozygous plants containing the disrupted gene. Therefore, we are phenotyping heterozygous plants paying special attention to ovules and siliques. As *AtRLI2* counts with a paralog, *AtRLI1*, we are currently using CRISPR/Cas9 to explore the knockout of *AtRLI* genes. We plan to challenge the knockout plants with *Turnip mosaic virus* to see if virus infection is affected by the abscence of the RNA silencing suppressor.

P4 - -EXPRESSION ANALYSIS OF SEX-RELATED CANDIDATE GENES IN PISTACHIO

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Pistachio, sex expression, dioecious, RNAseq

The great majority of flowering plants are hermaphrodites, but a large proportion of angiosperm families include dioecious species, with separate male and female individual plants like pistachios. Pistachio (*Pistacia vera* L.) is a dioecious species that has a long juvenility period. For commercial pistachio orchards, approximately one male tree is needed for 11-20 females. As a result, 5-10% of a typical pistachio orchard will not produce nuts. Our previous studies showed that pistachio has ZW/ZZ sex determination system. In this study, we aimed to find sex-determining genes in pistachio. We sampled weekly buds from male and female trees during the growing season and before flowering. In the first step, we examined the buds by scanning electron microscope with the objective of determining floral differentiation. Stamen and carpel primordia are initiated in both male and female flowers in the spring, and then development of organs of the opposite sex was arrested at the primordial stage. RNAseq study was performed during that stages and candidate genes were determined. These candidate genes have been testing by expression analysis at various developmental stages of buds of different male and female pistachio cultivars, and the results will be presented in the meeting.

P5 - -IMPLEMENTATION OF A REGENERATION AND TRANSFORMATION PROTOCOL FOR *VITIS VINIFERA* VARIETIES AND ROOTSTOCK TO INDUCE GENE SILENCING AGAINST GFLV-GLRaV VIRUS

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Vitis vinifera, gene silencing, virus resistance, GFLV-GLRaV

Grapevine cultivation is penalized by pathological problems with significant impact on production, quality and related costs. This species is affected by numerous viral diseases, such as “fanleaf (GFLV)” and “leaf roll (GLRaV)” diseases, which are the most diffused in Europe. The application of rigorous certification criteria is the only strategy available to control the diffusion of viruses. Traditional breeding techniques are limited in that there is reduced genetic resources and an increase in variability that is unacceptable for the preservation of traditional grapevine clones. Post Transcriptional Gene Silencing (PTGS) has emerged as alternative tool to induce resistance to virus in several plant species, even by using rootstocks as a source of RNAi controlling plant virus infection. For the application of this technology in grapevine it is really important to have efficient regeneration and transformation protocols for the most important cultivars and rootstocks.

For this aim, the regeneration and transformation protocol via organogenesis (Mezzetti et al., 2002) was optimized for different grapevine cultivars (Vermentino, Albana, Pignoletto, SanGiovese), in comparison with the efficient table grape cultivar Thompson Seedless, and rootstocks (1103 Paulsen, 110 Richter and Kober 5BB). The meristematic bulks created for each clone were used as explants for *Agrobacterium*-mediated genetic transformation protocols with a gene construct that express the e-GFP as marker gene. Genotypes having the highest regeneration and transformation efficiency were also used for transformation experiments using a hairpin gene construct designed to silence the RNA-dependent RNA polymerase (RpRd) of the GFLV and GLRaV3, which would induce multiple virus resistances.

P6 - -SOLUTION STRUCTURE OF THE FIP37 AND WTAP, THE ADAPTOR PROTEINS OF PLANT AND MAMMALIAN M6A METHYLTRANSFERASE COMPLEXES.

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RNA modification, N6-methyladenosine, FIP37, WTAP, solution structure

Recently, widespread N6-methyladenosine (m6A) modification of messenger RNA (mRNA) and non-coding RNA was discovered in yeast, flies, mammals and plants. It was shown that this modification could affect mRNA fate within the cell: changing its splicing pattern, promoting transport to the cytoplasm, increasing degradation rate and enhancing translation. Moreover, m6A modification of primary miRNA transcripts promotes miRNA biogenesis in human cell lines. At the functional level m6A modification plays an important role in many biological processes, including: stem cell differentiation, circadian clock in mammals, functioning of the nervous system and sex determination in flies, meiosis in yeast and development in plants.

In mammals two methyltransferases: METTL3 and METTL14, together with the adaptor protein WTAP are required for m6A methylation. In plants MTA and FIP37, in yeast IME4 and MUM2 were identified as functional homologs of METTL3 and WTAP, respectively. From structural studies it is known how methyltransferase subunits cooperate with each other for substrate recognition and methyl group transfer from the S-adenosylmethionine cofactor but little is known about the role of WTAP and FIP37 proteins in m6A methylation of mRNA at the molecular level.

Here we present the results of SAXS studies of WTAP and FIP37 proteins in solution. We showed that WTAP and FIP37 proteins form in solution high-order oligomers of various molecular weight. Ab-initio modeling revealed an elongated shape of oligomers of both proteins. This experiments have also shown that the degree of oligomerisation is concentration dependent. We observed similar behavior for a shorter fragment of FIP37 encompassing the coiled-coil domain, showing that this region is required for the FIP37 oligomerisation. Using yeast two-hybrid system we also shown that coiled-coil fragment encompassing residues from 100 to 200 is sufficient for the oligomerisation. At the the functional level the oligomerisation of WTAP and FIP37 proteins may facilitate recognition of mRNA and engagement of the methyltransferase complex for catalytic activity and formation of functional speckles within the nucleus.

This work was supported by the KNOW RNA Research Centre in Poznan (No. 01/KNOW2/2014) and MAESTRO grant (2013/10/A/NZ1/00557) from the National Science Centre.

P7 - -THE IDENTIFICATION OF SPATIAL AND TEMPORAL ROLE OF LUS-MIR172E AND LUS-MIR396C

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Linum usitatissimum, miRNA, Northerrn blot

Detection and quantification of miRNA expression is a key step in understanding their role in gene regulation. Northern blot analysis was used to confirm the expression level of lus-miR172e and lus-miR396c in selected tissues and different developmental stages of tested flax genotypes. For the study, flax genotypes with different content of alpha-linolenic acid (Amon, Libra and Raciol) and control genotype (Bethune) were used. Since flax is an interesting model of genomic research, two families of *Linum usitatissimum* micro RNAs have been selected, lus-miR172e which is incorporated in the metabolism of cell wall and lus-miR396c which is involved in controlling cell proliferation during leaflet development. Lus-miR172e which had a relatively high expression in stems of 2-week plants, moderately high in the roots and the lowest expression was shown in the leaves. Differences in expression were also noted between individual genotypes within selected plant organs. In stem and whole plant tissues (leaves and stems), the genotypes of Raciol and Bethune showed higher expression compared to the Amon and Libra genotypes. The highest expression of lus-miR396c was recorded in 1-week leaves and in 1-week plants (leaves and stems). Expression analysis by Northern Blot method confirmed accumulation of lus-miR172e in stems and lus-miR396c in whole plants and leaves of flax with decreasing age of plants.

Acknowledgement: This work has been supported by European Community under project no 26220220180: Building Research Centre „AgroBioTech“.

P8 - COMPARISON OF TRANSFORMATION EFFICIENCIES BETWEEN MERLOT (cultivar) AND RICHTER 110 (rootstock) SOMATIC EMBRYOS FOR POSSIBLE APPLICATION OF RNAI TECHNOLOGY IN GRAPEVINE.

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Grapevine's special sensory attributes sets it apart among fruit crops. Majority of world production is represented by a relatively small number of well-known elite cultivars and their landraces, which are subject to significant disease pressures. Genetic improvement of grapevine for disease resistance and abiotic stress tolerance is of utmost importance for the sustainability and profitability of the viticulture industry worldwide. Although better genetic resistance is required to reduce production losses, grapevine is difficult to improve through conventional breeding due to impediments imposed by its lifecycle. Advances in cell culture, gene insertion and computational technology have improved conventional breeding. Micro propagation, genetic transformation and plant regeneration have been the subject of intense study for more than two decades (Grey et al 2014). One of the most attractive features of cell cultures is that, plants obtained are predominantly normal and devoid of any phenotypic or genotypic variation, as they are derived from single cells hence, transformed somatic embryos are free of chimeras. Embryogenic cultures of Vitis vinifera Merlot (variety) and Richter (rootstock) were initiated from stamens and pistil of unopened flowers. The somatic embryos obtained from the cultures were used as explants for Agrobacterium-mediated transformation. Transient and stable expression of GFP in the transformed somatic embryos exhibited green fluorescence under the microscope using UV filter. This protocol can be utilised for transformation using a suitable gene construct for the possible application of RNAi technique to control diseases in grapevine. The efficiency of this method will be compared with that of organogenesis.

P9 - -MICRORNA IN TOMATO VARIETIES

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Tomato, microRNAs, fruit development

MicroRNAs are class of non-coding endogenous small RNA, usually consisting of ~20-24 nucleotides for plants that participate in posttranscriptional gene silencing and play fundamental roles in biology. They are involved in many biological processes in plants such as development, signal transduction, protein degradation, response to environmental stress and pathogen invasion and regulate their own biogenesis. Tomato (*Lycopersicon esculentum*) is second most consumed vegetable in the world. Over 75.000 accessions of cultivated and wild species of tomato are maintained in Genbank around the world. Tomato is a model plant for the study of fleshy fruit ripening and senescence owing to its genetic and molecular tractability. The findings suggest the important role of miRNAs in tomato fruit development starting from the initiation of the fruit until the final ripe stage. Previous studies revealed that for global identification of miRNA targets and comparing four different stages of tomato fruit development, a total of 119 target genes of miRNAs were identified. It is known that natural or induced variation in miRNA expression could be used on breeding programmes aimed of modifying ripening parameters in tomato. Fruit formation and fruit yield in tomato were affected by over-expression of miR156. The gene colorless non-ripening (CNR), a member of the squamosa-promoter binding protein (SBP) family, involved in fruit ripening, is targeted by miR156. It is known that miR156 is expressed at different fruit stages and their expression decreases at the red ripe stage. To determine exactly how much of the CNR transcripts are present as cleavage products, expression analysis by quantitative PCR can be used. Our intention is to explore the status of CNR transcripts in different ripening stages in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* with yellow and red fruits.

P10 - -INVESTIGATING THE RNA SILENCING SUPPRESSOR ATRLI2 OF ARABIDOPSIS THALIANA

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Arabidopsis thaliana, *AtRLI2*, *RNA silencing suppressor*

ATP-binding cassette sub-family E member 1 (ABCE1) is a highly conserved protein among eukaryotes and archaea. First identified as RNase L inhibitor, ABCE1 is currently recognized as an essential translation factor involved in several stages of eukaryotic translation and ribosome biogenesis. We have demonstrated that AtRLI2, the homolog of ABCE1 in *Arabidopsis thaliana*, is an endogenous suppressor of RNA silencing. Mutational analysis of AtRLI2 shows that the N-terminal domain with two iron-sulfur clusters is important for the suppression function. Also nucleotide binding domains seem to be related to the suppressor function.

Interestingly, ABCE1 is crucial for the viability of several organisms (knockouts in yeast, *C. elegans* and *Trypanosoma brucei* are lethal). We have determined that *AtRLI2* is ubiquitously expressed, showing an increased expression in flowers and siliques. At the moment we are characterizing one *Arabidopsis thaliana* T-DNA insertional line of *AtRLI2* and have not been able to obtain homozygous plants containing the disrupted gene. Therefore, we are phenotyping heterozygous plants paying special attention to ovules and siliques. As *AtRLI2* counts with a paralog, *AtRLI1*, we are currently using CRISPR/Cas9 to explore the knockout of *AtRLI* genes. We plan to challenge the knockout plants with *Turnip mosaic virus* to see if virus infection is affected by the abscence of the RNA silencing suppressor.

P11 - PHO2 (PHOSPHATE2) INTERACTS WITH NLA (NITROGEN LIMITATION ADAPTATION) TO MAINTAIN PHOSPHATE HOMEOSTASIS IN BARLEY

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Barley, PHO2, phosphate homeostasis,

Appropriate inorganic phosphate (Pi) concentration in soil is one of the most important factors for proper plant growth and development. Pi is an available form of phosphorus which is transported from soil to root by plasma membrane phosphate transporters (PHT1). The soil Pi concentration influences plants Pi-related genes expression. Barley *PHT1;1*, *MIR399*, *MIR827* are up-regulated during Pi starvation. In the same condition barley *PHO2* (*PHOSPHATE2*, encoding E2 ubiquitin-conjugating enzyme) gene expression is decreased due to the post-transcriptional silencing by miRNAs399. We found that expression of barley Pi-related genes is also affected by heat stress (under Pi sufficient condition). In roots the expressions of *PHT1;1*, *PHT1;4*, *PHT1;6* and *PHO2* gene expression was down-regulated, while the *NLA* (Nitrogen Limitation Adaptation, E3 ubiquitin-protein ligase) gene expression was up-regulated. It is known that in *Arabidopsis thaliana nla* mutant accumulates Pi similarly to *pho2* mutant. It is due to the role of PHO2 (E2 enzyme) and NLA (E3 ligase) proteins in the ubiquitin transfer to the target proteins. The ubiquitinated proteins are then degraded by 26S proteasome. Using two-hybrid system we found direct interactions between barley PHO2 and NLA proteins. The results were validated by a pull-down assay. We look also for new PHO2 interacting proteins in barley. For this purpose, we constructed transgenic barley lines overexpressing tagged PHO2 protein.

This work was funded by National Science Centre (Poland) grant, DEC-2013/11/B/NZ9/01761, and supported by KNOW Poznan 01/KNOW2/2014 and GDWB-06/2016.

P12 - THE SOCIOECONOMIC BENEFITS OF BIOLOGICAL CONTROL OF WESTERN CORN ROOWORM *DIABROTICA VIRGIFERA VIRGIFERA* AND WIREWORMS *AGRIOTES SPP.* IN MAIZE AND POTATOES FOR SELECTED EUROPEAN COUNTRIES

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Diabrotica virgifera virgifera, Wireworms Agriotes spp., Integrated Pest Management (IPM), biological control agents, socioeconomic gain.

Innovative biological pest control of the western corn rootworm (WCR) *Diabrotica virgifera virgifera* and Wireworms *Agriotes spp.* in maize and potato cultivation in Europe is driven by (1) the economic damages caused and (2) the restrictions on chemical pesticides.

We analyze the efficacy of biological control agents for WCR and Wireworms based on European field trails. A partial equilibrium displacement model is used to estimate the changes in producer and consumer surplus for France, Italy, Spain, Germany, Austria and Romania given different adoption- ceiling and speed. Furthermore, the benefit of a potential reduction in pesticide use due to biological control application is evaluated. The results suggest a total annual welfare gain of ca. €190 million from biocontrol of WCR in maize production for the countries under consideration at an adoption- ceiling and speed of 30 % and 2.41, respectively. In potato production, an annual welfare gains of over €2 million may be recorded in ecological and/or organic cultivation. Overall, the biological control methods provide an economical alternative in maize and can contribute to increase the competitiveness of European Union (EU) agriculture, while they look promising for certified organic potato production at current level of control efficiency.

P13 - DOUBLED HAPLOIDS OF SPRING BARLEY WITH PARTIAL WDV REPLICATION PROTEIN

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WDV, barley, androgenesis, Agrobacterium-mediated transformation

Wheat dwarf virus (WDV) is considered to be one of economically most affecting viral diseases in numerous countries in the world. Winter wheat and barley, which are of high economic importance, are affected substantially by WDV. Yield loses caused by this pathogen are considered to be limiting factor of crop production. Biomass production as well as grain yield decrease significantly in affected plants. Based on the principle silencing of target gene by pathogen derived resistance (PDR), the modified viral sequences of Rep gene carrying several mutations were transformed into spring barley cv. Golden Promise. Regenerated plants were then planted in soil and DNA was isolated from the leaves. The presence of the transgenic transcript was verified by PCR. Androgenesis was exploited to quickly obtain stable homozygous line that can be used for large scale testing. The anthers were isolated, when majority of microspores were in the mid-to late uninucleate stage, and then cultivated *in vitro*. The ploidy level of the regenerated transgenic plants from anthers was determined by flow cytometric estimation of DNA content. The progeny of all doubled-haploid plants exhibited presence of the transgene.

P14 - THE EFFECT OF LEAD AND *A. PISUM* ON EXPRESSION LEVELS OF PHENYLALANINE AMMONIALYASE AND CHALCONE SYNTHASE GENES IN PEA SEEDLINGS

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Lead, Acyrthosiphon pisum, flavonoid biosynthesis enzymes; defense responses, Pisum sativum

The aim of this study was to determine the effect of an abiotic factor, i.e., lead at various concentrations (low causing a hormesis effect and high causing the toxic effect), on the expression levels of genes encoding enzymes of the flavonoid biosynthesis pathway (phenylalanine ammonialyase and chalcone synthase in pea (*Pisum sativum* L. cv. Cysterski) seedlings and then during infestation by the pea aphid (*Acyrthosiphon pisum* Harris). Phenylalanine ammonialyase (PAL) is an enzyme initiating phenylpropanoid metabolism, and chalcone synthase (CHS) catalyses the first committed step in the flavonoid biosynthetic pathway. Semi-quantitative RT-PCR analysis revealed that pea aphid feeding alone (+aphids), lead administration at the high and low concentrations in the medium (0.5 mM Pb^{2+} and 0.075 mM Pb^{2+}) as well as the cross-talk of lead and *A. pisum* infestation (0.5mMPb^{2+}+aphids and 0.075mMPb^{2+}+ aphids) upregulated mRNA levels for PAL and CHS in relation to the control. However, these stress factors much more strongly upregulated CHS than PAL. Very high upregulation of mRNA for the CHS genes were observed as a result of the impact of lead or the cross-talk of lead and *A. pisum*, especially at the toxic concentration of lead. The obtained resultes indicate involment of these enzymes in regulation of flavonoids syntesis and in the defence strategy of pea against stresses.

P15 - THE NOVEL NUCLEAR BODIES CONTAINING MIRNA PRECURSORS IN THE PLANT CELL NUCLEUS

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miRNA, Arabidopsis thaliana, nuclear bodies

MicroRNAs (miRNAs) are short, non-coding RNA engaged in the regulation of gene expression. MiRNA is transcribed by RNA Polymerase II (RNAPII) as a pri-miRNA which are next processed to mature miRNA by muti-subunits complex called Microprocessor. The plant complex engaged in miRNA biogenesis contains endorybonuclease DCL1 and additional proteins like SERRATE and HYPONASTIC LEAVES 1 (HYL1). We have discovered nuclear bodies containing pri-miRNA163 by using Fluorescence *in situ* Hybridization. These pri-miRNA-containing nuclear structures are different from dicing bodies that have been previously describe in plant miRNA biogenesis. The new bodies observed by us do not contain also any Cajal bodies markers. The number of nuclear bodies containing pri-miRNAs ranged from one to three in each nucleus. The presence of pri-miRNA-containing nuclear structures were also confirmed for: pri-miRNA156, pri-miRNA393a and pri-miRNA393b.

P16 - EXPLORING NGS, HIGS AND SI-RNA TECHNOLOGIES FOR THE CONTROL OF *FUSARIUM* EAR BLIGHT IN WHEAT

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Fusarium ear blight (FEB) is a major problem in most small-grain cereal growing regions and now threatens global food security. Currently strategies to control FEB are not very effective, fungicides give partial protection and development of genetic-based resistant cultivars has proven to be difficult. Therefore, new ways to control FEB are urgently required. Here, we describe the framework and initial results from a bilateral UK-Brazil project that is using a bespoke whole genome sequencing, *in planta* transcriptome and reverse genetics guided approach to understand and pinpoint the *Fusarium* genes and pathways required to cause disease in wheat heads. Our intention is to identify a new suite of *Fusarium* genes for intervention that can simultaneously be targeted via host induced gene silencing (HIGS). HIGS constructs are being tested for efficacy in *Arabidopsis*, lettuce and wheat, and promising HIGS constructs have been stably transformed into a commercial moderately-FEB resistant Brazilian wheat in preparation for field testing in southern Brazil during 2018 and 2019. By taking a *Fusarium* genome/reverse genetics guided approach, this is enabling the development of flexible new ways to control FEB disease in wheat crops grown in Brazil. The main prerequisites needed to apply this approach in other wheat growing regions will be addressed, as well as additional scientific, societal and industry benefits that could potentially emerge when using HIGS technologies for plant disease control.

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P17 - CAREAL GRASS JUICE-MEDIATED NF-κB REGULATION IN HUMAN CELLS

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Cereal grass juice, NF-κB, anti-oxidant, microRNAs

Natural products and traditional medicines are of great importance. Cereal grass juices, due to their remarkable anti-oxidant ability, were shown to act as co-suppressors of many health disorders such as inflammation, heart diseases, obesity and cancer. Recent studies demonstrated cereal grass juice-mediated improvement in wound healing, however cellular and molecular mechanisms underlying these processes were not fully characterized.
In the first step of our study, we show for the first time, that in normal fibroblasts (BJ cell line) low dose cereal grass juices exhibit strong adaptive response through hormetic mechanism mediated by NF-κB/HO-1 and insulin/IGF-1 anti-oxidant pathways. As consequence, the process of wound healing is significantly upregulated. In cancer cells (ES-2 cell line), on the other hand, despite ant-oxidative defense mechanism activation, the levels of ROS and RNS are elevated. That leads to enhanced O-GlcNAcylation, DNA damage and cell cycle arrest and as a result in impaired wound healing. Our study provides insights into the underlying mechanisms through which cereal grass juices activate hormetic adaptation response in normal fibroblasts and induce cytotoxic and genotoxic events in cancer cells. Further questions that arise from this study: are the microRNAs involved in the NF-κB-mediated regulation in this case? If yes, could it be miRNAs derived directly from cereal grass juices? The controversial data appearing in scientific papers for last several years, shows that this kind of regulation is highly possible. In cereal grass juices there is a unique concentration of all cell ingredients, so probably also miRNAs. Therefore, the next ongoing step in our analysis is cereal juice-derived miRNAs purification and sequencing. If the miRNAs concentration is high, the potential risk of exogenous gene regulation is increasing, thus, not only from directly consumed plants but especially juices and other plant extracts.

P18 - INTRODUCTION TO THE iPLANTA COST ACTION AND RNAi RESEARCH AND DEVELOPMENT IN RELATION TO CROP PROTECTION.

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Methods to exploit plant defence mechanisms or changing plant metabolism by RNA silencing show great potential. Interfering RNA can be used to improve plant composition while enhancing levels of beneficial nutrients, and to improve plant productivity by suppressing undesirable traits and switching resources to more beneficial quality and yield traits. The HORIZON2020 iPLANTA network (http://iplanta.univpm.it/), will define and coordinate the most important research tasks for the development of these novel transgenic strategies across 31 EU and nearby countries with inputs from cooperating researchers in Associated countries (N and S America, Australasia etc..). The project has the following main tasks: a) Evaluation of the efficacy of the RNA molecules for the induction of disease and pest resistance and metabolic changes. b) Examination of the specificity of the selected miRNAs and siRNAs and their impacts on both target and non-target/off-target systems. c) Developing specific risk assessment and risk management guidelines which relate to the data requirements and specific effects of the miRNAs and siRNAs on food, feed and the environment. d) Understanding the modes of transmission, uptake, systemic spread and degradation of dsRNAs, mi- and siRNAs. f) Determining the environmental and socio-economic impacts of plant RNAi technology and products and developing a public dissemination strategy.

P19 - NBS-LRR DERIVING SRNAS UNIQUELY IN NUCLEAR-REPLICATING VIRUS-INFECTED TOMATO PLANTS TARGET COMPONENTS OF THE PHOTOSYNTHETIC MACHINERY.

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Plant viruses modify gene expression in infected tissues by altering the micro (mi)RNA- mediated regulation of genes. Among conserved miRNA targets there are transcripts coding for transcription factors, RNA silencing core and disease resistance proteins. Paralogs in these gene families are widely present in plant genomes and are known to respond differently to miRNA-mediated regulation during plant virus infections. Using genome-wide approaches applied to Solanum lycopersicum infected by a nuclear- replicating virus we highlighted miRNA-mediated cleavage events that could not be revealed in virus-free systems: among them, we confirmed the targeting of one of the two Argonaute1 paralogs, seven transcriptional factors from five different families cleaved by miR156, miR160, miR166, miR169 and miR172 and one RX-Coiled-coil (RX-CC), nucleotide binding (NBS), leucine rich (LRR) mRNA cleaved by miR6024. Interestingly, in most cases short indels close to the target sites discriminated cleavage of duplicates, indicating a functional significance of short indels in fine-tuning gene expression in plant- virus interaction. miR6024-mediated cleavage, uniquely in virus-infected tissues, triggers the production of several unique 21nt secondary siRNAs. These secondary siRNAs, rather than being involved in the cascade regulation of other NBS-LRR paralogs, explained cleavages of several mRNAs annotated as defence related proteins and components of the photosynthetic machinery. Outputs of these data explain part of the phenotype plasticity in plants, including the appearance of yellowing symptoms in the viral pathosystem.

P20 - CRISPR/CAS9 MODIFICATION OF SYMBIOSIS CANDIDATE GENES AND IMPROVEMENT OF PATHOGEN RESPONSE IN POPLAR

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Plant immune response, Melampsora, mycorrhiza, PAMP, Lysin-Motif-receptor

Plant immune response is triggered by "pathogen associated molecular pattern" (PAMP) after perception of fungal chitin. Lysin-Motif-receptor-like proteins (LysM-RLP) and Lysin-Motif-Receptor-like-Kinases (LysM-RLK's) are the main components to provoke plants defence. In collaboration with project partners of the Chito-Pop consortium, genes encoding LysM-RLK in poplar and LysM-effectors in the mycorrhizal fungus *Laccaria* will be functionally characterized. The objective of the consortium project is to generate poplar lines with an enhanced resistance against *Melampsora* and improved mycorrhization potential.

The Thünen Institute of Forest Genetics is analysing the LysM-RKL's which probably have an impact on the formation of mycorrhizal symbiosis. In poplar are 4 homologues of the rice LysMRLK11 and LysMRLK2 genes. The predicted genes are Potri.005G128400, Potri.007G032300, Potri.008G160600 and Potri010G078700. The analyses for the modification by the CRISPR/Cas9 were performed for Porti.005G128400 and the paralog pair Potri.008G160600 and Potri.010G078700. For the modification in Potri.005G128400, 7 homozygous independent insertions or deletions were detected. After the modification by CRISPR/Cas9 in Potri.008G160600 and Potri.010G078700, none of the regenerating plants revealed a homozygous mutation in both genes. The knockout of all genes resulted in two regenerated lines with homozygous modifications. *In silico* analyses indicates that the consequences of the modifications are premature stop-codons. These findings will be verified by applying transcript sequencing of the modified genes. Promising lines will be tested for changes in mycorrhization behaviour. Those genes which cause a reduction in mycorrhization will be used for overexpression experiments to improve mycorrhization afterwards.

To analyse the function of different LysM-RLK's during infection with *Melampsora* spp., various poplar genotypes and clones will be infected. Infection assays of different poplar clones were conducted and are still ongoing to build-up a collection of wildtypes which are resistant or susceptible against *Melampsora spp*. In future, perspective *Melampsora* infection assays will be performed with transgenic poplar, modified in LsyM-RLK genes that presumably are involved in resistance against fungal pathogens. Afterwards, transgene poplar lines modified in symbiosis genes will also be analysed for differences in resistance against *Melampsora spp*.

https://www.thuenen.de/index.php?id=6789&L=1

P21 - POLYMER MEDIATE DELIVERY OF DSRNA IN LEPIDOPTERA FOR RNAI-BASED CONTROL OF PEST INSECTS

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Confocal microscopy, CF203, uptake, dsGFP. fluorescence intensity

The confocal fluorescence microscope is a powerful tool which has vast applications in biology. It creates sharp images of a specimen providing a window into the physiology of living cells at sub-cellular levels. This is attained by the confocal pinhole, which allows the exclusion of most of the light from the specimen that is not from the microscope's focal plane.

Using this technology we ambition to analyze the uptake of polymer coated dsRNA (polyplex) in *Choristoneura fumiferana* midgut cells (CF203). To investigate whether the polyplex is uptaken as a complex by cells and if cellular uptake is maximized when using the polyplex compare to the dsRNA, simultaneous experiments were setup. First, the ability of the synthesized polymer to condensate the dsRNA was investigated by agarose gel electrophoresis. Once condensation was confirmed, labeled dsRNA was produced by Label IT® siRNA Tracker Intracellular Localization Kit, Cy®3 (Mirus) and polymer G23 was labelled with FITC. The polyplex and dsRNA were incubated for 4 hour at 27^{o} C in CF203 cell culture. After incubation the nucleus of the cells where stained using Hoechst 33342 . Images where taking using Nikon A1R inverted confocal microscope and 40-60x microscope lens.

Our initial results suggest that the polyplex is mostly uptaken by the cell as a complex, however free dsRNA can also be seen inside the cells. By using LysoTracker™ Deep Red and labeling early endosome we ambition to confirm the location of de polyplex inside the cell.

P22- CHARACTERIZATION OF NOVEL MIRNAS IN BARLEY

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Hordeum vulgare, miRNA, drought response, degradome

MiRNAs are small ribonucleic acid molecules usually 21 nucleotides in length that takes crucial part in transcriptional and post-transcriptional regulation of gene expression when incorporated into multiprotein complex- RISC (RNA-induced silencing complex) in all biological processes occuring in plants.

Currently there are only 71 barley mature miRNAs annotated in MirBase, much less than in other crop species such as wheat (119 miRNAs), rice (713 miRNAs) or maize (321 miRNAs). That suggests a large portion of miRNA molecules still undetected therefore vast part of regulatory mechanisms unexplored. We decided to overcome these differences, identify and validate novel miRNAs in barley.

In order to complete this task we prepared and sequenced small RNA libraries from five barley developmental stages and extracted a ranking list of potentially new miRNAs in barley, which was then subjected to verification.

Using Northern blot we proved the presence of 9 new miRNAs, for which precursor structures have been identified. With 5' and 3' RACE we identified structure of miRNA- coding genes and using degradome data we determined their target mRNAs. Our data show the accumulation of miRNAs in 68^{th} day of development (spike stage) and interesting changes in expression in particular flower organs such as stamen, which suggests involvement in the development and function of this organ.

We also analysed expression level of novel miRNAs in seven different stresses and found that 7 miRNAs are down-regulated upon drougth conditions which correlated with up-regulation of their targets.

Overall our data provide important information about the molecular mechanisms underlying the development of barley and its response to environmental stress expanding the current state of knowledge.

P23 - ARE BARLEY MICRORNA PRECURSORS FROM MIR444 FAMILY ASSOCIATED WITH RIBOSOMES?

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Barley, microRNA, pri-miRNA, splicing

MicroRNAs from the *MIR444* family have been found exclusively in monocots. The exceptionally feature of the MIR444 gene structure is the presence of an intron that separates two halves of pre- miR444 structure and encodes one half containing miR444 in one exon while the another half containing miR444* is encoded in the neighbour exon. Based on data from NGS Illumina small RNA sequencing we deduced that there are three *MIR444* genes in barley genome. RACE and RT-PCR experiments show that all three pri-miRNAs undergo extensive alternative splicing generating multiple pri-miRNA444 isoforms which can be functional or non-functional (microRNA cannot be generated).

Bioinformatics analysis revealed the presence of short potential open reading frames (ORFs) in almost all of the pri-miRNAs444. Others previous studies revealed that plant pri-miRNAs encode regulatory peptides [Lauressergues et al., 2015]. That prompted us to analyse cytoplasmic fraction for the presence of pri-miRNA. We carried out polysome profiling and using RT-PCR we identified that both, non-functional and functional pri-miRNAs444 are associated with ribosomes. As a negative control we used U3 snoRNA and we did not find it to be associated with the ribosomes. As a positive control we used spliced CBP20 mRNA that is dominant in the cytoplasmic fraction. Our results show that we indetified splice isoforms from all *MIR444* family genes are exported from the nucleus to the cytoplasm and play yet unknown role in plant metabolism.

P24 - GLOBAL ANALYSIS OF SMALL RNAS LEVEL CHANGES IN BARLEY ROOTS AND SHOOTS DURING PHOSPHATE STARVATION

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Barley, small RNAs, microRNA, degradome, gene silencing

Inorganic phosphate (Pi) is an important factor for plant growth and development as an available form of phosphorus (P) - the fundamental macronutrient for the structural and metabolic needs of plants. In plants, Pi level is controlled and phosphate homeostasis is maintained by the regulatory network of Pi signaling pathways. Small RNAs: microRNA399 and microRNA827 play an important role in Pi level regulation. These microRNAs target barley mRNAs encoding PHOSPHATE2 (ubiquitin-conjugating E2 enzyme) in the case of miR399 and NLA (Nitrogen Limitation Adaptation, E3 enzyme in Arabidopsis) and SPX-MFS (implicated in Pi sensing or transport) in the case of miR827. We analyzed changes in small RNAs expression profiles in barley roots and shoots during Pi starvation. We performed degradome analysis to find a correlation between small RNAs level changes and sequences that could be recognized by small RNAs and next cut by Ago proteins. Firstly we selected small RNAs which level was significantly changed during Pi starvation (EDGE test: Bonferroni and FDR P-value correction). Then small RNA sequences were mapped to miRbase and to barley sequences deposited in Ensembl Plants database (release 37). We found that most of changes were detected for reads derived from non-translating RNAs and rRNA sequences in roots and tRNAs in shoots. We identified new sites within 5'UTR of *PHO2* mRNA which are recognized by microRNA399 as well as novel targets for microRNA399 and microRNA827. To identify the role of small RNAs in abiotic stress response we plan to introduce a small RNA sequence into the barley microRNA166 or microRNA167 precursor backbone sequence and transform barley using the construct via Agrobacterium transformation. Herein, we present the barley Pi-response network composed of small RNAs, their targets and protein products.

P25 - THE EFFECT OF LEAD AND A. PISUM ON EXPRESSION LEVELS OF PHENYLALANINE AMMONIALYASE AND CHALCONE SYNTHASE GENES IN PEA SEEDLINGS

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Lead, Acyrthosiphon pisum, flavonoid biosynthesis enzymes; defense responses, Pisum sativum

The aim of this study was to determine the effect of an abiotic factor, i.e., lead at various concentrations (low causing a hormesis effect and high causing the toxic effect), on the expression levels of genes encoding enzymes of the flavonoid biosynthesis pathway (phenylalanine ammonialyase and chalcone synthase in pea (*Pisum sativum* L. cv. Cysterski) seedlings and then during infestation by the pea aphid (*Acyrthosiphon pisum* Harris). Phenylalanine ammonialyase (PAL) is an enzyme initiating phenylpropanoid metabolism, and chalcone synthase (CHS) catalyses the first committed step in the flavonoid biosynthetic pathway. Semi-quantitative RT-PCR analysis revealed that pea aphid feeding alone (+aphids), lead administration at the high and low concentrations in the medium (0.5 mM Pb^{2+} and 0.075 mM Pb^{2+}) as well as the cross-talk of lead and *A. pisum* infestation (0.5mMPb^{2+}+aphids and 0.075mMPb^{2+}+ aphids) upregulated mRNA levels for PAL and CHS in relation to the control. However, these stress factors much more strongly upregulated CHS than PAL. Very high upregulation of mRNA for the CHS genes were observed as a result of the impact of lead or the cross-talk of lead and *A. pisum*, especially at the toxic concentration of lead. The obtained resultes indicate involment of these enzymes in regulation of flavonoids syntesis and in the defence strategy of pea against stresses.

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