

***1st COST iPLANTA CONFERENCE  
CREATING A PLANT RNAI RESEARCH  
NETWORK  
COST Action CA15223***

CONFERENCE OPEN TO ALL SCIENTISTS, TECHNICAL EXPERTS,  
REGULATORS, POLICY MAKERS WITH AN INTEREST IN NEW  
BIOTECHNOLOGY APPLICATIONS

***BOOK OF ABSTRACTS***

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Wild type

Grosshans H, Filipowicz W. *Nature*. 2008

## **INTRODUCTION OF IPLANTA COST ACTION**

Modern agriculture requires a continuous and fast expanding stream of novel scientific and technological innovations to tackle issues regarding quantity and quality of plant production for the benefit of the farmer, the consumer and the environment.

Recently, methods to exploit plant defence mechanisms or changing plant metabolism, by RNA silencing, have been shown to be promising. RNA silencing is a sequence-specific mechanism of gene expression regulation present in eukaryotes that is based on the action of micro- and small interfering RNA molecules (miRNAs and siRNAs) derived from double-stranded RNA precursors. These small RNAs can trigger post-transcriptional gene silencing (PTGS), since they induce the sequence-specific cleavage of a target RNA and/or the inhibition of translation. RNA silencing-based strategies represent useful tools for functional genomics and crop biotechnology.

RNA interference (RNAi) can be used in a 'within species' mode to improve plant composition by removing or reducing anti-nutrients, allergens and toxins while enhancing levels of beneficial nutrients, and to improve plant growth and productivity by suppressing undesirable traits and thus switching resources to more beneficial traits such as quality and yield. In addition, gene expression in pathogens (particularly viruses and fungi), invertebrate pests and parasitic plants can be targeted using a 'cross-species' or 'host-induced' silencing approach. Plants can be genetically modified to produce double-stranded RNAs which trigger silencing and thus affect essential physiological functions in pest or disease-causing organisms. RNA silencing functions also as a defence mechanism against viral infection, and RNA silencing-based technologies have been successfully applied to induce virus resistance in different plant species, such as fruit trees.

Therefore, this technology has the potential to introduce novel pest and disease resistance, quality and nutritional improvements, and changes in metabolism which will increase crop productivity and/or reduce post-harvest losses. However, it is important to consider that the methods used are based on siRNA and miRNA initiating silencing of a target gene in a very precise manner.

The new COST Action 15223 iPlanta has the main objective to examine the scientific challenges of RNAi techniques for disease and pest control, and metabolic enhancement of plants. It will identify the most advanced knowledge available for this tool and the more important applications for the improvement of agriculture, forestry and food sector. For all major applications it will consider the best practices for risk assessment/management and the socio-economic impact of new products from RNAi technology.

## **AIM OF THE CONFERENCE**

1. Review existing knowledge and state of the art to provide a solid background for new developments, applications and research information and to support risk assessments. Identify knowledge and data gaps.
2. A study of the current situation on the development and application of RNAi in GM crop plants worldwide.
3. Collect scientific information about biosafety aspects linked to RNAi GM plants and promote research collaborations in this area in order to reduce uncertainty about potential non-target and off-target effects of RNAi.
4. Review the social and economic impacts of GMP RNAi.

**PARALLEL SESSIONS – WG MEETINGS  
NATIONAL RESEARCH COUNCIL**

**Session 1 – RNAi Technology (WG1)  
ORAL**

**Disarming plant defense by RNAi: The use of DCL knock-down *Nicotiana benthamiana* plants for infection and overexpression studies**

K. Katsarou<sup>1</sup>, A. Oulas<sup>2</sup>, E. Mitta<sup>3</sup>, E. Dadami<sup>4</sup>, I. Vlatakis<sup>3</sup>, K. Kalantidis<sup>1,3\*</sup>

<sup>1</sup>*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas*

<sup>2</sup>*Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), HCMR*

<sup>3</sup>*Department of Biology, University of Crete, Heraklion, Crete, Greece*

<sup>4</sup>*RLP AgroScience GmbH; iPlanta-Institute for Plant Research; Neustadt, Germany*

\*e-mail: [kriton@imbb.forth.gr](mailto:kriton@imbb.forth.gr)

Dicer-like (DCL) proteins are key components in the miRNA and siRNA biogenesis and are involved in developmental regulation, epigenetic modifications, stress responses and viral defense. Understanding the role of each DCL in these processes is of major concern for the scientific community. *Nicotiana benthamiana* has four DCL proteins. DCL1 is associated with the production of miRNA and small RNAs from endogenous inverted repeats. DCL2 generates siRNAs from natural cis-acting antisense transcripts, is involved in transitivity and viral resistance. DCL3 generates 24nt-mers related to RNA-directed DNA methylation. DCL4 is the major antiviral protein, but DCL4 involvement in tasiRNA metabolism, transcription termination and production of specific miRNAs have been well documented. We created transgenic knock-down *N. benthamiana* plants by suppressing each and every of the four DCL proteins (DCL1i, DCL2i, DCL3i, DCL4i and DCL2/4i) using RNAi technology. Plants were crossed to each other producing all possible DCLi combinations. In addition, knock-out plants using the CRISPR/Cas9 technology were produced. These plant lines were molecularly and phenotypically analyzed. By using infection with various virus and viroids (ex. TRV, CMV, PSTVd) as well as overexpression of an exogenous protein (GFP) we show that these plants are excellent tools for i) studying the role of each DCL protein, ii) overexpressing proteins in the absence of silencing components and iii) studying of the crosstalk between silencing mechanism and pathogens.

## **MicroRNA 444.1 expression controls barely tillering in response to heat stress**

Z. Szweykowska-Kulinska<sup>1</sup>, A. Pacak<sup>1</sup>, K. Kruszka<sup>1</sup>, A. Swida-Barteczka<sup>1</sup>, P. Nuc<sup>1</sup>, W. Karlowski<sup>2</sup>

<sup>1</sup>*Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland*

<sup>2</sup>*Department of Computational Biology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland*

\*e-mail: zofszwey@amu.edu.pl

*Hordeum vulgare, microRNAs, target genes, RNAi silencing, tillering*

Plant microRNAs undergo extensive transcriptional and posttranscriptional regulation in plants. We identified three *MIR444* genes in barley genome. In all of these genes a long intron separates two fragments of stem and loop structure of which one contains miR444 and the other one - miR\*444. Thus splicing must take place at the level of the primary miR444 transcripts in order to produce mature miRs444. Barley miR444.1 is induced transcriptionally under heat stress and proper splicing is required for its accumulation. We found that miR444.1 targets MADS box 57 TF that acts as a repressor of transcription. MADS box 57 mRNA level is dramatically downregulated during heat stress when miR444.1 is induced. Moreover, plants stop to produce tillers. We generated transgenic barley plants using RNAi technology to silence MADS box 57 TF. These plants do not produce tillers as well. Transcriptome analyses of barley plants subjected to heat stress allowed us to identify genes that are upregulated in these conditions. Several of these genes are involved directly in gibberellin synthesis. Moreover, one of these genes contains two MADS box 57 TF binding motives in its promoter region. We generated barley transgenic plants overexpressing MADS box 57 TF fused to c-Myc tag. Using CHIP assay we proved that MADS box 57 binds specifically to the promoter region of the gene involved in the gibberellin biosynthesis and containing MADS box 57 binding motives. Selected gibberellins level measurements show their increase during heat stress. We conclude that heat-induced miR444.1 accumulation is responsible for inhibition of tillering in barley. Moreover, this pathway seems to differ from the pathway described in rice.

## **ABCE1 is a highly conserved endogenous suppressor of rna silencing**

C. Sarmiento<sup>1</sup>, K. Kärblane<sup>1</sup>, M. Toompuu<sup>1</sup>, J. Gerassimenko<sup>1</sup>, E. Truve<sup>1</sup>

<sup>1</sup>*Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 19086 Tallinn, Estonia*

\**e-mail: cecilia.sarmiento@ttu.ee*

*RNA silencing, suppressor, endogenous, ABCE1, AtRLI2*

RNA silencing is a sequence-specific mechanism regulating the gene expression in eukaryotes that in the case of plants acts as an important antiviral mechanism. In order to exploit RNA silencing pathways to improve crop quality, a deep understanding of the interplay between the pathogen and its host is needed. In addition, it is also of pivotal importance to know how RNA silencing pathways are finely tuned at the endogenous level. Suppressor proteins that are able to inhibit RNA silencing in the host have been identified in most plant viruses and also in oomycete and in bacterial plant pathogen. In addition to suppressors encoded by pathogens, genomes themselves encode endogenous silencing suppressors. One of this proteins is ABCE1, or AtRLI2 in the case of *Arabidopsis thaliana*, that belongs to the ABCE subfamily of ABC proteins. ABCE1 is a very conserved protein among eukaryotes and archaea and has been described as essential for the viability of several organisms. It is involved in the regulation of translation and in ribosome biogenesis and recycling. Via agroinfiltration experiments in *Nicotiana benthamiana*, we have demonstrated that siRNA levels are reduced in the presence of ABCE1 (human or Arabidopsis protein). Especially the 24 nt long siRNA levels are reduced and accordingly the suppression at the systemic level is strongly abolished. Mutational analysis shows that the N-terminal domain with 2 iron-sulfur clusters is important for the suppression function. Making use of HEK293 cells, we interestingly found that ABCE1 is important for cell cycle progression at S phase and that its depletion impairs DNA synthesis and histone expression levels, independently of its influence on total translation. Using co-immunoprecipitation and mass spectrometry, we identified putative ABCE1 interactors, some of which are related to transcriptional or post-transcriptional silencing and are conserved among different organisms. Further studies on endogenous suppressors may enlighten ways to make use of RNA silencing pathways.

## **Induction of silencing in plants by high-pressure spraying of in vitro-synthesized small RNAs**

A. Dalakouras<sup>1</sup>, M. Wassenegger<sup>1</sup>, J.N. McMillan<sup>2</sup>, V. Cardoza<sup>2</sup>, I. Maegele<sup>1</sup>, E. Dadami<sup>1</sup>, M. Runne<sup>1</sup>, G. Krczal<sup>1</sup> and M. Wassenegger<sup>1,3</sup>

<sup>1</sup>*RLP AgroScience GmbH, AIPlanta-Institute for Plant Research, 67435 Neustadt, Germany*

<sup>2</sup>*BASF Plant Science, 26 Davis Dr., Research Triangle Park, NC 27709*

<sup>3</sup>*Centre for Organismal Studies (COS) Heidelberg, University of Heidelberg, 69120 Heidelberg, Germany*

\**e-mail: michael.wassenegger@agrosience.rlp.de*

We describe a method for the delivery of small interfering RNAs (siRNAs) into plant cells. In vitro synthesized siRNAs that were designed to target the coding region of a GREEN FLUORESCENT PROTEIN (GFP) transgene were applied by various methods onto GFP-expressing transgenic *Nicotiana benthamiana* plants to trigger RNA silencing. In contrast to mere siRNA applications, including spraying, syringe injection and infiltration of siRNAs that all failed to induce RNA silencing, high pressure spraying of siRNAs resulted in efficient local and systemic silencing of the GFP transgene, with comparable efficiency as was achieved with biolistic siRNA introduction. High-pressure spraying of siRNAs with sizes of 21, 22 and 24 nucleotides (nt) led to local GFP silencing. Small RNA deep sequencing revealed that no shearing of siRNAs was detectable by high-pressure spraying. Systemic silencing was basically detected upon spraying of 22 nt siRNAs. Local and systemic silencing developed faster and more extensively upon targeting the apical meristem than spraying of mature leaves. However, we failed to induce systemic silencing of an endogenous gene, supporting the finding that transgenes are more prone to silencing than endogenes are.

Dalakouras A., Wassenegger M., McMillan J., Cardoza V., Maegele I., Dadami D., Runne M., Krczal G. and Wassenegger M. (2016). Induction of silencing in plants by high-pressure spraying of in vitro-synthesized small RNAs. *Front Plant Sci* 7: 1327.

**RNAi –mediated Knockdown of the voltage-gated sodium ion channel (Paralytic A) causes mortality in the insect storage pest *Tribolium castaneum***

A.M.R. Gatehouse, M.G. Edwards

*School of Biology, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK*

*\*e-mail: a.m.r.gatehouse@ncl.ac.uk*

*Voltage-gated ion channels, TcNav, insect pests, Tribolium castaneum, RNAi*

The voltage-gated sodium ion channel (VGSC) belongs to the largest superfamily of ion channels. Since VGSCs play key roles in physiological processes they are major targets for effective insecticides. RNA interference (RNAi) is widely used to analyse gene function, but recently, it has shown potential to contribute to novel strategies for selectively controlling agricultural insect pests. The current study evaluates the delivery of dsRNA targeted to the sodium ion channel paralytic A (TcNav) gene in *Tribolium castaneum* as a viable means of controlling this insect pest. Delivery of TcNav dsRNA caused severe developmental arrest with larval mortalities up to 73% post injection of dsRNA. Injected larvae showed significant ( $p < 0.05$ ) knockdown in gene expression between 30–60%. Expression was also significantly ( $p < 0.05$ ) reduced in pupae following injection causing 30% and 42% knockdown for early and late pupal stages, respectively. Oral delivery of dsRNA caused dose-dependent mortalities of between 19 and 51.34%; this was accompanied by significant ( $p < 0.05$ ) knockdown in gene expression following 3 days of continuous feeding. The majority of larvae injected with, or fed, dsRNA died during the final larval stage prior to pupation. This work provides evidence of a viable RNAi-based strategy for insect control, which can be delivered *in planta* or as a biopesticide.

**Silencing genes involved in the interaction between the whitefly *Bemisia tabaci* and Tomato yellow leaf curl virus**

M. Ghanim, S. Kanakala

Department of Entomology, the Volcani Center, HaMaccabim Road 68, PO Box 15159, Rishon LeZion 7505101, Israel

\*e-mail: [ghanim@volcani.agri.gov.il](mailto:ghanim@volcani.agri.gov.il)

*Bemisia tabaci*, TYLCV, transmission, gene silencing

Tomato yellow leaf curl virus (TYLCV) is an important plant virus transmitted by the whitefly *Bemisia tabaci* in a persistent-circulative and propagative manner. TYLCV is transovarially and sexually transmitted and is associated with the whitefly for its entire life. The virus further reduces fecundity, fertility and lifespan of *B. tabaci* and alters the expression of stress- and immunity-related genes. Those interactions suggest the involvement of insect proteins in the response to the virus and in the transmission process. We have used several approaches including gene expression analyses upon virus acquisition, antibody interference and transmission experiments, fluorescent in situ hybridization, co-immunolocalization with TYLCV and RNA interference experiments in planta, to search for insect proteins that have a role in TYLCV transmission by *B. tabaci*. We have identified several proteins that have a role in the transmission of TYLCV by *B. tabaci* such as Cyclophilin B, a peptidyl-prolyl isomerase protein that facilitates protein folding, and Heat Shock Protein 70, that have many roles as a chaperone and response upon stress. Those insect proteins were silenced by expressing them in plants for feeding experiments. The silencing has significantly influenced the transmission of the virus and in some cases has cause high mortality rates. The involvement of both proteins and others in TYLCV - *B. tabaci* interactions was verified using numerous techniques. Those results and their implications in developing better means for vector-borne disease control will be presented.

## **RNA silencing as a tool for studying genes involved in fruit set**

B. Molesini<sup>1</sup>, G.L. Rotino<sup>2</sup>, T. Pandolfini<sup>1</sup>

<sup>1</sup>*Department of Biotechnology, University of Verona, Verona, Italy*

<sup>2</sup>*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di ricerca per l'Orticoltura (ORL), Montanaso Lombardo (Lodi), Italy*

\**e-mail: tiziana.pandolfini@univr.it*

*Fruit set, crop improvement, regulation of ovary growth*

In Angiosperms, fruit usually develops from the ovary, the basal part of the pistil. The first visible sign of fruit development, named fruit set, represents the transition of a quiescent ovary to a rapidly growing young fruit. In the current model for fruit set control ovary growth is maintained in a repressive status before anthesis and only after successful completion of pollination and fertilization, this constriction is released. Genes involved in fruit set and/or early fruit development are often transcriptionally regulated before and after fertilization. Consequently, the analysis of the expression pattern of a target gene might be indicative for its putative role during fruit development. By using hairpin constructs to elicit RNA silencing, we characterized the function of two genes involved fruit initiation in both tomato and *Arabidopsis*. We identified a gene family (*Aucsia*), whose suppression determined the development of the fruit without fertilization and its function is linked to auxin metabolism. A second one is the NAOD gene, whose knock down provoked an increased number of aborted fruits. We proved that the NAOD gene codes for an N-acetylmethyltransferase, which is involved in arginine and polyamine metabolism.

## **A comparison of target gene suppression by RNAi-guided Cas9 nuclease**

T. Lawrenson, O. Shorinola, M. Smedley, S. Hayta, P. Hundleby, W. Harwood  
*John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK*

*\*e-mail: wendy.harwood@jic.ac.uk*

*Gene editing, CRISPR/Cas9, RNAi, Cas9 nuclease, transgene insertion.*

The use of RNA interference silencing cassettes in crop plants can lead to different levels of suppression of target gene activity but rarely complete silencing. In our experience, reductions in target gene activity up to 50% are typically achieved. RNA-guided Cas9 techniques on the other hand more commonly lead to complete knock-out of target gene function. This has been achieved in a range of crops including barley, Brassica oleracea, wheat, tomato and potato and in many cases transgene free plants containing mutations in the target gene have been recovered. We will compare and contrast these two techniques in a range of crops focusing on new developments in the technologies and how this might impact future applications.

**Reversible male sterility induction system based on artificial-microRNA mediated inactivation of two general transcription factors in eggplant (*Solanum melongena* L.)**

L. Toppino<sup>1</sup>, M. Kooiker<sup>2</sup>, M. Lindner<sup>2</sup>, M. Kater<sup>2</sup>, G.L Rotino<sup>1</sup>.

<sup>1</sup> CREA-ORL, Unità di ricerca per l'Orticoltura, 26836 Montanaso Lombardo, (LO), Italy

<sup>2</sup> DBS, Dipartimento di Bioscienze Università degli Studi di Milano, via Celoria 26, Milano, Italy

\*e-mail: [Laura.toppino@crea.gov.it](mailto:Laura.toppino@crea.gov.it)

*Solanum melongena*, microRNA, silencing, TAF

Plant male sterility is considered since decades a powerful tool for biological containment to prevent unwanted self-pollination during the breeding process and for hybrid seed production. Furthermore, inhibition of pollen dispersal could also answer to the raising concerns regarding the transgene flow via pollen from GM crops and the strong demand for implementing technologies that will allow co-existence of GM and non-GM agriculture. However, for crops that are not usually vegetatively propagated, an irreversible system is undesirable; therefore, strategies for making the male sterility trait reversible, yet tightly controllable, are needed. We developed a conditional system that prevents gene transmission *via* pollen in eggplant. The strategy for inducing male sterility is based on the anther-specific artificial microRNA-mediated silencing of TAF10 and TAF13, two endogenous general transcription factors known as acting during pollen development. The amiRNA constructs target a specific sequence of TAF genes under control of TA29 and NTM19 promoters, expressed in the tapetum and microspore, respectively. The ability to complement potential eggplant male sterile TAF-amiRNA lines to restore fertility is based on ethanol-inducible expression of a RNAi-insensitive form of the TAF target gene retrieved from tomato. Transgenic eggplants result completely male sterile and fertility can be fully restored by short treatments with ethanol, confirming the efficiency but also the reliability of the system in view of open field cultivation. By combining this system with induced parthenocarpy we also provided a novel example of complete transgene containment in eggplant, which might lead to the acceptance of transgenic applications in crop plants.

## **Investigating the efficacy of RNA silencing-mediated epigenetic modifications in virus infected plants**

Y. Fei, A. Molnar

*University of Edinburgh, Institute of Molecular Plant Sciences, Rutherford building, Mayfield Road, Edinburgh, EH9 3BF, United Kingdom*

*\*e-mail: attila.molnar@ed.ac.uk*

*RNA silencing, virus, RNA-directed DNA methylation, environment*

In plants, virus infection is monitored and controlled by an RNA surveillance system referred to as RNA silencing. The sequence-specificity of RNA silencing relies on small (21-24 nucleotides) non-coding RNA (sRNA) molecules. Virus-derived sRNAs (vsRNA) are generated from viral RNA precursors by the coordinated and hierarchical action of antiviral nucleases, called Dicers. vsRNAs then associate with and guide effector nucleases, namely Argonautes, to cleave viral RNAs in a sequence-specific manner in the cytoplasm. However, the activity of vsRNAs is not restricted to the control of viral RNA accumulation. They can regulate host gene expression if host mRNAs share sequence complementarity with vsRNAs. Intriguingly, vsRNAs are also able to target and methylate homologous DNA sequences in the nucleus suggesting that vsRNAs have the potential to regulate endogenous genes at the transcriptional level by modifying the epigenetic status of their promoter sequences. Since DNA methylation is heritable, virus-induced transcriptional gene silencing (ViTGS) may open new avenues to modulate gene expression in viruses-infected crops and their progenies. However, very little is known about the efficacy of different plant viruses to induce ViTGS and how environmental factors affect the initiation and stability of ViTGS. We will present data to address these questions.

## **The control of cortex anatomy via miRNA target mimicry**

G. Di Ruocco, G. Bertolotti, E. Pacifici, L. Polverari, S. Sabatini, P. Costantino and R. Dello Ioio

*Dip. Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma*

\*e-mail: [raffaele.delloioio@uniroma1.it](mailto:raffaele.delloioio@uniroma1.it)

Through secondary growth of the cortex, roots control air quantity in plants growing on wet soils (aerenchyma), and store nutrients in plants growing under adverse weather and nutritive conditions (storage parenchyma). Root cortex anatomy, basically the number of cortex layers, thus varies between different plant species.

Comparing the development of *Arabidopsis thaliana* (one cortex layer) and *Cardamine hirsuta* (two cortex layers), very recently we identified a novel mechanism where the spatial distribution of a miRNA (miR165/6) in the root ground tissue controls the localization of a transcription factor (PHABULOSA, PHB) that in turn controls the number of cortex layers (Di Ruocco et al, in preparation). This mechanism is most likely conserved in (related) crop species such as turnip (*Brassica rapa*).

We will reduce the activity of miR165/6 in the turnip cortex by miRNA target mimicry, i.e. by tissue-specific expression of MIM165/6, a DNA fragment that mimicks the miR165/6 target sequence: this will increase the number of cortex layers, thus increasing turnip storage capability and performance.

**POSTERS**

**Exploring the miRNAome in *Haberlea rhodopensis* – a model plant for drought tolerance**

M. Topalov, G. Yahubyan, M. Gozmanova, E. Daskalova, E. Apostolova, V. Baev

*Department of Plant Physiology and Molecular Biology, University of Plovdiv, 24 Tsar Assen St, 4000 Plovdiv, Bulgaria*

*\*e-mail: vebaev@plantgene.eu*

*miRNAs, Haberlea rhodopensis, NGS*

*Haberlea rhodopensis* is a paleolithic tertiary relict species best known as resurrection plant with remarkable tolerance to desiccation. *H. rhodopensis* exposed to severe drought stress shows ability to maintain the structural integrity of the photosynthetic apparatus which reactivates easily upon rehydration. Profiling of miRNAs by NGS has progressed rapidly and is a promising field for applications in RNAi. Here, we present the small RNA-seq analysis of 3 libraries derived from control (HRH), dehydrated (HRD) and rehydrated (HRR) plants. So far, the miRNAome of *Haberlea rhodopensis* remains unknown. We have identified 131 conserved miRNAs in the NGS datasets based on homology search with miRBase database. Most abundant miRNAs in the libraries were miR159a, miR396a-5p, miR159a, miR166a-3p and miR166e. The differential expression analysis showed a specific miRNA profiles upon dehydration and rehydration stage and revealed changes in expression level of several miRNAs, which are observed also in other plant species as key players in abiotic stress response. For example, miR396a-5p was found in tobacco to act as stress-responsive gene by increasing the tolerance to drought and cold, which we also confirmed to be upregulated in the process of dehydration in *Haberlea rhodopensis*. Upon rehydration process, most deregulated miRNAs were miR396a-5p, miR159a, miR168a and miR168b. Furthermore, we are going to experimentally validate selected miRNAs which can hold potential as drought tolerance biomarkers or can be even used as an artificial siRNA in plant molecular engineering.

## **Plant viruses for delivery of RNAi triggers for pest control: possibilities and obstacles**

L. Swevers<sup>1</sup>, J. Vontas<sup>2</sup>, K. Kalantidis<sup>3</sup>

<sup>1</sup>*Institute of Biosciences & Applications, NCSR "Demokritos", Aghia Paraskevi, Athens, Greece.*

<sup>2</sup>*Pesticide Science Lab, Agricultural University of Athens, Athens, Greece.*

<sup>3</sup>*Department of Biology, University of Crete, Heraklion, Greece.*

\*e-mail: swevers@bio.demokritos.gr

*plant virus, biopesticide, pest control, Hemiptera*

RNAi shows enormous potential for development as a safe and specific method for pest control but for many pests the efficiency of dsRNA delivery remains an important obstacle. The successful application of insecticidal RNAi seems to be affected by two main factors: (1) stability of dsRNA in environment, gut and insect tissues; and (2) efficient cellular uptake of dsRNA and processing by the insect RNAi machinery. Transgenic plants producing dsRNA were developed and shown to induce silencing in targeted insects but production of unprotected dsRNA in plant cells inevitably results in interaction with nucleases and plant RNAi which will reduce its yield. Here we explore the potential of plant viruses to function as delivery vehicles of RNAi to insects. Many plant feeders, most notably Hemiptera (aphids, whiteflies, planthoppers, leafhoppers, scale insects), transmit plant viruses in non-persistent and persistent (including circulative and propagative) fashion and therefore could be considered as targets for this approach. Plant viruses are predicted to function as specific and effective vectors while they provide natural protection against degradation and (in some cases) a natural amplification mechanism. However, for exploitation of plant viruses as delivery vectors of RNAi, many important issues need to be addressed, such as: (1) how do (wild-type and recombinant) plant viruses affect the physiology of the plant hosts?; (2) what defense mechanisms are triggered by plant viruses in the insect vectors?; (3) can efficient production systems for different classes of plant viruses be realized?; among many others. In this presentation an overview of the literature is presented regarding possibilities and obstacles for the use of plant viruses in RNAi-mediated pest control.

## **MicroRNA and tobacco species: Macedonian story yet to be told**

M.Terziki<sup>1</sup>, E.Shukarova Stefanovska<sup>1</sup>, E. Miskoska Milevska<sup>2</sup>, Z. Popovski<sup>2</sup>, Z.Arsov<sup>2</sup>, D. Plasheska Karanfiliska<sup>1</sup>

<sup>1</sup>*Research Center for Genetic Engineering and Biotechnology “Georgi D. Efremov”, Macedonian Academy of Sciences and Arts, Skopje, R. of Macedonia*

<sup>2</sup>*Faculty for Agricultural Sciences and Food, University of Ss Cyril and Methodius, Skopje, R. of Macedonia.*

\*e-mail: marijamirc2003@yahoo.com

*Tobacco microRNA, nicotine biosynthesis, etheric oils in Tobacco*

Micro- RNA molecules (miRNAs), as class of small endogenous regulator molecules deriving from double-stranded RNA precursors, are proposed to impact RNA silencing and have an effect on gene expression in eukaryotic cells. The influence of different miRNAs on gene regulation has been proven in a wide variety of plant metabolic and biological processes, such as: leaf development, root growth, organ maturation or tolerance to environmental stress. Determination of miRNAs expression promises to be a useful tool for functional genomics and crop biotechnology. However their silencing effect is still not completely cleared and more studies should be performed. Tobacco plant (*Nicotiana tabacum*) is an important economic and agricultural crop in the R. of Macedonia, with cca. 13 000ha of areas planted, yearly production of over 25 000t and around 150 000 people living from its cultivation. The most abundant and economically important alkaloid in tobacco is nicotine. Other valuable quality related feature is the etheric oils content in the aromatic tobacco species. Eleven miRNAs have already been published as molecules that have an influence on tobacco growth and development.

Having all the necessary infrastructure and human resources this is an attempt to initiate miRNA research in plant species in our country and through this COST action to learn from the experts with great experience who are deeply involved in this thematics. The intention of the RCGEB “Georgi D. Efremov” is to examine the influence of microRNAs and their target genes involved particularly in the nicotine biosynthesis and the etheric oils content in the autochthonous tobacco species in the R. of Macedonia. Since this is the first time ever to tackle this matter in our country, we expect to have some initial findings and in that way to contribute to the worldwide database on this subject, as well as to put the R. of Macedonia on the scientific map in the area of plant miRNA.

## **Selection and validation of novel targets for RNAi-based control of *Colorado potato beetle***

M. Petek, K. Gruden

*National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, 1000 Ljubljana, Slovenia*

*\*e-mail: marko.petek@nib.si*

*Leptinotarsa decemlineata, RNA interference (RNAi), insecticides, NGS, target specificity*

In the last decade double stranded RNAs (dsRNAs) have shown the potential to become environmentally acceptable insecticidal agents. In Colorado potato beetle (CPB), a serious potato pest, RNAi-mediated gene silencing is effectively triggered by dsRNA ingestion. Insecticidal dsRNA could therefore also be applied in potato fields by spraying. It is crucial that these dsRNAs act specifically on the pest species and that the mechanism of its action is well understood. To select good RNAi target genes, we have integrated the CPB transcriptome annotations with gene expression data and the publicly available *Drosophila melanogaster* and *Tribolium castaneum* RNAi screening datasets. Using an *in-silico* approach we narrowed down the target list by excluding genes with potential effect on other species (conserved nucleotide sequence) and genes present in the NCBI patent database. We have selected three target genes and used CPB feeding trials to test the efficiency of designed dsRNAs. Experiments are underway to explore changes induced by one of the selected dsRNA on the transcriptome of CPB guts and its effect on gut metagenome using next generation sequencing.

## **Expression analysis of candidate genes responsible for bud abscission in pistachio**

S. Kafkas<sup>1</sup>, E. Kafkas<sup>1</sup>, M.Güney<sup>2</sup>, M. Zarifikhosroshahi<sup>1</sup>, H. Karci<sup>1</sup>, N. Çoban<sup>3</sup>, H. Topçu<sup>1</sup>, M.A. Gündeşli<sup>4</sup>, S. Eti<sup>1</sup>, Ş.Karabiyik<sup>1</sup>, N.Aslan<sup>5</sup>, S. Arpacı<sup>6</sup>

<sup>1</sup>University of Çukurova, Faculty of Agriculture, Department of Horticulture, 01330, Balcalı, Adana, TURKEY

<sup>2</sup>University of Bozok, Faculty of Agriculture, Department of Horticulture, Yozgat, TURKEY

<sup>3</sup>Eastern Mediterranean Agricultural Research Institute, Adana, TURKEY

<sup>4</sup>East Mediterranean Transitional Zone Agricultural Research of Institute, K.Maraş, TURKEY

<sup>5</sup>Pistachio Research Institute, G.Antep, TURKEY

<sup>6</sup>Fig Research Institute, Aydın, TURKEY

\*e-mail: salihkafkas@gmail.com

*Pistachio, Pistacia vera, alternate bearing, bud abscission*

Alternate bearing is one of the most economically important problem in nut crops particularly in pistachios. This is a common phenomena in pistachio, and all cultivars in the production have tendency to alternate bearing. Bud abscissions severely occur in On year trees, and the trees may not have nuts in the following year, while Off year pistachio trees have less bud abscission with a good crop load in the next year. In this study, we aimed to find genes related to bud abscission which causes alternate bearing in pistachio. We did bud sampling from “On” and “Off” year trees of “Uzun” cultivar in 2015 growing season by 10 day intervals with three replicates. Transcriptom sequencing was performed in buds from On and Off year trees with three replicates for the first five sampling dates based on scanning electron microscope results. A total of 30 RNAs were sequenced and 4Gb clean data were obtained from each sample. We had a few candidate up-regulated and down-regulated genes from DEG analysis. We also sampled leaves, shoots, buds (from On and Off year trees), peduncles, nuts and kernels (from On year trees) by 15 days intervals from early spring to late harvesting during 2016 season for gene expression analysis. We tested eight candidate genes in all samples by RT-PCR analysis. Gene expression analysis showed that all candidate genes had higher expression levels in On year buds than Off year bud samples. The genes were highly expressed in the other tissues as well.

## **Seed priming, a tool for plant priming?**

E. Karalija

*Department for biology, Faculty of natural sciences and mathematics, University of Sarajevo Zmaja od bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina*

*\*e-mail: [erna.karalija@gmail.com](mailto:erna.karalija@gmail.com)*

*Seed priming, plant priming, biological priming*

One of the most critical stages in plant life is the emergence of the seedling. The seed priming is intended for improvement of the uniformity and germination rate by partially hydrating the seeds. On the other hand, plant priming can give a plant a chance for faster response to stress. By combining these two methods we could accomplish plant priming through seed priming. It is difficult to correlate phenotypic changes to priming effects, but with molecular aspects of primed state (seeds and emerged seedlings) would allow us to determine which factor is responsible for the primed state. The use of the knowledge of transcriptomics, proteomics and metabolomics should provide the tools for the identification of mechanisms involved in biological priming. After identification of the responsible genes it will be possible to conduct constitutive priming by silencing and enhancing the responsible genes. Beside the importance of this technique for agriculture in the light of the productivity it is also important to emphasize the importance of biological seed treatments in contrast to use of agrochemicals. Biological priming is a method with great prospects not only for its environmental safety but also socioeconomic aspect.

## MicroRNA-based molecular markers in plant research

K. Ražná<sup>1</sup>, L. Hlavačková<sup>1</sup>, J. Nôžková<sup>1</sup>, J. Moravčíková<sup>2</sup>

<sup>1</sup> Slovak University of Agriculture in Nitra, Faculty of Agrobiological and Food Resources, Department of Genetics and Plant Breeding, Slovak Republic

<sup>2</sup> Institute of Plant Genetics and Biotechnology SAS, Nitra, Slovak Republic

\*e-mail: katarina.razna@uniag.sk

*flax, microRNA, miRNA markers, genome mapping*

Molecules microRNAs play important role in the regulation of gene expression within various biological, metabolic and developmental processes as well as under various biotic and abiotic stresses at the post-transcriptional level. The miRNA-based molecular markers represent a type of functional markers reported so far in very few plants. The stability, polymorphism, functionality and transferability of miRNA-based markers make it possible to apply selected families of miRNAs (miR156, miR168, miR171 and miR408) for our purposes. The research was focused on (1) the identification of the functional markers as an interface between selected morphological traits and miRNA-based markers and (2) genotyping analysis of several flax (*Linum usitatissimum* L.) genotypes considering different micro environmental conditions, developmental stages connected to flower and seed formation and various content of alpha-linolenic acid. The analyses were done by miRNA-based molecular markers system using the touchdown PCR and qualitative Real-Time PCR. By means of miRNA marker assay have been recorded the unique profiles of miRNA loci as well as some functional markers. By mutually linking of molecular miRNA-based markers and morphological markers was possible to determine the unique genotypes of flax. The increase of miRNA activity has reflected the individual developmental stages from flower bud formation up to boll development. Results based on the average value of threshold cycle of miRNAs (miR156 and miR168) and the reference gene UBE2 (Ubiquitin-conjugating enzymes E2), value  $2^{-\Delta\Delta CT}$  was calculated. Results have suggested significant differences in the miR156 and miR168 activity of flax genotypes with different content of alpha-linolenic acid.

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## Session 2- RNAi Applications (WG2)

### ORAL

#### **RNAi-mediated strategy to control the African Sweetpotato weevils *Cylas puncticollis* and *Cylas brunneus* (Coleoptera, Brentidae)**

K. Prentice<sup>1,2,3</sup>, O. Christiaens<sup>2</sup>, I. Pertry<sup>3</sup>, M. Ghislain<sup>4</sup>, G. Gheysen<sup>2</sup>, G. Smagghe<sup>1</sup>

<sup>1</sup> Department of Crop Protection, Ghent University, B-9000 Ghent, Belgium

<sup>2</sup> Department of Molecular Biotechnology, Ghent University, B-9000 Ghent, Belgium

<sup>3</sup> VIB, Institute of Plant Biotechnology Outreach, B-9052 Ghent, Belgium

<sup>4</sup> International Potato Center (CIP), Genomics and Biotechnology Program, Nairobi 00603, Kenya

\*e-mail: [katterinne.prenticemuro@ugent.be](mailto:katterinne.prenticemuro@ugent.be)

*African sweetpotato weevil, Cylas puncticollis, Cylas brunneus, RNAi, dsRNA, microinjection, oral feeding*

The African sweetpotato weevils *Cylas puncticollis* and *Cylas brunneus* (SPW) are considered the major threats to sweetpotato production in Sub-Saharan Africa (SSA). RNA interference (RNAi) technology can add genetic resistance against SPW in sweetpotato. The presence of an efficient RNAi response and the identification of suitable target genes are important prerequisites to use RNAi as pest control strategy. In this study, we confirmed the functionality of the RNAi mechanism in SPW by silencing *laccase2* through microinjection, a gene which is involved in the sclerotization of the exoskeleton. Subsequently, we performed a screening for lethality of 24 potential target genes by microinjection. Twelve and fourteen dsRNAs showed high toxicity with more than 90% mortality for both *C. puncticollis* and *C. brunneus*, respectively. Based on these results, the three most lethal dsRNAs were chosen for oral delivery assays. These revealed that dsRNA by oral exposure could elicit a significant toxicity on both insects, although less sensitive for *C. puncticollis*. *Ex vivo* assays confirmed that dsRNA uptake is affected by degradation in *C. puncticollis* digestive system. In this research, we proved that RNAi has the potential to control sweetpotato weevils, especially for *C. brunneus*, while the delivery of dsRNA and possible protection against degradation might have to be optimized in *C. puncticollis*.

## **Host-induced gene silencing for the control of *Fusarium* head blight in wheat fields**

K. Kanyuka<sup>1</sup>, A. Machado<sup>1</sup>, M. Urban<sup>1</sup>, W. S. Lee<sup>1</sup>, N. Brown<sup>1</sup>, R. King<sup>1</sup>, E. Yamazaki Lau<sup>2</sup>, C. Sparks<sup>1</sup>, A. L. V. Bonato<sup>2</sup>, C. S. Tibola<sup>2</sup>, N. F. Martins<sup>3</sup>, F. J. Aragão<sup>3</sup>, J. West, J. M. C. Fernandes<sup>2</sup>, K. E. Hammond-Kosack<sup>1</sup>

<sup>1</sup>*Plant Biology and Crop Science Department, Rothamsted Research, Harpenden AL5 2JQ, UK  
(kostya.kanyuka@rothamsted.ac.uk)*

<sup>2</sup>*Centro Nacional de Pesquisa em Trigo, Embrapa, Passo Fundo, RS, Brazil*

<sup>3</sup>*Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil*

\*e-mail: kostya.kanyuka@rothamsted.ac.uk

*Fusarium graminearum, Fusarium culmorum, wheat, HIGS, disease resistance*

Fusarium head blight (FHB) is one of the most serious and hazardous crop diseases worldwide. The main consequence of FHB is that trichothecene mycotoxins, such as deoxynivalenol (DON), accumulate in the grain, presenting a health risk to humans and animals. In Southern Brazil, where 90% of Brazilian wheat is grown, severe FHB epidemic years occur at a minimum of every 4 or 5 years. Legal limits have been set on the DON levels permitted in harvested grain used for different purposes. However, even moderate FHB years are highly problematic causing the lack of available safe grain for use either on farms or for sale into the market. For low-income Brazilian farmers, FHB disease reduces the standards of living of farmer's families and that of their local communities. There is a pressing need to develop novel and effective FHB control options. Here we will introduce our new bilateral BBSRC-Embrapa collaborative project (started November 2016) in which we intend to take a novel whole fungal genome and disease modelling guided approach to develop a pipeline of genetically modified wheat genotypes harbouring hairpin T-DNA constructs, which can silence *Fusarium* spp. genes critical for wheat infection via host-induced gene silencing (HIGS). We also intend to determine the plant and fungal mechanisms that control the HIGS phenomenon.

## **Field trials for *Plum pox virus* resistance of honeysweet transgenic plum in Romania**

I. Zagrai<sup>1</sup>, M. Ravelonandro<sup>2</sup>, R. Scorza<sup>3</sup>, L. Zagrai<sup>1</sup>, N. Minoiu<sup>1</sup>

<sup>1</sup>*Fruit Research & Development Station Bistrita, Romania*

<sup>2</sup>*Institut National de la Recherches Agronomique Bordeaux, France*

<sup>3</sup>*Appalachian Fruit Research Station USDA-ARS, Kearneysville, WV, USA*

\*e-mail: [izagrai@yahoo.com](mailto:izagrai@yahoo.com)

*Plum pox virus*, resistance, transgenic plum, CP gene

*Plum pox virus* (PPV), known as the most detrimental viral pathogen of stone fruits, makes fruit production very problematic. There is a paucity of naturally high level resistance to PPV in plum, so genetic engineering remained an alternative approach for plum protection against PPV. According to our experience, the use of genetically-based plant resistance to PPV represents the most viable virus control strategy. 'HoneySweet' transgenic plum was developed by inserting the PPV-coat protein (CP) gene into the plum genome to obtain Pathogen Derived Resistance. The result was a protection against PPV based on RNA interference. 'HoneySweet' plum trees were the subjects of different field trials for PPV resistance undertaken in several European countries, Czech Republic, Poland, Romania and Spain. The first experiment in Romania, that ran for a period of 8-10 years, addressed the behavior of 'HoneySweet' under high natural PPV infection pressure in three experimental field plots. While the rate of conventional plums infected continuously increased, none of the 'HoneySweet' trees became naturally infected by aphids, demonstrating a high and durable resistance to PPV in endemic areas. A second experiment was related to the stability of PPV resistance in 'HoneySweet' artificially inoculated with PPV alone or in combination with two heterologous viruses [*Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV)]. Regardless of singular (PPV) or mixed (PPV+PDV, PPV+PNRSV) inoculation, PPV can be artificially transferred to the 'HoneySweet' plants by grafting. However the infection remained close to the inoculation site and did not systemically spread, indicating an effective inhibition of PPV genome replication. A third field trial is currently underway with the main aim of gaining further information on the agronomic and phenotypic performance of 'HoneySweet' under PPV endemic and geo-climatic conditions of Romania.

## **RNAi-mediated resistance to the quarantine virus causing sharka disease in stone fruit**

V. Ilardi<sup>1</sup>, E. Di Nicola<sup>1</sup>, S. Monticelli<sup>2</sup>, A. Gentile<sup>2</sup>, R. C. Garcia-Almodovar<sup>3</sup>, L. Burgos<sup>3</sup>, M. Tavazza<sup>4</sup>

<sup>1</sup>CREA-Centro di ricerca per la patologia vegetale, Rome, Italy

<sup>2</sup>CREA-Centro di ricerca per la frutticoltura, Rome, Italy

<sup>3</sup>Departamento de Mejora de Plantas, CEBAS-CSIC, Murcia, Spain; <sup>4</sup>ENEA Casaccia, Rome, Italy

\*e-mail: [vincenza.ilardi@crea.gov.it](mailto:vincenza.ilardi@crea.gov.it)

*Plum pox virus, PPV, transgenic plants, RNA silencing*

Sharka, the most important diseases of stone fruits, is caused by the quarantine pathogen *Plum pox virus* (PPV). At least seven PPV strains have been defined, of which PPV-D, -M and Rec are the most agronomically important. To identify the best RNAi construct capable of conferring PPV resistance in plum, we initially transformed *Nicotiana benthamiana* plants with four different PPV-M sequences arranged to express self-complementary hairpin RNAs, and covering the PPV 5'UTR region, P1 and HC-Pro genes. Each construct, when stably expressed in plants, conferred resistance/immunity to viral isolates of PPV-D, -M and Rec strains. Importantly, transgenic plants harboring 5'UTR/P1 sequences were also highly resistant to the distantly related PPV-EA and PPV-C strains, to PPV infection conducted at low and high temperature, and when previously challenged with *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) or *Artichoke mottled crinkle virus* (AMCV). Thus, the 5'UTR/P1 construct confers a wide and stable PPV resistance under abiotic and biotic stress conditions known to potentially have a detrimental impact on RNAi-mediated virus resistance thus supporting it as the best candidate to produce PPV-resistant transgenic plums. To this end, the plum, cv Stanley was independently transformed, in Italy and Spain, with the 5'UTR/P1 obtaining a total of 12 transgenic clones which were *in vitro* grafted onto PPV-D infected 'GF305' rootstocks. Nine out of 12 clones were resistant to the heterologous strain of PPV. Moreover, the Spanish PPV-resistant transgenic plants were further evaluated for PPV resistance under greenhouse conditions confirming the results obtained *in vitro*. Overall, our data suggest that 5'UTR/P1 construct can be profitably used to generate transgenic stone fruit trees having a robust and wide spectrum of resistance to distinct PPV strains.

## **The bottlenecks in obtaining an efficient transformation protocol for RNAi-based sharka resistant peach**

S. Sabbadini<sup>1</sup>, C.O. Limeri<sup>1</sup>, B. Molesini<sup>2</sup>, T. Pandolfini<sup>2</sup>, O. Navacchi<sup>3</sup>, B. Mezzetti<sup>1</sup>

<sup>1</sup>*Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, Ancona, IT*

<sup>2</sup>*Dipartimento di Biotecnologie, Università di Verona, Strada le Grazie 15, Verona, IT*

<sup>3</sup>*Vitroplant Italia, Cesena IT*

\*e-mail: [b.mezzetti@univpm.it](mailto:b.mezzetti@univpm.it)

*Peach, genetic transformation, PPV, gene silencing, rootstock*

Stone fruits, especially peach (*Prunus persica*), are among the most important tree species grown in the Mediterranean basin subjected to viral infections, in particular caused by Plum Pox Virus (PPV), the etiologic agent of Sharka disease, which leads to significant agronomic and economic losses. At the moment, there are no means of direct struggle against such infection but only means of prevention, which are often not effective and associated to environmental sustainability issues and costs for farmers. For these reasons, many programs of genomic studies and traditional breeding are aimed at understanding the mechanisms of resistance. A possible integrative strategy to classical breeding techniques is represented by the use of genetic engineering technology to obtain transgenic plants of cultivars and rootstocks bearing resistance genes. *Prunus*, in particular peach, is considered one of the most recalcitrant species for what concern *in vitro* regeneration and transformation, especially when the starting plant material originates from mature tissues. The study here described has the main objective of transferring and adapt the protocol previously developed in grape (Mezzetti et al., 2002) to the *in vitro* regeneration, via organogenesis, and *Agrobacterium*-mediated transformation of the peach rootstock GF677 (*Prunus persica* x *Prunus amygdalus*). The ultimate purpose is the introduction of an anti PPV RNAi construct, named ihp35S-PPV194.,

**POSTER**

**Investigation on the transgenic plum *Prunus domestica* L., clone c5 (cv. Honeysweet) for protection against sharka disease**

J. Polák<sup>1</sup>, J. K. Kundu<sup>1</sup>, P. Komínek<sup>1</sup>, E. Beoni<sup>1</sup>, And T. Neubauerová<sup>1</sup>

<sup>1</sup>*Division of Crop Protecting and Plant Health, Crop Research Institute, Drnovská 507, 16106 Prague, Czech Republic*

\*e-mail: polak@vurv.cz

*Plum pox virus, resistance, transgenic plum, RNA interference, C5, HoneySweet*

Sharka caused by *Plum pox virus* (PPV) is the most harmful disease of stone fruits in Europe and elsewhere in the world. There is no highly PPV resistant cultivar of plum. Biotech approach has led to the development of resistance through genetic engineering. In this study, we evaluated a transgenic plum *Prunus domestica* L., clone C5 (cv. HoneySweet), where the PPV resistance is based on RNA interference (RNAi). Resistance in C5 plums has been evaluated for PPV, Prune dwarf virus (PDV), and *Apple chlorotic leaf spot virus* (ACLSV) in a regulated field trial in the Czech Republic for twelve years (2002-2013). Even under high and permanent infection pressure introduced through graft inoculation of the viruses, PPV has been detected in C5 trees only in several leaves situated close to the point of inoculum grafting in the first nine years. Mild symptoms of PPV disappeared year by year. No PPV symptoms were observed in the following three years and results of ELISA detection were negative. Similar results were obtained, when RT-PCR was used for PPV detection. There was severe attack of transgenic plum trees by *Monilia* sp. In the twelfth's year. Mild PPV symptoms has appeared again in several leaves in the next year after the *Monilia* sp. infection. The presence of PPV was confirmed by ELISA and RT-PCR not only in symptomatic leaves, but also in several fruits showing no symptoms. No natural infection of PPV by aphids was recorded in C5 plums, even if PPV infected plants were growing in the same experimental field in close vicinity. Co-infections of PPV with PDV and/or ACLSV had practically no influence on stability of resistance in C5 trees. Twelve years of field testing in the Czech Republic clearly demonstrated the high level of resistance of C5 either to PPV infection by graft inoculation or natural infection through aphid vectors.

## **Applications of genome engineering technologies to rewrite disease resistance system**

E. Cappetta, G. Andolfo, M.R. Ercolano

*Department of Agricultural Sciences, University of Naples 'Federico II', Via Università 100, 80055Portici (Naples), Italy*

*\*e-mail: ercolano@unina.it*

*Defense system, RNAi, Genome-Editing, Effector, R-gene.*

The genome engineering technologies are signing the beginning of a new era, in which the synthetic biology lays the base for obtaining a reinforced plant defense system. Various research applications, based on RNA interference (RNAi) technology, were able to silence the gene expression with an high degree of specificity. This approach proved to be effective for obtaining resistance against phytopathogens controlling gene expression involved in the resistance process. Recently, a gene editing technology named Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 (CRISPR/Cas9) has received wide acceptance for its versatility in genetic applications. Unlike RNAi, the CRISPR/Cas9 system is capable to introduce heritable precision insertions and deletions in the plant genome. Several gene-editing applications can be used to modify players of plant immunity to obtain pathogen-resistant crops. RNAi cannot be used for such precision genome engineering applications since it primarily acts on transcript degradation machinery. However, RNAi and CRISPR/Cas9 can be used as useful tools for various scientific purposes. Both present advantages and disadvantages to improve the plant innate immunity system. A comparison of these two technologies (RNAi and CRISPR/Cas9) is reported suggesting strategies and targets for improving plant disease resistance.

## **Antisense RNA silencing of the TROL protein involved in plant photosynthetic energy partitioning**

H. Fulgosi, S. Jurić

*Laboratory for Plant Molecular Biology and Biotechnology, Division of Molecular Biology, Institute Ruđer Bošković, Bijenička cesta 54, HR-10 000 Zagreb, Croatia*

\*e-mail: fulgosi@irb.hr

*Arabidopsis thaliana, agroinfiltration, ferredoxin NADP<sup>+</sup> oxidoreductase*

Although basic mechanisms of plant photosynthesis have been established, regulatory pathways of energy partitioning are still largely unknown. We have proposed that At4g01050 gene product (TROL protein) from *Arabidopsis thaliana* (L.) Heynh., due to its catalytically inactive rhodanese domain and FNR binding, participates in regulation of photosynthetic electron partitioning (1). Many known catalytically inactive rhodanese domains are implicated in regulation, most commonly linked with signalling processes. Here we present reverse genetic approach to study the functions of the At4g01050 gene by using antisense RNA silencing. The At4g01050 cDNA was inserted into pGPTV-BAR binary vector in the antisense orientation, with respect to CaMV 35S promoter. *Arabidopsis* plants were subsequently transformed with pGPTV-antiTROL construct by using agroinfiltration. Plants with silenced TROL were subsequently analysed on molecular, biochemical, and cell biology levels (2).

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## **Transformation of spring barley with partial WDV replication protein**

P. Cejnar<sup>1</sup>, L. Ohnoutková<sup>2</sup>, M. Kostková<sup>2</sup>, T. Vlčko<sup>2</sup>, J. K. Kundu<sup>1</sup>

<sup>1</sup>*Crop Research Institute, Division of Crop Protection and Plant Health, Drnovská 507/73, 16106 Prague 6, Czech Republic*

<sup>2</sup>*Institute of Experimental Botany AS CR & Palacký University, Laboratory of Growth Regulators, Centre of the Region Hana for Biotechnological and Agricultural Research, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

\*e-mail: [jiban@vurv.cz](mailto:jiban@vurv.cz)

### *WDV, barley, Agrobacterium-mediated transformation*

*Wheat dwarf virus* (WDV) is one of the most serious viral diseases in cereals in the Czech Republic. It affects economically important species, mainly winter wheat and winter barley. The virus is transmitted by the leafhopper (*Psammotettix alienus*). The characteristic symptoms of WDV in infected plants include dwarfing, mottling, streaking of leaves, severe stunting, and even plant decline (principally in wheat, barley, and oat crops). Genetic engineering allows us to create transgenic plants with resistance to the virus. In this regard, expression vector pIPKb was prepared with a segment of replication protein of WDV. We used *Agrobacterium*-mediated method for the transformation of immature zygotic embryos of the spring barley variety Golden Promise. Explants and resulting calli were cultivated on selection medium for 8 weeks and later transferred to regeneration medium. Regenerated plants were then planted in soil and DNA was isolated from the leaves. The presence of the transgenic transcript was verified by PCR.

## **Down-regulation of endogenous peanut genes via RNAi for the reduction of peanut allergenicity**

O. Launer, O. Shoseyov

*The Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot 76100, Israel*

*\*e-mail: ofri.launer@mail.huji.ac.il*

*Peanut, RNAi, Ara h 1, Ara h 2, Ara h 3, Ara h 6*

Among the many types of food allergies currently known, peanut allergies are undisputedly one of the most severe and frequent, causing approximately 200 deaths/year in the USA alone. Due to the extensive use of peanuts and their by-products in the food industry, avoidance may not be a realistic solution for people suffering from allergies to peanuts. Four of the well-known *Arachis hypogaea* (peanut) allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6 are, ironically, seed storage proteins and therefore abundant in the seed. Furthermore, the Ara h 2 and Ara h 6 proteins were shown to contain cores that are highly resistant to proteolytic digestion, making them an even greater threat if unconsciously consumed.

In this study, we attempt to down-regulate the expression of the Ara h 1, 2, 3 and 6 genes using RNAi. We aim to generate *Arachis hypogaea* plants with reduced levels of all four proteins, and predict significant decrease in allergenicity of such plants. Initial scanning of the transformed plants will be performed using dot-blot. The more promising candidates will be scanned using PCR, RT-PCR and Western blotting to confirm the genetic transformation was indeed successful in DNA, mRNA and protein levels, respectively. Generation of the described plants will be followed by optimization of the silenced phenotype via self-fertilizations. The described plants could have great potential if we could demonstrate significant decrease in their allergenicity.

**SESSION 3 - RNAi biosafety (WG3)**

**ORAL**

**Environmental fate of double-stranded RNA (dsRNA)-mechanistic studies on dsRNA adsorption and degradation in laboratory systems**

K.M. Parker,<sup>1</sup> B. Mateescu,<sup>2</sup> M. Sander<sup>1</sup>

<sup>1</sup>*Institute of Biogeochemistry and Pollutant Dynamics (IBP), Department of Environmental Systems Science, ETH Zurich, 8092 Zurich, Switzerland*

<sup>2</sup>*Department of Biology, ETH Zurich, 8092 Zurich, Switzerland*

*\*e-mail: kimberly.parker @usys.ethz.ch*

*Environmental fate, adsorption, degradation, risk assessment, exposure modeling*

Genetically modified crops using RNA interference (RNAi) express double-stranded RNA (dsRNA) to alter plant metabolism or provide protection against disease and pests. Limited information is currently available on the processes that govern the fate of plant-released dsRNA in environmental matrices including agricultural soils, resulting in uncertainty in exposure analysis required for environmental risk assessment of RNAi crops. To reduce these uncertainties, we have developed approaches to evaluate the adsorption and transformation of dsRNA in environmental matrices including soils, sediments and surface water bodies. Experiments investigated the impact of microbial and enzymatic degradation, abiotic transformation, and adsorption to particle surfaces on dsRNA fate in these environmental matrices. The contribution will highlight the application of new experimental approaches to overcome methodological and conceptual challenges associated with studying dsRNA fate. Overall, this work leads to a more complete understanding of the stability and distribution of dsRNA in environmental matrices and informs exposure analysis in risk assessment models.

## **Regulating GMOs in Europe: how science law is moving to laws of fears. And why it matters**

K. Purnhagen

*Wageningen University, Law and Governance Group*

\*e-mail: [kai.purnhagen@wur.nl](mailto:kai.purnhagen@wur.nl)

*GMO regulation, Co-existence, Laws of Fears. Behavioural Economics*

Since 2010 EU co-existence rules gradually changed to allow Member States more freedom to determine GMO free territory. Art. 26b Directive 201/18/EC now explicitly allows Member States to opt out of a license for GMO cultivation. This Article aims to assess the reasons and possible consequences from a socio-legal perspective. It will first map the underlying legal regime and illustrate the corresponding principles and value judgments. It will then determine where the recent changes impose challenges to the underlying legal regime. Subsequently it will place these changes in the broader concept of behavioural economics. It will argue that these changes on the one hand do not reflect current (natural scientific) backup, but can be explained by finding from behavioural economics. In order to bring natural science and economic arguments to the forefront, long-term investments in a stronger framing of decisions is needed.



***iPlanta***

## **Canadian regulatory perspectives on genome engineered crops**

Stuart J. Smyth

*Department of Agricultural and Resource Economics, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8, Canada*

*\*e-mail: stuart.smyth@usask.ca*

*biotechnology, gene editing, GM apples, GM potatoes, innovation, regulation, risk*

New breeding techniques in plant agriculture exploded upon the scene about two years ago, in 2014. While these innovative plant breeding techniques, soon to be led by CRISPR/Cas9, initially appear to hold tremendous promise for plant breeding, if not a revolution for the industry, the question of how the products of these technologies will be regulated is rapidly becoming a key aspect of the technology's future potential. Regulation of innovative technologies and products has always lagged that of the science, but in the past decade, regulatory systems in many jurisdictions have become gridlocked as they try to regulate genetically modified (GM) crops. This regulatory incapability to efficiently assess and approve innovative new agricultural products is particularly important for new plant breeding techniques as if these techniques are classified as genetically modified breeding techniques, then their acceptance and future will diminish considerably as they will be rejected by the European Union. Conversely, if the techniques are accepted as conventional plant breeding, then the future is blindingly bright. This article examines the international debate about the regulation of new plant breeding techniques and then assess how the Canadian regulatory system has approached the regulation of these technologies through two more public product approvals, GM apples and GM potatoes, then discusses other crop variety approval and those in the regulatory pipeline.

**RNAi-based control of the pollen beetle *Meligethes aeneus*: risk assessment of using trap crops as a delivery platform**

H.M.T. Hokkanen, I. Menzler-Hokkanen

*Department of Agricultural Sciences, University of Helsinki, Finland*

\*e-mail: [heikki.hokkanen@helsinki.fi](mailto:heikki.hokkanen@helsinki.fi)

*pest management, pesticide resistance, IPM, dsRNA, exogenous application*

The pollen beetle, *Meligethes aeneus*, is a key insect pest of oilseed rape (OSR), *Brassica napus*, throughout Europe. Currently, insecticides are the primary means to control the pollen beetle, but in recent years, this insect has been observed to display widespread and high resistance to pyrethroids, which is the main insecticide class used against it. Novel and integrated strategies are necessary for improved and continuing pest control of the pollen beetle below economic thresholds. Molecular biology and genomics research provides a foundation of knowledge for a more informed approach to behavioral and ecological research. Very little, however, is known about the pollen beetle at the molecular/genomic level.

Currently work is being carried out on transcriptomics and functional genomics of the pollen beetle, including targeting various vital genes for RNA interference aiming at disruption of gene expression. Transgenic OSR is one approach in administering the RNAi product to the pollen beetle, but it may be a very lengthy and uncertain strategy in bringing this technology to the market, at least in Europe. An alternative is explored within the ERA-NET project IPM4Meligethes, focusing on feeding the pollen beetles with the appropriate dsRNA in the field. This could be done in an innovative way by using trap crops with exogenous dsRNA either in the spring (before maturity feeding of the female beetles), or in the autumn (before overwintering), depending on which vital gene(s) are targeted. Trap crops could be treated with the dsRNA-product either by spraying, or more elegantly, by using entomovectoring (honey bees or bumble bees). This would not involve using transgenic plants, which should facilitate an easier regulatory approval of the strategy.

Risk assessment to non-target organisms is mandatory also in this context. Variables in such an assessment include (i) the choice of trap crop plants, (ii) organisms visiting the trap crops and feeding on the pollen containing the dsRNA product, (iii) timing of application, and (iv) method of application. These variables will narrow the spectrum of non-target organisms that might be at risk, and which should be included in the safety assays.

## **Models for investigating the functional transfer of plant-derived RNA in mammalian cells**

V. Barragan-Borrero<sup>1</sup>, D. Van Leeuwen<sup>1</sup>, K. Sostar<sup>1</sup>, B. Mateescu<sup>1</sup>

<sup>1</sup>Group Cell biology of RNA silencing and Extracellular RNAs, ETH Zürich, Zurich, Switzerland

\*e-mail: bogdan.mateescu@biol.ethz.ch

*small RNA, double-stranded RNAs, extracellular RNAs, cross-kingdom RNA transfer*

The possibility to **exploit plant RNA silencing** mechanisms, including the use of **RNA interference (RNAi)**, now represent a highly promising approach to improve crop nutrient properties, productivity or resistance to pests. A fascinating aspect of RNA silencing is its ability to **cross cell boundaries**, as illustrated in *C.elegans* and *A.thaliana*, where the process is key to antiviral defense, epigenetic silencing, developmental patterning and stress responses. Interestingly, accumulating evidences indicate that such mechanisms also operate in mammalian cells, and some studies even suggested functional transfer of **plant-derived small RNA (e.g. miRNAs)** following **dietary intake** in mouse and human *in vivo*. Although highly controversial and though provoking, these claims raise considerable **biosafety concerns** in the era of RNAi engineered crop.

As part of the NIH common fund program on extracellular RNAs (exRNA.org), we are already engaged in developing different techniques/models to explore this hypothesis. For example, we readily established a collection of mammalian cell lines expressing genetically encoded fluorescent sensors capable to detect **functional transfer of specific extracellular small RNAs** originating from mammalian or **plant sources**. In addition, these sensor cells also have the unique capacity to detect functional transfer of **long double-stranded RNA (dsRNA) precursors** (>200 nt), that are generally used to elicit the primary RNAi response in plant. In parallel, we also developed mouse models and biochemical fractionation techniques allowing us to selectively purify small RNAs (**including potential plant-derived RNAs**) that are actively **engaged in mammalian RNA silencing complexes**, starting from biofluid (e.g. serum) or specific cell type (e.g. mouse enterocytes) *in vivo*.

In this first phase of the project, we will challenge these innovative experimental systems with **native extracts** isolated from **different established transgenic RNAi plants** (edible or model system), in order to unambiguously validate (or infirm) the possibility of plant-to-mammal trans-kingdom RNA functional transfer both *in vitro* and *in vivo*.

**SESSION 4 - RNAi socio-economy (WG4)**

**ORAL**

**New techniques, scientific and regulatory aspects**

K. M. Nielsen<sup>1, 2</sup>

<sup>1</sup>*Department of Life Sciences and Health, Oslo and Akershus University College, Oslo, Norway*

<sup>2</sup>*Genøk-Center for Biosafety, Tromsø, Norway*

\*e-mail: [kaare.nielsen@hioa.no](mailto:kaare.nielsen@hioa.no)

*New plant breeding techniques*

Site directed nuclease technology offers new opportunities for targeted genome modifications. No or minor vector sequences in the final product may not allow detection of a particular modification, in particular for undeclared modifications. A key question is if new gene modification processes / products are sufficiently consistent as a group, and distinguishable from current GMOs that can warrant a different regulatory status. The new techniques still rely on recombinant DNA technology, although the final products may or may not have carry a particular genetic characteristic commonly associated with the current product range of plant GMOs. Current GMOs are regulated as a single group suggesting similar risk profiles. Moreover, regulation in the EU is process triggered. Here I explore to what extent there are relevant and consistent differences for new techniques in plant breeding that can set them apart in a regulatory approach. An international consensus has not yet been established. EFSA Guidance documents are available for cis-intragenics and SDN-3 technologies. [www.efsa.europa.eu/en/gmo/gmoguidance.html](http://www.efsa.europa.eu/en/gmo/gmoguidance.html).

**Production risk in expected gross margin based on new methods of plant pest and disease resistance and improving crop quality and yield based of using analytic network modelling**

A.Mihnea<sup>1</sup>, D. Nikolov<sup>2</sup>

<sup>1</sup>*Bucharest University of Economic Studies, Cladirea Mihail Moxa, 5-7 Mihail Moxa Str., (Bucuresti), Romania*

<sup>2</sup>*Institut of Agricultural Economics (IAE), 125 Zarigradsko shose, Blvd. Bl.1, 1113 (Sofia), Bulgaria*

\*e-mail: *dnik\_sp@yahoo.com, adraiana.agapie@yahoo.com*

*Fragaria, genetic mapping, microsatellites, EST, functional genomics*

In farm operational management systematic Gross Margin's (GM) computation operates as proxy for the profit's dynamics. Development of new methods of plant pest and disease resistance and improving crop quality and yield are required to confront challenges of future food supply and security. Interfering with RNA can be used to improve plant growth and productivity by suppressing undesirable traits and thus switching resources to more beneficial traits such as quality and yield. When GM is computed by farmers themselves, this action can yield potential benefits which go beyond the simple result. Collection of the GM's estimations done by the farmers themselves reveals the perceptions about the specific risk channels induced by the current variable cost estimations. Starting from a very simple example of GM this paper illustrate how a simple Analytic Network Model (ANP) can be built so that local knowledge transfer about the potential variations of the variable costs incurred can be embedded, without the inclusion of additional numerical data about the environmental conditions. Using this model every farmer can perform a sensitivity analysis in order to identify the magnitude of variation in the present GM calculations depending on the specific risks considered.



***iPlanta***

## **New breeding solutions for new farmers' challenges**

D. Piovan

*Confagricoltura, farmer, ITALY*

*\*e-mail: dbpiovan@gmail.com*

*Sustainable agriculture, NBTs, breeding, farming challenges, innovation*

Farmers all over the world are facing dire challenges: a growing population; the loss of arable land; the urge to produce more food and at higher safety standard without putting more pressure on the environment; an alarming attack from part of the civil society to farmers and to science.

To answer these challenges we need to apply constant innovation to traditional farming. Genetics and new breeding solutions are probably the most important source of innovation that must be made available to all farmers.

We need crops with an improved efficiency in the usage of fertilizers and of water. We need to reduce the amount of pesticides applied to crops. Several researchers are working on these issues and are gaining remarkable results.

We are asked to improve the quality of both our process and our product. We firmly believe that a close cooperation of scientists and farmers can bring great results, not only in the fields but also in communication with civil society.

## **The formation of GM-free and GM coasean clubs: will they form and if so how much can they achieve?**

M. J. Punt<sup>1</sup>, J. Wesseler<sup>2</sup>

<sup>1</sup>*Management and Economics of Resources and the Environment (MERE), University of Southern Denmark, Niels Bohrsvej 9, 6700 Esbjerg, Denmark*

<sup>2</sup>*Agricultural economics and rural policy group, Wageningen University, Hollandse Weg 1, 6706 KN Wageningen, Netherlands.*

\*e-mail: [punt@sam.sdu.dk](mailto:punt@sam.sdu.dk)

*Coexistence, club formation, coalition formation, ex-post liability, game theory*

The unintended presence of traces of genetically modified (GM) crops in the harvests of nonGM crops plays a prominent role in the debate over the coexistence of GM and nonGM crops. One way to address the issue is the formation of GM-free or GM-only clubs. We model the decisions of individual farmers to cultivate either GM or nonGM crops and combine this with a game theoretic model of club formation to investigate the feasibility of clubs. We consider two liability regimes: GM farmers are liable or they are not. We consider two benchmarks: Nash equilibrium without negotiations and the efficient allocation and compare those with partial cooperation through Coasean clubs. We find that relatively large clubs can form but they are not always necessary to reach the efficient allocation. In more than 90-95 percent of the cases an efficient allocation is reached if farmers can freely decide under profit maximization what to cultivate. This holds independent of the property rights system and provides strong support for coexistence policies based on ex-post liability such as in the US and Spain.

## **A socioeconomic assessment tool for innovative biotechnology in agricultural pest management**

E. O. Benjamin<sup>1</sup>, G. Buchenrieder<sup>1</sup>, H. Strasser<sup>2</sup>, J. Wesseler<sup>3</sup>

<sup>1</sup>*Technical University of Munich, Alte Akademie 12, 85354 Freising, Germany  
(emmanuel.benjamin@tum.de)*

<sup>2</sup>*University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria. (Hermann.Strasser@uibk.ac.at)*

<sup>3</sup>*Wageningen University, Hollandseweg 1, 6706KN Wageningen, Netherlands. (justus.wesseler@wur.nl)*

\*e-mail: *Emmanuel.benjamin@tum.de*

*Dibrotica, Integrated Pest Management (IPM), Biotechnology, Risk Assessment*

The Western Corn rootworm (WCR) *Diabrotica virgifera virgifera* is causing economic damages in maize production in parts of Central and Eastern Europe. The European Union has directed Member States to consider Integrated Pest Management (IPM) as a strategy for controlling WCR, which may increase biotechnology activities in Europe. The co-formulation of *Metarhizium brunneum*, under real farm conditions as an innovative direct biotechnological method in maize production is perceived to be viable. However, a certain degree of uncertainty and irreversible effects may arise from such IPM innovation. Uncertainty is associated with the efficacy and development of pest resistance over time depending on the control strategy, while irreversible effects are long term impacts (externalities) of the control strategy on non-target organisms and the environment. Thus, the socioeconomic evaluations of innovative biotechnology, here the IPM of WCR, based on a simple cost-benefit, grossly neglects uncertainty and irreversibility, and are inadequate. This contribution outlines a comprehensive conceptual framework for socioeconomic evaluation of innovative biotechnology. The conceptual framework consists of a threshold value for the irreversible costs (externalities) which consider the uncertainty surrounding the effectiveness of a proposed biotechnology (known as MISTIC: *Maximum Incremental Social Tolerable Irreversible Costs*). Furthermore, the dynamics of pest resistance (rise and break down) and the implications for control strategies should be considered.

## **Local resistance to genetically modified organisms in food production diffusion patterns and the GMO-free network**

U. Hartung, J. Tosun, S. Schaub

*Heidelberg University, Bergheimer Strasse 58, 68259 Heidelberg, Germany*

*\*e-mail: ulrich.hartung@ipw.uni-heidelberg.de*

*Diffusion ; EU ; Germany ; GMO-free regions; municipal level*

The European Union's (EU) governance of genetically modified organisms (GMO) for food and feed has provoked societal and political resistance on all levels of political decision-making. The local level, however, has achieved only scant attention in the academic research on the GMO multi-level regulatory system. This research gap is surprising, since local level actors are much more directly affected by the commercial cultivation of GM crops and/or test plots than actors on any other political level. Hence, this study sheds light on the decisions of German local governments to join the GMO-Free Network, a transnational community of subnational entities opposing the EU's liberal stance towards GMOs. In Germany, 352 municipalities and rural districts have declared themselves GMO-free since 1999 and thus advocate for practices of food production which abstain from utilizing GMOs. In terms of theory, this piece of research builds on the literature on policy diffusion. Thereby it scrutinizes several diffusion mechanisms in order to reveal the underlying factors behind local policy decisions to become GMO-free. The research questions are the following: i) Can diffusion be observed?; ii) Which mechanisms can explain the diffusion on the local level? The findings show that learning from earlier adopters was an important driver of the network's growth, whereupon it was favoured by the partisan affiliation of mayors to the Green party. Besides, the study provides evidence of intra-state diffusion, since notably in the Eastern German states, fewer local governments joined the network. This result as well as the finding of a non-significant interrelation between the GMO affectedness and the membership in the network leaves considerable space for future research.

## **Socio-economic aspects of novel breeding technologies in agriculture – perspectives and current work in Germany**

H. Kehlenbeck, J. Saltzmann

*Julius Kuehn-Institute, Institute for Strategies and Technology Assessment, Stahnsdorfer Damm 81, D-14532 Kleinmachnow*

*\*e-mail: [hella.kehlenbeck@julius-kuehn.de](mailto:hella.kehlenbeck@julius-kuehn.de)*

*Cost benefit analysis, value chain, genome editing, socio-economic impact assessment*

Novel breeding technologies in agriculture provide a technological innovation for breeding by altering traits of plants in a very efficient and targeted manner towards improved agricultural production of plants. These innovations can change and enhance agricultural production systems thus affecting different socio-economic levels. Changes are expected concerning the economic situation across the entire agricultural value chain: (1) at farm-level, (2) on agricultural markets with respect to supply and demand in the food and non-food sector, (3) in industries for operating consumables, (4) for consumers, (5) for the society as a whole (e.g. food security, employment, science, discussion process and legal aspects) and (6) for environmental economic impacts.

New varieties derived by the new methods may e.g. increase agricultural efficiency, help to adapt plants to climate change, increase opportunities to produce on marginal lands, improve the resistance towards diseases and pests or decrease requirements for land use. Despite positive traits of the new products the technologies are subject to very controversial public debates. In Germany a transdisciplinary research project addressing these aspects with specific respect to genome editing just started to provide scientific based knowledge and to enhance a more objective discussion. The methodological approach on the socio-economic aspects comprises systematic reviews on socio-economic impacts of novel breeding technologies on different socio-economic levels, as well as cost benefit analysis and scenario techniques. A close linkage with legal and ethical aspects finally allows for a comprehensive and interdisciplinary evaluation of the novel methods which can be taken into the discussion with stakeholders.

## **Regulation of New Plant Breeding Techniques: The Case of Rapeseed in the EU**

T. J. Venus, D. Drabik, J. Wesseler

*Wageningen University, Agricultural Economics and Rural Policy Group, Department of Economics, Hollandseweg 1, 66706kn, The Netherlands*

*\*e-mail: Thomas.Venus@wur.nl*

*New Plant Breeding Techniques, GMO, labeling, coexistence, identity preservation, regulation, vertical product differentiation*

We analyze the market and welfare effects of regulating crops derived by New Plant Breeding Techniques (NPBTs) as genetically modified (GM) or conventional products. We consider the EU mandatory scheme for labeling GM products and a voluntary non-GM scheme for labeling livestock products derived from non-GM feed. We develop a partial equilibrium model that explicitly takes into account both the coexistence costs at farm-level and the segregation and identity preservation costs at downstream level. By applying the model to EU rapeseed, we find that regulating NPBTs as GM (as compared to non-GM) in combination with mandatory and voluntary labeling increases prices and makes consumers overall worse off and producers better off. We also show that higher coexistence costs make the price increasing effect even stronger. Voluntary non-GM labeling applied to feed makes consumers in this sector overall worse off but benefits farmers and rapeseed oil consumers overall as long as segregation costs are low. Consumers of biodiesel and industrial products such as lubricants produced from GM rapeseed benefit from high segregation costs. We show that the effects of farm-level coexistence costs largely differ from the effects of downstream market segregation costs.

## **POSTER**

### **Structure of the seed industry in Sweden**

C. Morfi, K. Karantininis, H. Andersson

*Swedish University of Agricultural Sciences (SLU)*

*\*e-mail: karantininis.konstantinos@slu.se*

*Seed Industry, Sweden, Value chain*

This study provides an overview of the Swedish seed industry. Using a supply chain approach we document the stages of production and the actors involved, from plant breeding to seed multiplication and distribution channels. We identify the variety owners for major crops which we classify based on the origin of the breeding station. In this way we can estimate the number of varieties that were produced in Sweden and those been licenced from abroad. This ratio of local varieties/imported varieties is of special significance for a country with long history in plant breeding and rough Nordic climate.

We also provide a statistical summary of the industry in Sweden compared to EU countries and a historical review of the development of imports and exports of seed. We focus on the Farm Saved Seeds (FFS) channel. In Sweden FFS accounts for 20% of cereal production. Though most European countries charge farmers on the basis of self-reported use of FFS, the Swedish Seed Association in collaboration with the Board of Agriculture, and the Farmers Union have developed a unique mechanism; they estimate the approximate use of FFS and charge farmers who haven't declared their use FFS accordingly.

## **SESSION 5 - RNAi Communication and public acceptance (WG5)**

### **ORAL**

#### **Framing a communication strategy for RNAi GM plants based on experiences from past EU and national research projects**

K. Minol, J. Freitag And K. Sinemus

*Genius GmbH - Science and Dialogue, Robert-Bosch-Str. 7, 64293 Darmstadt, Germany*

*\*e-mail: klaus.minol@genius.de*

*communication strategy, risks and benefits, media, stakeholder engagement, public perception*

The vast majority of the general public is scared and anxious about GM plants. Demonstrations, destroyed field trials, and heavily biased news reports tell a never-ending story of rejection, concern and neglect. Does the problem actually stem from a lack of information? Are scientists (as well as companies and organizations) not active enough in providing comprehensive information about the real risks and benefits of GM products, and about new applications of this knowledge? Are the media also unable, or unwilling, to consider this information and convey it to the broader public? Experiences gained from social science studies and major research projects, such as the EU-funded GRACE project (GMO Risk Assessment and Communication of Evidence; 2012-2015) and the German communication initiative on biosafety research (bioSicherheit.de; 2002-2012) funded by the German Federal Ministry of Research and Education, clearly indicate that a lack of information on its own does not necessarily lead to a lack of acceptance. This has more to do with the manner in which a topic is addressed and by whom, as well as the basic attitude of politicians and citizens towards innovations. Over the past few years, the social consensus that new scientific processes are indispensable for the progress of humanity has been crumbling. Therefore, scientific evidence plays only a very minor role in the current discussion, which is dominated by negative emotional debates encouraged by critical NGOs. This situation calls for significant changes in future communication strategies for GM plants. The active engagement of stakeholders in good science governance and communication activities is becoming increasingly important. The presentation will summarize experiences (best practices and major failures) drawn from past communication initiatives and will provide proposals for suitable communication channels, targets and messages, as well as stakeholder engagement strategies to increase the relevance and recognition of GMO research programmes, including from a broader societal perspective.

## **The COST action FP0905 experience on scientific dissemination**

F. Migliacci<sup>1</sup>, D. Paffetti<sup>2</sup>, C. Vettori<sup>3</sup>

<sup>1</sup>*National Research Council (CNR), Institute of Chemistry of Organometallic Compound (ICCOM), Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy (fabio.migliacci@iccom.cnr.it)*

<sup>2</sup>*Department of Agrifood Production and Environmental Science, University of Florence, P. le delle Cascine 28, 50144, Florence, Italy (donatella.paffetti@unifi.it)*

<sup>3</sup>*National Research Council (CNR), Institute of Biosciences and Bioresources (IBBR) – UOS Florence, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy (cristina.vettori@cnr.it)*

*\*e-mail : fabio.migliacci@iccom.cnr.it, donatella.paffetti@unifi.it, cristina.vettori@cnr.it*

The EU COST Action FP0905 (2010-2014) aimed at collecting and evaluating the scientific knowledge on genetically modified trees (GMT) related to biosafety accessible and published in the last 30 years. All obtained results have to be coordinated in order to provide the correct information regarding the use of GM forest trees. The existing or new information from various European countries will provide a basis for future EU policy and regulation recommendations regarding the use of GM forest trees.

To fulfill these aims, the COST Action FP0905 was divided in four Working Groups (WGs). The Working Group 4 (WG 4) focused on the increase of public awareness on the use of GM forest plantations and environmental protection through science-based information management of the [www.cost-action-fp0905.eu](http://www.cost-action-fp0905.eu) dynamic website (WG4).

For spreading information relevant to this COST Action, the website (<http://www.cost-action-fp0905.eu/>) was set up to provide communication between participants of the Action, and to disseminate the activities, knowledge and technology produced beyond the COST Action network.

The Web 2.0 technologies were used to support the aims of the COST Action, with specific positive (YouTube and Twitter) but sometimes also negative communication experiences (i.e., hacker's attacks) of the Action are reported.

**IPLANTA CONFERENCE  
SAPIENZA UNIVERSITY**

**Session 1 – RNAi Technology (WG1)**

**Fruit-derived nanovesicles as carrier of mRNAs, microRNAs and bioactive compounds with effects on bone health**

S. Avnet, A. Massa, N. Baldini

*Alma Mater Studiorum - Università di Bologna & Istituto Ortopedico Rizzoli Bologna, Italy*

*\*e-mail: nicola.baldini@ior.it*

In the nutraceutical field, strawberries have been gaining a growing interest for their high nutritional quality and high content of bioactive compounds, like folate, vitamin C, and several phytochemicals with antioxidant properties. In particular, vitamin C is a master regulator of bone metabolism, as it stimulates procollagen, enhances collagen synthesis, promotes the osteogenic marker alkaline phosphatase and improves the biological features of mesenchymal stromal cells (MSC) by protecting them from apoptosis and by up-regulating their stemness. As a result, patients with vitamin C deficiency have an increased risk of bone frailty. However, studies of dietary vitamin C intake and the relation with bone mineral density have been conflicting. This is possibly due to the limited absorption and biological half-life of vitamin C dietary supplementation. Under this context, recent discoveries on the existence of nanovesicles that are released by edible plants and that might influence mammalian host cells through interspecies communication are very encouraging. These plant-derived nanovesicles share several features with animal and human extracellular vesicles, exosomes (EXO) that have an endocytic origin and a diameter of 30-100 nm. EXO have been found in many biological fluids since they are released by a variety of cells. EXO serve as biological nanocarrier to mediate cell-cell communication with a cargo of proteins, mRNAs, microRNAs, and lipids that can be transferred to recipient cells, and have been thus explored as drug-carriers. Indeed, EXO-based drug delivery might solve several major problems, such as non-specific biodistribution and short half-life of drugs in the systemic circulation. Similarly, EXO-like nanovesicles that are directly isolated from fruits might be proposed as carriers of nutraceutical bioactive compounds. EXO-like nanovesicles have been already shown to be released by grape, grape fruit, ginger and carrot, and to mediate interspecies communication by inducing mRNA expression of cytokines with anti-inflammatory and anti-oxidant activities. Given the effect of vitamin C on bone, we aim to investigate the potential of EXO-like nanovesicles isolated from strawberries as a nutraceutical approach to improve bone health.

## **The challenges of RNAi-mediated insect pest control and the search for novel delivery methods**

O. Christiaens, G. Smagghe

*Department of Crop Protection, Ghent University, Coupure Links 653, 9000 Gent*

*\*e-mail: olchrist.christiaens@ugent.be*

*RNAi, delivery, insects, efficiency*

Over the past decade, RNA interference (RNAi), the sequence-specific posttranslational suppression of gene expression, has proven very useful for molecular research in many insect species. It showed its usefulness to analyze gene function and its potential to manage pest populations and reduce disease pathogens. However, a significant variability in RNAi efficiency is observed between different insect orders, species and even between different laboratory colonies. Several factors have been identified that can influence RNAi efficiency, such as dsRNA persistency in the insect body, availability of the RNAi core gene machinery, uptake and systemic transport of the dsRNA, the dsRNA molecule itself, the influence of viral infections, and more. Here, we present an overview of what is known in literature concerning the different challenges that need to be overcome to achieve efficient RNAi-silencing in different insect orders.

## **Exploiting RNA biology to reduce the late blight disease potato**

K. Persson-Hodén, J. Fogelqvist, A. Åsman, C. Dixelius

*Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala BioCenter, Linnean Center for Plant Biology, Box 7080, S-75007 Uppsala, Sweden*

*\*e-mail: Christina.Dixelius@slu.se*

*Phytophthora, plant resistance, potato, small RNA*

Many breakthroughs have been made in identifying different classes of small RNAs of various origins, as well as elucidating their biogenesis pathways and functions in diverse plants species and pathogens that cause plant disease. Work is spanning from RNA interference (RNAi) which involves sequence-specific gene regulation by small non-coding RNAs, host-induced gene silencing (HIGS) where hairpin constructs are expressed in plants aiming to target essential pathogenicity genes in diverse pathogens and genome editing applying CRISPR/Cas9 systems. All relatively new and powerful approaches giving high expectations of crop improvements.

We are studying RNA biology in order to understand the interactions between potato and the late blight pathogen, *Phytophthora infestans*. We have characterized the canonical gene silencing pathways in *P. infestans*, the Argonaute proteins, and verified functional plant host mediated gene silencing targeting several genes in this oomycete pathogen. The long-term aim of our work is to understand the short durability of the late blight resistance genes in potato and to edit the potato genome to avoid or slow down this process. Present work build on our knowledge base on this plant-pathogen system and includes; development of a CRISPR/Cas9 system in *P. infestans*, verification of small RNA transfers to the host via pull-down followed by “Ion Proton” sequencing, trans-kingdom transport events and gene function analysis affecting plant resistance genes.

## **Inducing RNAi mechanisms as a way of tackling of the Plum pox virus genome in woody perennial plants**

M. Ravelonandro<sup>1</sup>, R. Scorza<sup>2</sup>, I. Zagrai<sup>3</sup>, C. Dardick<sup>2</sup>, A. Callahan<sup>2</sup>, L. Zagrai<sup>3</sup>, P. Briard<sup>1</sup>

<sup>1</sup>*UMR-1332, Biologie du Fruit et Pathologie, INRA-Bordeaux, France*

<sup>2</sup>*Appalachian Fruit Research Station, ARS, USDA, Kearneysville, 25430 West-Virginia, USA*

<sup>3</sup>*Fruit Research and Development, Bistrita, Romania*

*\*e-mail: michel.ravelonandro@inra.fr*

*Perennial plants, silencing, RNAi, resistance*

Plum pox virus (PPV), the causal agent of sharka disease reduces yield, market access and profitability for stone fruit growers. There is neither cure nor treatment against PPV. To save the fruit-tree industry, a variety of preventive measures (diagnosis, nursery survey, eradication...) have been employed. While conventional breeding techniques are still challenging due to the few sources of resistance including hypersensitivity and virus tolerance, plant biotechnology has efficiently tackled the PPV genome replication via silencing. Utilized as models for resistance, transgenic plum lines ('HoneySweet', B14, C1738 and P1CP1) transcribe a dsRNA-precursor, which through the action of a plant dicer, generates siRNA that specifically target PPV RNA for destruction. Exploiting the seasonality of plum-tree development, the regulation between the virus transgene and a few endogenous genes was assessed by evaluating their methylation status. The results in both greenhouse and field experiments showed a changes in the methylation state of endogenous genes and similarly too in the virus transgene. Mechanisms to understand why the engineered virus transgene conferred a stable and durable virus resistance, and its relationship to variable methylation status like that of the other endogenous genes will be presented and discussed.

## Session 2- RNAi Applications (WG2)

### RNAi in forest tree

H. Häggman<sup>1</sup>, M. Fladung<sup>2</sup>

<sup>1</sup>University of Oulu, Faculty of Science, Genetics and Physiology Unit, P.O.Box 3000, 90014 Oulu, Finland

<sup>2</sup>Thuenen-Institute of Forest Genetics, Genome Research, 22927 Grosshansdorf, Germany

\*e-mail: [hely.haggman@oulu.fi](mailto:hely.haggman@oulu.fi), [matthias.fladung@thuenen.de](mailto:matthias.fladung@thuenen.de)

*Populus, miRNA, artificial miRNA, small interfering RNA, functional genomics*

For long time, RNA was believed to only act as a messenger between DNA and protein, however, discoveries in the past ten years suggest that RNA is also involved in the regulation of genome organization and gene expression. Evidence has been obtained that regulatory RNA molecules play a central role for RNA in evolution and ontogeny in complex organisms including tree species. Regulatory RNA comprises all types of small RNA molecules including micro- and small interfering RNAs (miRNAs, siRNAs) that mediate the silencing effect.

There are several examples of RNA interference (RNAi) in tree species (mainly poplar) to modify gene expression. As early as in 2004, a set of different vectors has been proposed for poplar generating RNAs capable of duplex formation of sequences specific for the beta-glucuronidase (GUS) reporter gene system (Meyer et al. 2004). Examples of successful RNAi-mediated suppression in poplar are the p-coumaroyl-CoA 3 $\beta$ -hydroxylase gene involved in lignin deposition (Coleman et al. 2008), the PoGT47C gene to reduce the glucuronoxylan content (Lee et al. 2009), isoprene biosynthesis (Behnke et al. 2009), the PtFAD2 gene to change freezing tolerance (Zhou et al. 2010), the 4CL1 gene (Tian et al. 2013), and poplar plasma membrane intrinsic proteins (Bi et al. 2015). Stability of RNAi-mediated transgene suppression has been confirmed in the field (Li et al. 2008).

Few years ago, microRNAs (miRNAs) were discovered including all types of non-coding RNAs acting at the post-transcriptional level and, thus, playing a key role in the regulation of gene expression. In several tree species including poplar, pine, eucalypt, genome-wide expression profiling of miRNAs in several tissues, different developmental stages and under stress revealed an uncountable number of these small RNA molecules (e.g. Li et al. 2013, Song et al. 2013, Ding et al. 2014). Finally, miRNAs and also artificial miRNA genes (amiRNAs; can be designed at <http://wmd3.weigelworld.org/cgi-bin/webapp.cgi>) have already been genetically transferred into different tree species to specifically silence single or multiple genes of interest.

Examples of successful RNAi, miRNA and amiRNAs applications in forest tree species will be provided. Advantages and disadvantages of these technologies in respect to the particular features of forest tree species (longevity, extended vegetative phases) will be discussed.

## **Characterization of the role of a *lectin* gene in the susceptibility of strawberry fruits to *Colletotrichum acutatum***

M. Guidarelli<sup>1</sup>, S. Sabbadini<sup>2</sup>, F. Negrini<sup>1</sup>, B. Mezzetti<sup>2</sup>, E. Baraldi<sup>1</sup>

<sup>1</sup>Università degli Studi di Bologna, Dipartimento di Scienze Agrarie (DipSA), Viale Fanin 46, 40127, Bologna, Italy

<sup>2</sup>Università Politecnica delle Marche, Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Via Brecce Bianche 10, 60131, Ancona, Italy

\*e-mail: elena.baraldi@unibo.it

The fungal pathogen *Colletotrichum acutatum*, the causal agent of strawberry *Fragaria × ananassa* anthracnose, can infect strawberry fruit hosts at pre-harvest unripe stages becoming quiescent until fruit ripen, causing anthracnose symptoms only on red ripe fruit. In order to understand the molecular basis of the low susceptibility of white unripe fruits, the role of a gene encoding for a lectin protein that becomes overexpressed in white fruits upon *C. acutatum* inoculation, has been investigated by developing an *Agrobacterium*-mediated transient and stable transformation experiment, for silencing the lectin gene in white unripe strawberry and for producing strawberry plants silencing this gene respectively. Exploiting the potential of plant species to silence genes when recognize intron-containing constructs encoding self-complementary 'hairpin' RNA, a construct containing the partial sense and corresponding antisense sequences of *FaMBL* (*Fragaria × ananassa* Mannose Binding Lectin) gene separated by an intron was generated. Unripe strawberry fruits transiently silenced with *Lectin*-RNAi appeared more susceptible to *C. acutatum* with respect to control fruits suggesting that this gene could play important role in regulating the different level of susceptibility of ripe and unripe strawberry fruits to *C. acutatum*. For this reason, the *FaMBL*- silencing construct was used to produce strawberry plants silencing this gene. Genetic transformation was performed in the cultivar Sveva and the selection of regenerating shoots, as well as the identification of selected lines capable to proliferate on the kanamycin selective agent, is described.

## **Resistance of cassava expressing dsRNAs against coding sequences of geminiviruses**

H. Vanderschuren<sup>1,2</sup>

<sup>1</sup>*Plant Biotechnology, Biology Department, ETH Zurich, Switzerland*

<sup>2</sup>*Plant Genetics, AgroBioChem Department, University of Liège, Belgium*

\*e-mail: [herve.vanderschuren@ulg.ac.be](mailto:herve.vanderschuren@ulg.ac.be)

*RNAi, dsRNA, geminivirus, resistance, cassava*

Cassava Mosaic Disease (CMD) is a major constraint to cassava production in Africa and in the Indian sub-continent. CMD is caused by cassava geminiviruses (CGMs), which are bipartite ssDNA viruses. At least 9 CGM species are infecting cassava and CGM diversity is particularly high on the African continent. In order to engineer resistance against *African cassava mosaic virus* (ACMV), we developed transgenic cassava expressing dsRNA against the ACMV Rep (AC1) sequences that are conserved amongst CGMs. Transgenic dsAC1 cassava lines were selected based on the accumulation of transgene-derived small RNAs. We established a rapid and high-throughput agroinoculation method in order to assess virus resistance in cassava. Selected transgenic cassava lines displayed robust resistance against increasing loads of ACMV delivered by gene gun and by agroinoculation. We also observed a fast recovery phenotype in transgenic lines following graft inoculation of ACMV. Best performing dsAC1 cassava lines were also inoculated with *South African cassava mosaic virus* (SACMV) and *East African cassava mosaic virus* (EACMV). Recovery from SACMV and EACMV infections in transgenic cassava lines was delayed as compared to the recovery phenotype observed with ACMV infection. Collectively, our results suggest that high similarity between transgene-derived dsRNAs and target virus sequences is required to engineer transgenic plants rapidly recovering from virus infection. Selected transgenic dsAC1 cassava lines also showed delayed development of CMD symptoms in the field. Molecular analysis of CGM species in the field is being performed in order to better characterize the level of resistance of dsAC1 lines against multiple CGM species.

## **Plant mediated RNA interference (RNAi) to block P450 based detoxification of insecticides and plant allelochemicals in agricultural pests**

J. Vontas<sup>1,2</sup>, E. Pitsili<sup>3</sup>, N. Kryovrisinaki<sup>3</sup>, I. Moustaka<sup>3</sup>, A. Sandri<sup>3</sup>, E. Morou<sup>1,3</sup>, E. Siozou<sup>3</sup>, Riga M.<sup>1,3</sup>, Roditakis E.<sup>4</sup>, Van Leeuwen T.<sup>5,6</sup>, D'ambrosio C.<sup>7</sup>, Kalantidis K.<sup>1,3</sup>

<sup>1</sup>*Institute of Molecular Biology & Biotechnology, Foundation for Research & Technology Hellas, 100 N. Plastira Street, GR-700 13, Heraklion Crete, Greece*

<sup>2</sup>*Department of Crop Science, Agricultural University of Athens, 11855-Athens, Greece.*

<sup>3</sup>*Department of Biology, University of Crete, 71409 Heraklion, Greece*

<sup>4</sup>*Hellenic Agricultural Organisation - "DEMETER", Institute of Olive Tree, Subtropical Crops and Viticulture, Heraklion, Greece*

<sup>5</sup>*Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam (UvA), Science Park 904, 1098 XH Amsterdam, The Netherlands*

<sup>6</sup>*Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, B-9000, Ghent, Belgium*

<sup>7</sup>*Metapontum Agrobios, Metaponto, Italy*

\*e-mail: [vontas@imbb.forth.gr](mailto:vontas@imbb.forth.gr)

*Detoxification, Insecticides, allelochemicals, RNAi*

The control of major agricultural pests, such as the spidermite *Tetranychus urticae* and the whitefly *Bemisia tabaci* has been largely based on the use of insecticides. However, the pests evolve resistance to most chemicals rapidly, often via cytochrome P450 detoxification mechanisms. The same enzyme family is involved in the detoxification of phytotoxins, and the ability of those polyphagous pests to feed on numerous plants. By using a variety of approaches, including production and characterization of recombinant P450 proteins, and plant mediated RNA interference (RNAi), we try to understand the precise mechanisms involved in the detoxification process.

We generated transgenic plants to block P450-based detoxification, aiming to investigate the role of the P450s in the ability of *T. urticae* and *B. tabaci* to overcome insecticide toxicity as well as to cope with plant allelochemicals. The transformation was performed via the *Agrobacterium tumefaciens* route using appropriate binary vectors. Hairpin constructs were constructed using the Gateway plasmid system where the target sequences were placed through homologous recombination in sense and antisense orientation each side of an intronic sequence. Transgenic plants (both tobacco and tomato plants) were regenerated in tissue culture, rooted and transferred to soil. We determined the success and effect of gene-silencing by qPCR expression analysis and phenotypic determinations and in particular the effect of the cytochrome P450 silencing on whitefly / spidermite development on transformed plants and insecticide resistance.

### **SESSION 3 - RNAi biosafety (WG3)**

#### **Risk assessment of RNAi-based GM plants**

N. Papadopoulou, F. Alvarez, Y. Devos, A. Lanzoni, C. Paoletti, M. Ramon, E. Waigmann

*Department of Scientific Evaluation of Regulated Products Development, European Food Safety Authority, Parma, Emilia Romagna 43126, Italy*

*\*e-mail: nikoletta.papadopoulou@efsa.europa.eu*

*RNAi, risk assessment, genetically modified organisms, plants*

Genetically modified (GM) plants intended for market release can be designed to induce silencing of specific genes in planta or in target pests through RNA interference (RNAi). As part of the pre-market risk assessment (RA), the European Food Safety Authority (EFSA) evaluates any risks that GM plants may pose to the animal and human health and the environment. Potential risks associated with the use of RNAi in GM plants were considered at an international scientific workshop organised by EFSA. Experts from academia, RA bodies and the private sector discussed the biology underlying the RNAi mechanisms, current and future applications of RNAi-based GM plants, and RA approaches. The outcome of the workshop helped determine in which areas the existing approaches for RA are appropriate, and whether complementary or alternative RA strategies need to be developed, for RA of RNAi-based GM plants (EFSA, 2014; Ramon et al., 2014, Casacuberta et al., 2015). Additionally, current limitations in methods available to unequivocally identify potential non-target effects and the likelihood of exposure of humans, animals and the environment to those following RNAi in planta, were considered. Thus, EFSA commissioned three separate external scientific reports, to obtain a comprehensive literature overview on several of these issues that could further drive food and feed and environmental risk assessment of RNAi-based GM plants.

- 1) European Food Safety Authority, 2014. International scientific workshop 'Risk assessment considerations for RNAi-based GM plants' (4–5 June 2014, Brussels, Belgium). EFSA supporting publication 2014:EN-705, 38 pp.
- 2) Ramon M., Devos Y., Lanzoni A., Liu Y., Gomes A., Gennaro A. and Waigmann E., 2014. RNAi-based GM plants: food for thought for risk assessors. *Plant Biotechnology Journal*, 12 (9): 1271-1273.
- 3) Casacuberta J.M., Devos Y., du Jardin P., Ramon M., Vaucheret H., Nogué F. 2015. Biotechnological uses of RNAi in plants: risk assessment considerations. *Trends Biotechnology*, 33(3):145-147.

## **Possible environmental effects of RNAi-based GM plants**

I. Urru<sup>1</sup>, J.B. Sweet<sup>2</sup>, A. Dietz-Pfeilstetter<sup>3</sup>, S. Arpaia<sup>1</sup>

<sup>1</sup>*ENEA Centro Ricerche Trisaia, S.S. 106 Jonica, km 419.5, I-75026, Rotondella (MT), Italy*

<sup>2</sup>*J T Environmental Consultants, Cambridge CB24 5JA, UK*

<sup>3</sup>*Julius Kühn-Institut, Institute for Biosafety in Plant Biotechnology, Braunschweig, Germany*

\**e-mail: Isabella.urrui@enea.it*

*RNAi, pest control, biosafety, non-target organisms*

RNAi can cause specific post-transcriptional gene silencing in most eukaryotes, including insects. Ingestion of dsRNA can elicit RNAi activity, therefore this technique has potential for controlling crop pests. Genetically modified plants protected from herbivorous pests by expressing dsRNAs targeting vital insect genes have been developed, and are available for cultivation outside Europe. Possible adverse effects due to the expression of dsRNA in these plants may arise from the specific activity of RNA-based silencing molecules in crop plants to which biota are exposed along trophic webs. The potential adverse effects of RNAi can theoretically be ascribed to one of the following categories:

- a) Off target effects including possible silencing effects in the GM plants and in the non-target organisms that may be exposed to the dsRNA including fungi, beneficial insects and mammals including humans.
- b) Silencing the target gene in non-target organisms
- c) Degradation of non-targeted mRNA by transitive RNA silencing
- d) Effects resulting from immune stimulation
- e) Saturation of the RNAi machinery

Due to the peculiar mechanisms of action and the uncertainties pertaining to the possible uptake of dsRNA in non-target organisms, a tailored approach to environmental risk assessment (ERA) for RNAi-based genetically modified crops has been advocated. Arthropod toxicity tests on selected focal species through different exposure routes represent the most reliable data source for ERA. Bioinformatic analyses can detect complementarity between dsRNA/siRNA molecules and mRNA sequences in exposed organisms, and can support ERA through the identification of potential off target genes. However, such analyses are limited by several factors (e.g., not all relevant genomes are sequenced). The presentation will review the main findings in the existing literature and highlight research gaps that need to be addressed in order to support ERA of RNAi-GM plants.

**RNAi-based pest control: current understandings in terms of environmental safety and non-target effects**

O. Christiaens, G. Smagghe

*Department of Crop Protection, Ghent University, Coupure Links 653, 9000 Gent*

*\*e-mail: olchrist.christiaens@ugent.be*

*RNAi, delivery, insects, biosafety, risk assessment*

RNA interference (RNAi), a post-transcriptional gene silencing mechanism has shown great promise as a next-generation biopesticide. While the first RNAi-based products could enter the North-American market as early as the next few years, regulatory bodies elsewhere are investigating the possible implications of this novel technology on the environment and are looking at risk assessment requirements. Here, we give an overview on what is known in the literature in terms of species-specificity of dsRNA, the potential implications for non-target species, the environmental fate of dsRNA and potential requirements for risk assessment.

## **Food safety assessment strategies for crop plants derived through RNAi mediated gene silencing**

H.A. Kuiper<sup>1</sup> And E.J. Kok<sup>2</sup>

<sup>1</sup>Formerly RIKILT, Institute of Food Safety, Wageningen UR, The Netherlands

<sup>2</sup>RIKILT, Institute of Food Safety, Wageningen UR, The Netherlands

\*e-mail: [h.a.kuiper@kpnmail.nl](mailto:h.a.kuiper@kpnmail.nl)

*RNAi mediated food crops, food safety assessment, nutritional assessment, WHO/FAO and OECD risk assessment guidance, targeted compositional analysis, genomic and metabolomic profiling*

RNAi interference has gained an important role during the last 15 years in agriculture in the development and improvement of pathogen resistance in food plants, the safety and nutritional profile of food crops and yields. Specific RNAi mediated crops have been approved by regulatory authorities in various countries. This contribution is focussed on strategies for the safety and nutritional assessment of RNAi-plants and their derived foods and feed.

Risk Assessment Strategies for foods derived from modern biotechnology as designed by WHO/FAO and OECD, and various other National Risk Assessment Agencies will be described. Upon proper application of these guidelines, they will provide adequate protection of humans and animals ingesting these type of foods. The risk assessment is *comparative* and *not absolute*, i.e. the properties of the modified food crop are compared with those of an appropriate non-modified counterpart. Identified differences are assessed regarding their potential safety and nutritional impact. Current strategies are primarily focussed on the characteristics of newly expressed proteins and the assessment of their safety and functional profile. Furthermore specific attention is paid to possibly occurring *unintended alterations* in the modified food crop as result of the genetic modification.

The above mentioned guidelines are also applicable to the assessment of RNAi mediated plants. However no new proteins are expressed through targeted gene silencing, but particular attention should be given to potential unintended (non-targeted) alterations due to specific RNA mediators, which might negatively impact the safety/nutritional profile of the modified crop for humans/animals. The ubiquitous nature of si RNAs in plants is well known but is the biological spectrum of RNA mediators sufficiently characterised as well as the presumed history of safe consumption through foods? Examples of RNAi – mediated food crops will be presented and experiences of EFSA, FSANZ, and USA-FDA with the safety and nutritional assessment of RNAi mediated food crops will be given.

Further development of risk assessment strategies for RNAi mediated crops will be discussed. A *case-specific* approach is recommended for the identification and assessment of off-target alterations by applying a *Targeted Compositional Analysis* based on the results of the molecular characterization and bioinformatics. Furthermore development, validation and application of profiling methods using genomic and metabolomics approaches will be advocated as promising and powerful assessment tools.

## **SESSION 4 - RNAi socio-economy (WG4)**

### **Assessing socio-economic impacts of GMP RNAi-Technologies: Concepts and Methods**

J. Wesseler

*Agricultural Economics and Rural Policy Group, Sub-Department of Economics, Social Science Department, Wageningen University, Hollandseweg 1, 6706KN Wageningen, NL*

*\*e-mail: justus.wesseler@wur.nl*

*Cost-Benefit Analysis, Regulation, Uncertainty, Irreversibility, RNAi-Technology*

Assessing the socio-economic impacts of GMP RNAi- technologies has to consider a number of factors. First, there are uncertainties related to the future benefits and costs; second, the investment in developing the technologies includes in many cases a substantial amount of sunk costs that can be considered to be irreversible; third, benefits and costs are unevenly distributed among different stakeholders horizontally (between e.g. farmers or consumers) as well as vertically as (providers, users, consumers); and fourth, depends on the policy environment, influencing the first three issues mentioned.

In this contribution, the concepts and methods used for assessing the socio-economic impacts of new technologies will be discussed. The paper starts with the regulatory approaches, which logically results, independent of the legal system (civil law in comparison to common law), in the ex-ante regulation vs. ex-post liability comparison. For assessing the optimal level of a supra level regulation (optimal level of ex-ante regulation vs. ex-post liability) within such a framework, the effects of irreversibility and uncertainty need to be explicitly considered (Without uncertainty and irreversibility, a regulatory problem would not exist.) and the effects be discussed in more detail. Nevertheless, even if a new technology the supra level regulatory hurdle, and be expected to generate net-welfare gains, benefits and costs will unevenly distributed among stakeholders, some may gain and some may loose and models to assess the distribution of gains and losses be discussed including political economy considerations.

The paper concludes with implications and priorities for the socio-economic impact assessment of GMP RNAi technology.

## **The political economy of the European agri-food chain**

K. Karantininis<sup>1</sup>, S. Chatzopoulou<sup>2</sup>

<sup>1</sup>*Swedish University of Agricultural Sciences (SLU)*

<sup>2</sup>*Roskilde University, Denmark*

\*e-mail: [karantininis.konstantinos@slu.se](mailto:karantininis.konstantinos@slu.se)

*Value chain, Political economy, mergers, food*

The perpetual expansion of the EU food regulatory policy making the last decade, stimulated new governance demands in the food chain both within the EU and the global level. This article concentrates on the EU food chain regulatory framework as part of the ‘Better regulation’ agenda. The article aims to unpack the specific elements of the food chain rules (‘what’ is regulated / ‘who’ are regulated) but also the way these rules are assessed under the Fitness check. These processes are not always discernible and impact on the food chain governance.

Regulating the EU food constitutes a significant institutional innovation and determines sectoral structural and organisational changes with global dimensions. Unravelling the specific elements of the EU food regulations, which do not necessarily focus only on setting standards, allows a better understanding of their indirect link to sectoral structural changes and governance. This article is based on EU data on mergers and acquisitions in the food chain sector, a series interviews and EU documents on the food regulatory policy.

The EU regulations were introduced to deal with the market inefficiencies and failures (positive), but also aimed to respond to the requirements for more transparency, information diffusion and satisfaction of consumers’ demands for high quality, healthy and safe food (normative) after a series of food scandals and crises. Drawing on regulatory governance and organisation theories, the article claims that the EU food chain regulatory framework induced indirectly distinctive links among the different levels of the food chain that rendered them interconnected and interdependent. Furthermore, this interdependence and interconnection among all the levels in the food chain (from production to consumer) blurs the boundaries of responsibility among the actors and becomes even harder to trace who is accountable for what. This results in a complex administration and management process of the increasing food regulatory requirements for high quality and testing standards and intensifies competition in the sector with pressing consequences especially to the SMEs that cannot always afford new investments in advanced technology. The article shows that the sector responds to the new circumstances through vertical or horizontal cooperation and forms new modes of governance that are characterised by higher levels of concentration with global dimensions.

## **Regulation of New Plant Breeding Techniques: The Case of Rapeseed in the EU**

T. J. Venus, D. Drabik, J. Wesseler

*Wageningen University, Agricultural Economics and Rural Policy Group, Department of Economics, Hollandseweg 1, 66706kn, The Netherlands*

*\*e-mail: Thomas.Venus@wur.nl*

*New Plant Breeding Techniques, GMO, labeling, coexistence, identity preservation, regulation, vertical product differentiation*

We analyze the market and welfare effects of regulating crops derived by New Plant Breeding Techniques (NPBTs) as genetically modified (GM) or conventional products. We consider the EU mandatory scheme for labeling GM products and a voluntary non-GM scheme for labeling livestock products derived from non-GM feed. We develop a partial equilibrium model that explicitly takes into account both the coexistence costs at farm-level and the segregation and identity preservation costs at downstream level. By applying the model to EU rapeseed, we find that regulating NPBTs as GM (as compared to non-GM) in combination with mandatory and voluntary labeling increases prices and makes consumers overall worse off and producers better off. We also show that higher coexistence costs make the price increasing effect even stronger. Voluntary non-GM labeling applied to feed makes consumers in this sector overall worse off but benefits farmers and rapeseed oil consumers overall as long as segregation costs are low. Consumers of biodiesel and industrial products such as lubricants produced from GM rapeseed benefit from high segregation costs. We show that the effects of farm-level coexistence costs largely differ from the effects of downstream market segregation costs.

## **Naturally GM: the development of new breeding techniques through patent data analysis**

V. Ventura, D. Frisio

*Department of Economics, Management and Quantitative Methods (DEMM), University of Milan, via Celoria 2, 20129 Milan, Italy*

*\*e-mail: vera.ventura@unimi.it*

*NBTs, intellectual property (IP), patents, public research*

After decades of debate on GMOs, even mainstream media are realizing that a new wave of techniques is going to replace the “old” transgenic approach to plant breeding. They define plants derived from new breeding techniques (*NBT*) as *natural GMOs* or *PDO-GMOs* to underline the most innovative aspects of this set of techniques – basically, they imitate nature. The potential applications of *NBTs* are widespread and a wide range of plants and traits are currently close to commercialization. Nevertheless, despite the large production of scientific studies dealing with the technical aspects of *NBTs* as well as the implication of considering them *GMOs* or not in legislation, the economic evaluation of this field of research is still poorly investigated. In this context, the aim of this work is the identification of innovation pathways related to the development of *NBTs*. More specifically, the main objective is to investigate the nature of the innovation itself, the type of international actors involved (public VS private) and the different strategies adopted in terms of innovative products or processes. The selected methodology is the analysis of patent data, since empirical research has shown that patents are frequently a good predictor of economic performance, widely used in the scientific literature as indicator of innovation. A combination of keywords and *IPC/CPC* codes are used to identify and collect patent data from the *Espacenet* web source, focusing on the most important *NBTs* and the main Patent Systems (*USPTO*, *EPO*, *JPO*, *SIPO* and the *PCT* route). The results reveal that patent analysis is confirmed to represent a useful tool in understanding the evolutionary trajectories of the agbiotech supply chain. For the specific field of research, data shows that private sector is still prevalent, following a pattern that doesn't significantly differ from the traditional *GMOs* R&D. Nevertheless, alongside with the *Big Six* agbiotech companies, some emerging pure agbiotech firms are acquiring relevance. Patent data analysis also identifies significant differences in the role of private and public research among the *NBTs*, evidencing the specialization of some universities and the rising of Chinese research.

**SESSION 5 - RNAi Communication and public acceptance (WG5)**

**The opportunity of a fresh start**

G. Carrada

*Independent author and consultant, Rome, Italy*

*\*e-mail: giovanni.carrada@fastwebnet.it*

*Communication, New breeding techniques, public understanding of science, perception of biotechnology*

By fundamentally changing the nature and extent of the genetic modification of plants, the new breeding techniques can mimic the natural occurrence of genetic variation, without adding to a plant any genetic material other than the desired trait as in all the breeding methods developed since the rediscovery of Mendel's laws. The new breeding techniques therefore offer the opportunity of a fresh start in the public understanding and appreciation of modern biotechnology applications in agriculture.

## **Communicating to address perceptions around plant breeding technologies: experiences from the AMIGA GM study on potato engineered for late blight resistance**

E. Mullins

*Dept. Crop Science, Oak Park, Carlow, Ireland*

*\*e-mail: ewen.mullins@teagasc.ie*

*Cisgenic potato, late blight disease, field trials, public debate*

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. As part the EU funded 'AMIGA' (Assessing and Monitoring the Impact of GM crops on Agroecosystems, 2012-2016) project, we were tasked with completing an agronomic and environmental assessment of a potato line, previously cisgenically engineered ([www.DuRPh.nl](http://www.DuRPh.nl)) to resist the late blight pathogen *Phytophthora infestans*. This was the first field-based GM study completed in Ireland in almost 20 years and hence generated significant public interest. In response, we implemented a communication strategy that ran in tandem with the active research programme, with over 82 'knowledge transfer' events completed, across a range of forums and media outlets. As such we hosted open days, participated in stakeholder workshops and public debates and seminars which were attended by > 5000 people, over the lifetime of the study. Being able to deliver scientific fact in a non-scientific language was critical to ensure engagement through all events and providing context to answer the question 'why is the study necessary?' was imperative. More specifically, explaining what is a risk was an essential objective, achieved by challenging audiences to comprehend risk of a GM plant versus the risks they experience in their daily routines. By framing the discussion in an objective, impartial way, we ensured we answered every query that was made, accepted all invitations received and attended all relevant events, irrespective of event bias, time organised or day held. The communication strategy implemented was not about trying to convince that one system is the sole solution but rather the focus was on educating, explaining and providing answers so that participants decide for themselves, based on a position of information that is grounded in sound science.

## **New science dissemination opportunities on facebook platforms**

H-G. Opsahl Sorteberg

*Norwegian University of Life Sciences, Dept Plant Science, Ås, Norway*

*\*e-mail: hildop@nmbu.no*

*Dissamination, communicating science, GMO/New breeding techniques, Facebook, New media channels*

Food security is an increasing basic challenge, and might add to the world's instability and threaten peace and human welfare more than anything else. This demands food trade security and increased sustainable food production, depending on scientific progress and communication. Surprises such as Brexit and the newly elected US president show how new the media don't pick up new information channels and new trends changing our world. Even the pope in Rome encourages increased real content in the many media platforms, avoiding feeding people junk causing Coprophagy.

Technology and networking through Google and Facebook (FB) are changing news distribution and thereby our perception of the world. Science dissemination must adapt accordingly, and the need for knowledge-based information is more important than ever given complicated new scientific break through and fast changes. The new social media tools give us new opportunities through streaming, podcast, potentially reaching the whole world 24/7/365. This also shortens the distance to the central media, where a text message can make the same day's news headlines.

To profit from this we need to adapt, and differentiate between audiences, media channels, publications through Wikipedia and Facebook and interactive communication. Media training focuses on short news presentations of typically seconds rather than minutes and hours, with the need of keeping 1-2 clear messages focused at the time. Disagreements with e.g. NGOs can give us attention and time, if we remember that our audience and interest is to reach the broader public. Science depends on reaching out, through classical papers in Journals but even more to the broader public. There is even an emerging open access publication through recognized new FB platforms.

Examples will be given on how to use social media to reach out through FB groups directly and via media, and interactive in both directions. FB groups and collegiums can secure coverage of the global news picture and publication channels. The FB "Vi må snakke om GMO" has 850 members in little Norway, and a core active daily group of journalists, politicians, scientists and dedicated public with good discussions of GMOs in general covering new techniques for farming in a broad sense, based on a scientifically sound foundation. This has led to many focused newspaper articles, TV debate participation, shaping the main news, larger TV productions, participation in important meetings and general seminars and has influenced politicians/party programs. Science dissemination will be illustrated with TV productions, news coverage, newspaper headlines, back stage activities and FB backings.

***A tale of two trials: communicating recent research on GM crops at Rothamsted Research***

M. Tsalavouta

*Rothamsted Research, West Common, Harpenden, Hertfordshire, UK, AL5 2JQ*

*\*e-mail: [matina.tsalavouta@rothamsted.ac.uk](mailto:matina.tsalavouta@rothamsted.ac.uk)*

Engagement, public dialogue, genetic engineering, field trials

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. At Rothamsted Research through strategic communication about the research undertaken in the organisation, we aim to engage in discussion and listen to our stakeholders. The most notable cases exemplifying the impact that such approach can have in the research is the communication approach relating to field trials of two distinct experiments with GM crops since 2012. The proposition to conduct field trials concerning the development of aphid repellent wheat plants was met with strong opposition, threat of destruction and intrusion by an activist. As a consequence, the researchers engaged in a national and international extensive communication activity with all interested stakeholders, the media, online conversations and petitions, and participated in hundreds of debates/events organised by a multitude of organisations. The researchers always explained in an accessible manner the science underpinning the experiment, the risks and how they were managed, who may be benefit from this research. Conducting the experiment and allowing it to conclude gained public support. The trial was completed in 2013 successfully and the results, although they did not demonstrate that the wheat plants repelled aphids effectively in the field, were extensively and proactively communicated through the media and a variety of other channels to hundreds of thousands of people nationally and internationally. The lessons learnt from this experience informed the proactive communication strategy for a new experiment that initiated in 2014 at Rothamsted testing the efficacy of Camelina plants modified to produce omega-3 fish oils in the field. This field trials have not been met with opposition or threat and three successive trial seasons have been successfully concluded.