Deutsche Phytomedizinische Gesellschaft e.V.

Nederlandse Kring voor Plantenvirologie

Sixth Joint Meeting of the

DPG working Group "Virus Diseases of Plants" and the

"Nederlandse Kring voor Plantevirologie"

March 27. – 28. 2017

Rheinische Friedrich-Wilhelms Universität
Bonn, Germany
Meeting address:
Lecture hall Anatomy, Nussallee 10, 53115 Bonn, Germany

Program and Abstracts

This symposium has been sponsored by

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Conference Organization:

Tatjana Kleinow and Mark Varrelmann (DPG working group "Virus diseases of plants")

René AA van der Vlugt (Nederlandse Kring voor Plantevirologie)

Joachim Hamacher (local organizer; Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES), Phytomedizin, Rheinische Friedrich-Wilhelms Universität, Bonn)

Monday, 27. March 2017	
11:00 – 11:30	**Registration, Poster up**
11:30 – 11:45	**Opening session – Welcome and announcements** *Tatjana Kleinow, Mark Varrelmann, René AA van der Vlugt, Joachim Hamacher & Team*
11:45 – 13:20	**Section I: Virus characterization Chair** *Edgar Maiß*
11:45 – 12:25	**Keynote Lecture: Regulatory framework for international exchange of biological material with respect to the Nagoya Protocol** *Thomas Greiber*
12:25 – 12:45	**Near-atomic resolution structure of a plant geminivirus determined by electron cryo-microscopy** *Katharina Hipp, Clemens Grimm, Holger Jeske & Bettina Böttcher*
12:45 – 13:05	**Contributions of pararetroviruses to genome evolution and function** *Katja R Richert-Pöggeler, Osamah Alisawi, JS (Pat) Heslop-Harrison & Trude Schwarzacher*
13:05 – 13:20	**Award ceremony: DPG Prize for Young Academics for Pamela Akoth Ogada** *Johannes Hallmann*
13:20 – 14:30	**Coffee & Tea break and Poster viewing (70 min.)**
14:30 – 16:10	**Section II: Virus identification & characterization Chair** *Mark Varrelmann*
14:30 – 14:50	**Construction of an infectious full-length cDNA clone of beet chlorosis virus for agroinfection using Gibson Assembly** *Veronika Wetzel & Mark Varrelmann*
14:50 – 15:10	**Celery latent virus: Difficulties and pitfalls in sequence determination and construction of an infectious full-length clone** *Hanna Rose, Ines Döring, Heinrich-Josef Vetten, Wulf Menzel, Katja R Richert-Pöggeler & Edgar Maiß*
15:10 – 15:30	**Sequence variation in strawberry latent ringspot virus** *Annette Dullemans, Theo van der Lee, Peter Bonants, Martin Verbeek, Marleen Botermans, Marcel Westenberg, Christel de Krom, Annelien Roenhorst, Maarten de Kock, Iris JE Stulemeijer & René AA van der Vlugt*
15:30 – 15:50	**Emaraviruses in woody hosts - how far can you count?** *Susanne von Bargen, Marius Rehanek, Jenny Roßbach, Thierry Candresse, Hans-Peter Mühlbach, Artemis Rumbou, Michael Kube, Martina Bandte & Carmen Büttner*
15:50 – 16:10	**Characterization of a nucleorhabdovirus from *Physostegia*** *Wulf Menzel, Katja R Richert-Pöggeler, Stephan Winter & Dennis Knierim*
16:10 – 16:30	**Short Break (20 min.)**
16:30 – 18:55	**Section III: Diagnostics & Contributions from practice Chair** *Jan HW Bergervoet*
16:30 – 16:50	**Multiplex detection of plant pathogens** *Jan HW Bergervoet, René AA van der Vlugt, Henry van Raaij, Antje de Bruin, Margarit JEIM de Klein, Anke CM Clerkx, Willem Stol, Petra van Bekkum & Jose CM van Beckhoven*
16:50 – 17:10	**A multiplex Luminex xTAG-assay to distinguish between infectious and non-infectious cucumber green mottle mosaic virus on cucumber seeds** *René AA van der Vlugt, Henry van Raaij, Petra van Bekkum & Jan HW Bergervoet*
17:10 – 17:25	**Specificity of ELISA and PCR tests on related potyvirusses in lilium and tulip** *Iris JE Stulemeijer, Jiska van Lavieren, Veronika Wallner & Maarten de Koc*
17:25 – 17:40	**Legume viruses survey 2016 in Germany** *Yahya ZA Gaafar & Heiko Ziebell*

17:40– 17:55	**Viruses and viroids occuring 2016 in Austria** *Sabine Grausgruber-Gröger*
17:55 – 18:10	**Outbreak of potato spindle tuber viroid in vegetatively-propagated plants of *Capsicum annuum* in The Netherlands** *Christel E de Krom, JThJ Verhoeven, JGB Voogd, N Strik & Annelien* Roenhorst
18:10 – 18:25	**Occurrence and importance of soil-borne cereal viruses in Germany** *Ute Kastirr, Angelika Ziegler, Viktoria Fomitcheva, Dragan Perović, Peter Hübner, Lothar Böttcher, Hendrik Schwabe, Barbara Kempe, Birgit Post, Hilmar Cöster, Bettina Golecki, Volker Rohlf, Mark Heubach , Sabine Rode & Ralph-Peter Nußbaum*
18:25 – 18:40	**Highlights in virus diagnosis 2016** *Heiko Ziebell*
18:40 – 18:55	**Discovery of a new nanovirus obviously associated with a severe disease in parsley** *Heinrich-Josef Vetten*
19:30	**Get-together (including buffet but without drinks)** Harmonie, Frongasse 28-30, 53121 Bonn Endenich

Tuesday, 28. March 2017	
08:20 – 10:20	**Section IV: Virus characterization & gene functions Chair** *Björn Krenz*
08:20 – 09:00	**Keynote Lecture: A biological perspective on the tospoviral proteins and their roles during infection and dissemination in plants and thrips** *Richard Kormelink*
09:00 – 09:20	**Discovery and characterization of dimeric tospoviral S RNA molecules** *André Bertran, M Ciuffo, Paolo Margaria, Cristina Rosa, Renato Oliveira Resende & Massimo Turina*
09:20 – 09:40	**Effects of Ourmia melon virus movement protein mutants on virus infection, and their co-localization with plant cytoskeleton and organelles** *Paolo Margaria, Natali Ozber, Charles T Anderson, Massimo Turina & Cristina Rosa*
09:40 – 10:00	**Turnip mosaic virus P1 protein is a novel interaction partner of the stress granule component G3BP** *Susanna Krapp, Johannes Keseler, Eva Greiner, Uwe Sonnewald & Björn Krenz*
10:00 – 10:20	**Investigations on pseudorecombinants from beet soil-borne mosaic virus and beet necrotic yellow vein virus in *Beta vulgaris* subsp. *vulgaris*** *Sebastian Liebe, Jose Fernando Gil, Eugene Savenkov, Edgar Maiß & Mark Varrelmann*
10:20 – 11:00	**Coffee & Tea break and Poster viewing (40 min.)**
11:00 – 13:00	**Section V: Gene function, Resistance & Technologies Chair** *Richard Kormelink*
11:00 – 11:20	**Sequence variation of a full-length clone derived PLRV isolate over several passages** *Dennis Knierim & Stephan Winter*
11:20 – 11:40	**Players and mechanisms in antiviral pattern-triggered immunity in plants** *Ines Wyrsch, Thomas Boller, Manfred Heinlein & Annette Niehl*
11:40 – 12:00	***Ty-1*: a resistance gene against geminiviruses** *Corien Voorburg & Richard Kormelink*
12:00 – 12:20	**Use and misuse of methyl-cytosine assays in geminivirus research** *Holger Jeske*

12:20 – 12:40	**Controlled assembly of 2- and 3-dimensional virus crystals** *Veronika Rink, Mario Braun , M Ani, KaJohn Boonrood, Christine Müller-Renno, Gabi Krczal & Christiane Ziegler*
12:40 – 13:00	**Tobacco mosaic virus-derived nucleoprotein domains as building blocks for porous bio-functional materials** *Nana L Wenz, Sylwia Piasecka, Matthäus Kalinowski, Clemens Richert & Christina Wege*
13:00	**Wrap up of the meeting:** **Closing remarks joint part** *Tatjana Kleinow, Mark Varrelmann, René AA van der Vlugt, Joachim Hamacher & Team* **Societies business hour**
afterwards	**Departure** Possibility for lunch at the local Mensa

Overview Posters

Poster No. 1

Dispersal of plant viruses in irrigation water prevented by electrolytic disinfection of nutrient solution

Martina Bandte, Marlon Hans Rodriguez, Uwe Schmidt & Carmen Büttner

Poster No. 2

Electronmicroscopical observations of doubly infected *Hortensia*-leaves with Hydrangea ringspot- and eggplant mottled dwarf virus

Caro Sichtermann & Joachim Hamacher

Poster No. 3

Split GFP as a tool to study viral infection: Cauliflower mosaic virus

Beatriz Dáder, Jean-Luc Macia & Martin Drucker

Poster No. 4

Analysis of the complete genome sequence of Euphorbia ringspot virus, an atypical species in the genus *Potyvirus*

Dennis Knierim, Wulf Menzel & Stephan Winter

Poster No. 5

Strategies for the implementation of a certification program for virus-tested plant material in Colombia

Joseph Cutler, Juliane Langer, Marlon Hans Rodriguez, Orlando Acosta Losada, Fánor Casierra-Posada, Adriana Castañeda Cardena, Mónica Betancourt Vasquez, Wilmer Cuellar & Carmen Büttner

Poster No. 6

Labeling of beet mosaic virus (BtMV) with green and red fluorescent proteins and analysis of distribution in *Nicotiana benthamiana*

Hamza Mohammad, Ali Pasha & Edgar Maiß

Poster No. 7

Cell death triggering and effector recognition by the single dominant *Sw*-5 and *Tsw* resistance genes

Irene van Grinsven, Athos Silva de Oliveira, Dryas de Ronde, Octav Caldararu, Andrei-Jose Petrescu, Renato Oliveira Resende & Richard Kormelink

Poster No. 8

First report of mint vein banding-associated virus infecting *Mentha* x *gracilis* in Germany

Wulf Menzel & Stephan Winter

Poster No. 9

Symptom expression and virus accumulation in screen house *Nicotiana* hosts co-infected with cassava brown streak viruses

Esperance Munganyinka, Elijah M Ateka, Joseph Ndunguru, Agnes W Kihurani, Stephan Winter, Fred Tairo, Peter Sseruwagi, Marie Claire Kanyange & Beate Stein

Poster No. 10

Tobacco mosaic virus with defined discriminable coat protein domains: Manipulating RNA-directed self-assembly

Angela Schneider, Fabian J Eber, Nana L. Wenz, Klara Altintoprak, Holger Jeske, Sabine Eiben & Christina Wege

Poster No. 11

Transmission of tomato chlorotic dwarf viroid assisted by potato leafroll luteovirus by *Myzus persicae*

Thi Thu Vo & Joachim Hamacher

Poster No. 12

Complete sequence determination and construction of an infectious full-length clone of a celery mosaic virus isolate from Quedlinburg (Germany)

Hanna Rose & Edgar Maiß

Poster No. 13

Complete sequence and construction of an infectious full-length clone of a Panicum mosaic virus isolate from Aschersleben (Germany)

Hanna Rose, Frank Rabenstein, Katja R Richert-Pöggeler & Edgar Maiß

Poster No. 14

A novel virus is associated with the ringspot disease in common oak (*Quercus robur* L.)

Marius Rehanek, Susanne von Bargen, Martina Bandte & Carmen Büttner

Poster No. 15

Strawberry latent ringspot virus in lily is seed transmitted and localised in the embryo

Martin Verbeek & CCMM (Ineke) Stijger

Poster No. 16

Q-bank for sharing data and information on plant virus and viroid isolates in collections

Annelien Roenhorst, L Flint, Wulf Menzel, Stephan Winter & René AA van der Vlugt

Poster No. 17

Effects of pepino mosaic virus and cucumber green mottle mosaic virus on drought tolerance in *Nicotiana benthamiana*

AJ Westgeest & René AA van der Vlugt

Poster No. 18

Application of next generation sequencing for the study and diagnosis of plant virus diseases in agriculture

René AA van der Vlugt, Angelantonio Minafra, Antonio Olmos, Maja Ravnikar, Thierry Wetzel, Christina Varveri & Sebastien Massart

Poster No. 19

Studies on the identification of a silencing suppressor of celery latent virus

Simon Schiwek, Hanna Rose & Edgar Maiß

Poster No. 20

Characterizing cassava resistance against cassava brown streak viruses

Samar Sheat, Bettina Fürholzner, Beate Stein & Stephan Winter

Poster No. 21

Feasibility of cowpea chlorotic mottle virus-like particles in nanobiotechnology: potential VLP scaffold for epitope-based vaccines

Afshin Hassani-Mehraban, Sjoerd Creutzburg, Luc van Heereveld & Richard Kormelink

Poster No. 22

Novel insight in the interaction between REP, PCNA and SCE1, key factors in geminivirus replication

Francesca Maio, Harrold A van den Burg & Marcel Prins

Poster No. 23

Looking for elicitors and receptors of cauliflower mosaic virus (CaMV) transmission activation

Christiane Then, Jean Luc Macía, Gaël Thébaud & Martin Drucker

Poster No. 24

A proteomics approach in finding host factors essential for tospovirus replication

Tieme Helderman, Harrold A van den Burg & Marcel Prins

Poster No. 25

Viruses associated with diseased broad-leaved tree species in Europe

Susanne von Bargen, Martina Bandte, Artemis Rumbou, Thierry Wetzel, Thierry Candresse, Armelle Marais-Colombel, Chantal Faure, Maria Landgraf, Juliane Langer, Jenny Roßbach & Carmen Büttner

Poster No. 26

Phosphorylation of the begomovirus movement protein affects host range, symptom development, and viral DNA accumulation

Tatjana Kleinow, Gabi Kepp, Marc Nischang, Sigrid Kober, Alexander Beck, Ulrich Kratzer, Holger Jeske & Christina Wege

Poster No. 27

Full genome characterisation of tospoviruses by next generation sequencing

Annette Dullemans, Petra van Bekkum, Annelien Roenhorst, Richard Kormelink & René AA van der Vlugt

Poster No. 28

Direct identification of sugar beet soil-borne viruses in soil samples

Viktoria Fomitcheva & Thomas Kühne

Poster No. 29

Tobacco mosaic virus as an additive in hydrogels

Sabine Eiben, Sabrina Kleiser, Tina Lang, Günter Tovar, Christina Wege, Michael Schweikert & Alexander Southan

Poster No. 30

Molecular characterization of a new 'rule-breaking' tobacco rattle virus RNA2 and its stepwise degradation and finally loss in potato plants grown in a nematode-free environment

Kerstin Lindner, Inga Hilbrich & Renate Koenig

List of Participants

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Abstracts Oral Presentation

Section I

Keynote Lecture: Regulatory framework for international exchange of biological material with respect to the Nagoya Protocol

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Near-atomic resolution structure of a plant geminivirus determined by electron cryo-microscopy

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African cassava mosaic virus belongs to the begomovirus genus of the geminivirus family. The unique twin particles of incomplete icosahedra are joined at five-fold vertices building an unusual waist. The interaction within a half-capsid or across the waist was unknown so far. We have determined the virion structure by electron cryo-microscopy with a resolution of 4.2 Å and built an atomic model for its capsid protein. The inter-capsomer contacts mediated by the flexible N-termini and loop regions differed within the half-capsids and at the waist, explaining partly the unusual twin structure. Basic amino acid residues inside the capsid form a positively charged pocket next to the five-fold axis of the capsomer suitable to bind DNA. Within this pocket, density most likely corresponding to DNA was resolved.

Contributions of pararetroviruses to genome evolution and function

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Pararetroviruses comprise dsDNA viruses that use reverse transcription for genome replication. They are represented by the virus families *Hepadnaviridae* and *Caulimoviridae* infecting animal and plants respectively. The quasispecies nature of viruses together with the mobility of these infectious entities makes them ideal candidates for horizontal DNA transfer. Indeed, cytogenetics using fluorescent *in situ* hybridization (FISH) combined with improved sequencing technologies revealed that endogenous pararetrovirus sequences (EPRVs) are ubiquitously and in high diversity present in eukaryotic genomes. Propagation after genome invasion as well as repeated events of genome colonialization may both account for the high abundance of EPRVs found in some plants. Lateral DNA transfer mediated by pararetroviruses occurred at different time points during evolution and is still ongoing. It can be assumed that any infected somatic plant cell presents a target for horizontal DNA transfer.

However, only those integrations get manifested as EPRVs that are not deleterious for the host and these can be identified by current detection methods. EPRVs can be found degenerated as fossils as shown for rice, but also as functional sequences representing complete viral genomes triggering diseases as documented for banana and petunia. EPRVs can be suitable markers for monitoring dynamic processes during genome hybridisation such as during generation of *P. hybrida*. It may be advantageous for the host to preserve EPRV sequences to serve as triggers for RNAi in order to prevent virus infection. Recent studies discuss molecular functions for the host provided by integration of pararetroviral movement protein (MP)-like sequences. Based on their phylogenetic analyses and known MP functions in macromolecule trafficking a possible role of integrated MP in control of tissue differentiation is proposed. EPRVs will provide valuable tools to understand the mechanisms involved in lateral DNA transfer. The role of endogenous pararetrovirus sequences in spurring genome adaptation of sessile eukaryotes including plants to changing environmental conditions still awaits elucidation.

Section II

Construction of an infectious full-length cDNA clone of beet chlorosis virus for agroinfection using Gibson Assembly

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A French isolate of beet chlorosis virus (BChV-2a) was used for construction of a full-length cDNA clone of the virus using one-step cloning procedure Gibson Assembly in a binary vector to be used for *Agrobacterium*-mediated infection. As the complete virus genome sequence initially determined (Hauser et al., 2002) was lacking the extreme ends, it was required to identify the 3´terminal and 5´terminal end sequences by means of "rapid amplification of cDNA ends" (RACE). The full-length viral cDNA was cloned into a binary vector under control of the cauliflower mosaic virus 35S promotor and used for *A. tumefaciens* mediated infection of *Nicotiana benthamiana* and *Beta vulgaris* plants. Replication, systemic movement and virion assembly were detected six weeks post inoculation by means of specific RT-PCR, ELISA and Western blot. Recombinant virus from agroinfected *B. vulgaris* plants was successfully aphid transmitted by the natural vector *Myzus persicae* with an efficacy compared to the wild-type virus used for the construction. The host range of BChV was examined using naturally and cDNA clone infected *B. vulgaris* plants by means of aphid transmission. Both wild-type and recombinant virus led to systemic infection in B. vulgaris, *N. benthamiana* and *Beta macrocarpa*, whereas no infection was detectable in *Brassica napus*, *Nicotiana hesperis*, *Nicotiana clevelandii*, *Capsella bursa pastoris* and *Montia perfoliata*, which coincide with the literature. This full-length cDNA clone of BChV, next to cDNA clones of other *Luteoviridae*, can be used to study more about host specificity, host range, infection efficacy, as well as protein functions and resistance mechanisms.

Celery latent virus: Difficulties and pitfalls in sequence determination and construction of an infectious full-length clone

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Celery latent virus (CeLV) was first described by Brandes & Luisoni in 1966 and further characterized by Bos et al. in 1978 (Brandes und Luisoni 1966; Bos et al. 1978). CeLV-infected celeriac (*Apium graveolens* var. *rapaceum*) and celery (*Apium graveolens* var. *dulce*) plants do not show any symptoms in comparison to healthy plants. It was found that the virus could infect other hosts mainly in a latent manner. On *Chenopodium quinoa* it causes systemic symptoms and is seed-transmissible to rates up to 70 %. To date no potyvirus-like inclusion bodies were detected for CeLV and the vector is still unknown. In electron microscopy investigations CeLV appears as flexible rod-shaped particles resembling those of potyviruses with an estimated size of 885 nm.

First sequencing approaches using the classical gene-walking method combined with rapid amplification of cDNA ends (RACE) revealed a genome sequence of 11.220 nucleotides with a short 5'-UTR of 13 nt and a 3'-UTR of 284 nt with an additional 3'-polyA tail. The resulting polyprotein consists of 3640 amino acids with an N-terminal localized signal peptide. The polyprotein sequence shows weak similarities to members of the genus *Macluravirus* and *Bymovirus* especially to the cylindrical inclusion protein (CI) and nuclear inclusion protein b (NIb) domains (BLAST). Phylogenetic analyses suggest that CeLV is a member of a putative new genus within the *Potyviridae*. For the construction of an infectious full-length clone different amplification and cloning strategies were applied for example Gibson assembly or based on restriction sites with varying numbers and lengths of fragments. For a long time it was not possible to receive an infectious full-length clone due to single insertions or deletions in the coding sequence resulting in a frame-shift that ends up in a premature stop in translation. But also all other clones without any lethal mutations were not able to cause an infection in *Nicotiana benthamiana*. In order to get a deeper insight in the CeLV population and sequence composition in the plant, a Deep Sequencing approach was performed using random PCR fragments produced with primers designed by Froussard in 1992 (Froussard 1992). The results indicated that the determined 3'-end is longer than expected which could be proved in further experiments. Up to now it was not possible to detect a polyA-Tail which is known to be a typical feature of viruses in the *Potyviridae*. Finally, the additional information made it possible to establish an infectious full-length clone.

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Sequence variation in strawberry latent ringspot virus

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Background and Objectives

Strawberry latent ringspot virus (SLRSV) is an unassigned species in the family *Secoviridae*. SLRSV infections are reported in many mono- and dicotyledenous crops. SLRSV has a quarantine status for China and some other countries in South-East Asia. Since SLRSV occurs symptomless in lilies in the Netherlands, lily bulbs are routinely tested for export to these countries. So far testing for SLRSV has been performed by double-antibody sandwich (DAS) enzyme-linked immuno sorbent assay (ELISA). To replace DAS-ELISA by reverse transcription-polymerase chain reaction (RT-PCR), primers were

developed based on a limited number of SLRSV sequences. However, RT-PCR results were not in full accordance with DAS-ELISA results. Therefore, additional SLRSV sequences were determined from infected field samples of lily bulbs and from collection materials, to gain more insight in the sequence variation of this virus.

Materials and Methods

Total RNA extracts were used for preparation of cDNA libraries with individual MIDs suitable for Illumina HiSeq paired end sequencing (TruSeq Stranded Total RNA sample Preparation Kit with Ribo-Zero Plant (Illumina Inc, San Diego CA, USA)). and subsequently run in batch on a HiSeq 2500. After MID splitting individual datasets were fed into custom-designed workflows within CLC Genomics Workbench (Qiagen, Denmark). These workflows comprised 'de novo' and reference assemblies with and without substraction of plant-related reads. Resulting contigs were analysed by BlastN and BlastX against the NCBI database to identify SLRSV related sequences.

Results

Twenty one new SLRSV sequences were assembled, 12 from lilies and 10 from virus-collection materials. Most assemblies of the individual SLRSV datasets resulted in near full length RNA1 and RNA2 segments. Primer walking and additional conventional Sanger sequencing on a selection of isolates resulted in assemblies of full-length RNA sequences of five isolates.

Comparison of all available coat protein (CP) nucleotide sequences showed 4 groups with 60-80% sequence identity between groups against 85-99% identity within groups. CP amino-acid sequence identities were higher with 63-94% between and 97-99% identity within groups.

Conclusion

Comparison of SLRSV isolates revealed a considerable sequence variation within this species, which might explain the erratic results of RT-PCR in comparison with DAS-ELISA.

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Emaraviruses in woody hosts - how far can you count?

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European mountain ash ringspot-associated virus (EMARaV) is the type member of the genus *Emaravirus* comprising plant viruses with a segmented ss(-)RNA genome mainly infecting woody hosts. Emaraviruses consists of at least four conserved monocistronic genome segments within the enveloped spherical particle, encoding the replicase (RNA1), a glycoprotein precursor (RNA2), the viral nucleocapsid protein (RNA3), and a movement protein (MP, RNA4). Some members of the genus contain up to four additional genomic RNA molecules encoding proteins of unknown function. Until now, no genome segment has been identified in EMARaV encoding a 42 kDa protein which is orthologous to the functionally characterized MPs of related emaraviruses. Viruses within the genus are transmitted by eriophyid gall mites and have a narrow host range usually restricted to few related species. Recently, new tentative emaraviruses have been described, which are associated with diseases for instance in redbud and kiwifruit, by application of high-throughput sequencing techniques (di Bello et al. 2016, Zheng et al. 2016). Here, we report novel putative emaraviruses

affecting different broad-leaved tree species with chlorotic ringspots, veinbanding, mosaic, mottle and/or leaf deformation. Viral sequences were retrieved from contigs assembled from raw data of a standard-paired end 100 library generated by a metagenomic approach with random hexamers or applying generic primers during the preparation of cDNA-libraries for illumina RNASeq. Preliminary analyses of results provide first insight into the genome organization of four putative new emaraviruses discovered in important deciduous tree species of the European forest and urban green space.

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Characterization of a nucleorhabdovirus from *Physostegia*

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In July 2014, a *Physostegia* plant showing severe chlorotic mottle symptoms and leaf deformation was received from Austria. Following mechanical inoculation, the virus systemically infected *Nicotiana occidentalis* 37b. Bullet shaped particles were found in electron microscopical observations indicating the presence of rhabdoviruses. As expected for negative strand RNA viruses, dsRNA extraction failed to extract viral replication intermediates from infected *Physostegia* or *Nicotiana* plants. Furthermore, no PCR products could be obtained by using published sets of degenerate rhabdovirus oligonucleotide primers. This prompted us to use a total RNA extract as template for a next generation sequencing approach. Following selective depletion of ribosomal RNA (Invitrogen RiboMinus™ Plant Kit), a paired-end library was created from dscDNA (Illumina Nextera XT Library Preparation Kit) and sequenced on an Illumina MiSeq platform. *De novo* assembly of the one million reads obtained was done with Geneious software. One contig of 13,193 bases assembled of 40,376 reads was identified by blast as the putative genome (lacking the extreme 5'- and 3'-ends) of a nucleorhabdovirus, showing the highest nucleotide sequence identity with 70.7% to an eggplant mottled dwarf virus (EMDV) isolate, followed by 53% to a potato yellow dwarf virus isolate. The nt identity values between assigned nucleorhabdoviruses range from 38.4% to 58.6%. The lacking extreme 5'- and 3'-ends were determined by RACE, resulting in a total genome size of 13,320 nts. Following back-inoculation to *Physostegia*, Koch's postulates could be fulfilled. No sequence identity threshold value has been defined for species demarcation within the genus *Nucleorhabdovirus*. Seedlings grow from seeds of infected *Physostegia* plants remained all symsptomless, indicating the lack of seed transmissibility, which is consistent with previous observations of other rhabdoviruses. Due to the symptoms observed on the original host, the isolate was named Physostegia chlorotic mottle virus (PhCMoV) and is available at the DSMZ Plant Virus Collection under accession no. PV-1182.

Section III

Multiplex detection of plant pathogens

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Background and Objectives

Plant diseases pose an enormous threat to global food security and market access. While food production must increase to meet the demands of the world's growing population, plant pathogens still claim 10-16% of the global harvest (Strange 2005, Chakraborty, 2011). Large scale breeding programs introducing resistance genes or other traits in major crops increase production yield, but this is a time consuming and expensive route which usually improves production by 5%. Advances in phytosanitary methods have a direct effect on plant health and immediately can increase production up to 30%. The most effective method for plant diseases management is to remove the diseased plants. To detect diseased plant material at early stages, high-throughput diagnostic technologies are needed.

Materials and Methods

For large scale testing ELISA is still widely used, but this technology allows limited multiplex detection possibilities. Next to this, detection of fungi and viroid's using ELISA is next to impossible while the Luminex technology offers excellent possibilities for the multiplex detection of plant pathogens based both on immunology (Luminex xMAP®) and RNA and/or DNA based detection (Luminex xTAG®). Furthermore, multiplex testing reduces labour costs and the amounts of consumables and buffers to be used significantly.

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A multiplex Luminex xTAG-assay to distinguish between infectious and non-infectious cucumber green mottle mosaic virus on cucumber seeds

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Background and Objectives

Cucumber green mottle mosaic virus (CGMMV; genus *Tobamovirus*) is currently causing significant problems worldwide. The virus is mainly restricted to *Cucurbitacae* however, several weed species have been reported as possible host plants. CGMMV is clearly seed transmitted which is the suspected cause for recent outbreaks in the USA and Australia.

Several serological and molecular tests are available to detect the virus on seeds however none can distinguish between infectious ('alive') and non-infectious ('dead') virus. Only an elaborate, time-consuming and relatively insensitive bioassay can make its distinction.

We set out to develop a test that can make a more reliable distinction between infectious and non-infectious CGMMV on cucumber seeds.

Materials and Methods

Different seed batches from various origin and naturally infected with CGMMV were contributed by H. Koenraadt (Naktuinbouw, Roelofarendsveen, The Netherlands). A true multiplex Luminex xTAG assay, employing multiple RT-PCR primer sets in combination with 11 TSPE primers (van Brunschot et al, 2014) was designed and tested to detect CGMMV RNA from different CGMMV isolates and subjected to an internationally accepted validation protocol.

Results

All TSPE primers gave clear positive signals on the untreated seed batches. A standard dry-heat treatment on the different batches of CGMMV-infected seeds (B. Woudt, Syngenta Seeds, Enkhuizen, The Netherlands) reduced all TSPE signals to nearly background levels, indicating a significant breakdown of the viral RNA.

Conclusions

The developed multiplex xTAG assay successfully detected different CGMMV isolates in both leaf material and cucumber seeds and was capable to distinguish intact from degraded CGMMV RNA. It was also validated with respect to various performance criteria. Bioassay experiments to confirm the correlation between the absence of TSPE signals with the abolishment of virus infectivity will be reported.

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Specificity of ELISA and PCR tests on related potyvirusses in lilium and tulip

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In lilium and tulip crops, three highly related potyviruses exist that give color breaking symptoms on the flowers. In liliums, lily mottle virus (LMoV) and lily virus A (LVA) are known to cause these symptoms, while similar symptoms in tulip are caused by tulip breaking virus (TBV). However, there are observations that all three viruses can occur in both crops with a low frequency.

In 2016, we have performed a survey in lilium and tulip to study the distribution of LMoV, LVA and TBV in both crops. We have tested selected material parallel with dedicated ELISA and PCR tests. In addition, we have performed generic poty PCR's to confirm the identity of the virus. Besides insight in potyvirus distribution in plants with color breaking flowers, this approach also gave insight in the specificity of our ELISA and PCR tests.

Legume viruses survey 2016 in Germany

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Legumes are important crops for human and animal consumption. They can be infected by numerous plant viruses, some of which are seed-transmitted causing yield losses and economic impacts. Plants with virus-like symptoms were collected and sent to Julius Kuehn-Institute (JKI) for analyses.

The virus families that were diagnosed are *Betaflexiviridae*, *Luteoviridae*, *Nanoviridae* and *Potyviridae*. The most prominent virus family found was the *Luteoviridae* mainly due to pea enation mosaic virus (PEMV) of the genus *Enamovirus* (over 70% of samples analysed). *Nanoviridae*, in particular pea necrotic yellow dwarf virus (PNYDV) (over 50% of samples analysed), was found Nationwide in peas (*Pisum sativum*), faba beans (*Vicia faba*) and lentils (*Lens culinaris*) causing crop

losses. It was only reported in the last few years in green peas in Saxony and Saxony-Anhalt with no major economic impact.

In contrast to other plant viruses, nanoviruses infection can lead to total crop losses. They are transmitted by aphids in a circulative, persistent manner. Their alternative winter hosts are unknown however there are susceptible hosts have been identified such as clover and vetch species. Moreover, there are no resistant pea or faba bean accessions discovered till now. Thus, PNYDV is a potential threat for the legume crops extension in Germany and surrounding countries.

Viruses and viroids occurring 2016 in Austria

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Outbreak of potato spindle tuber viroid in vegetatively-propagated plants of *Capsicum annuum* in The Netherlands

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In 2016 potato spindle tuber viroid (PSTVd) was identified in vegetatively-propagated plants of sweet pepper (*Capsicum annuum*) at five locations. The nursery imported four seedless pepper selections from Israel and PSTVd was found in all progeny plants. Moreover, at the nursery the viroid had spread to some plants of other vegetatively-propagated selections, grown in the same compartment. Plants of both the Israeli and other selections were distributed to growers for fruit production and for demonstration of new varieties. The presence of PSTVd was confirmed at all these locations, even though infected plants did not show symptoms. All lots with infected plants were destroyed under official supervision of the NPPO.

PSTVd was identified by analysing the viroid genome sequences obtained after RT-PCR. The predominant sequence was identical to a sequence found in the same pepper selections in Israel and an isolate from *Solanum jasminoides* (GenBank Accession FM998548). This indicates that the infected pepper selections from Israel were the source of the outbreak in The Netherlands. Moreover, infected plants of *S. jasminoides* may have been the original source of inoculum of the vegetatively-propagated pepper plants.

This outbreak shows once more that symptomless hosts may act as pathway for introduction of PSTVd, which in combination with vegetative propagation pose a risk for unnoticed spreading.

Occurrence and importance of soil-borne cereal viruses in Germany

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Soil-borne cereal viruses are widespread in America, Asia and Europe and cause serious yield losses in winter cultures of wheat, rye and triticale. In Germany the furoviruses soil-borne wheat mosaic virus (SBWMV) and soil-borne cereal mosaic virus (SBCMV) and the bymovirus wheat spindle streak mosaic virus (WSSMV) occur in numerous cereal growing areas. These viruses are vectored by the endoparasitic protist *Polymyxa graminis* (Led.) of the order *Plasmodiophorida*.

The aim of our work was to study the epidemiology of soil-borne viruses and characterize the virus-vector pathogen populations in different types of grains.

The dissemination of the furoviruses in Germany was analysed. The SBWMV was identified in three German federal states. For the pathogen characterization sequences of different virus isolates were compared. A new strain of SBWMV (New York strain) was detected in Germany for the first time. This virus causes in wheat grain yield losses of 40% in infested fields.

For the SBCMV several virus types are known. Differences in aggressiveness between the O- and C-types of SBCMV were detected. In Germany the C-type arise in various federal states with a high aggressiveness to durum wheat, rye and triticale but not to bread wheat. In other European countries the O-type shows high aggressiveness to bread wheat too.

Comparison of WSSMV isolates has shown that most German isolates contained an extra three amino acids. This insertion is missing in French and American strains.

The *formae speciales* of *P. graminis* were differentiated, a set of sequences from 15 *P. graminis* isolates was submitted to database. The occurrence of the subspecies *P. g. temperata* (ribotyp I) and *P.g. tepida* (ribotyp II) was identified in different infested by soil-borne viruses regions and in several types of grain. These analyses demonstrated that the viruses of a pathogen population were not dependent for their transmission on specific small grain varieties or specific *P. graminis formae speciales.*

In the result of monitoring of soil-borne virus occurrence was verified that these virus diseases are widespread in the federal states Schleswig-Holstein, Lower-Saxony and Saxony-Anhalt. In seven other states these virus are detected until 2016 in several parts of cereal growing areas. The monitoring has to be increase in the southern states of Germany.

The cultivation of resistant varieties is the only way to control these virus diseases. The number of resistant wheat varieties in Germany is insufficient for the effective wheat production in virus infested regions of different geographically and climatically growing areas.

Highlights in virus diagnosis 2016

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Discovery of a new nanovirus obviously associated with a severe disease in parsley

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Section IV

Keynote Lecture: A biological perspective on the tospoviral proteins and their roles during infection and dissemination in plants and thrips

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Tomato spotted wilt virus (TSWV) presents the type species of the genus *Tospovirus* within the arthropod-borne *Bunyaviridae*, a large family of primarily animal infecting viruses that are classified into the four genera *Orthobunyavirus*, *Phlebovirus*, *Nairovirus* and *Hantavirus* and a fifth one, the

Tospovirus genus, containing the plant-infecting members. From all these viruses some are listed as potential agents for bioterrorism, or belong to the most devastating plant viruses worldwide (Plyusnin & Elliott, 2011; Kormelink et al., 2011). Like for all bunyaviruses, tospovirus particles are spherical and membrane bound (~80-120 nm) and contain a tripartite RNA genome tightly encapsidated by the nucleo(capsid)protein (N) and small amounts of the viral RNA-dependent RNA polymerase (RdRP, or L protein). The structural proteins N and two glycoproteins (Gn and Gc) play a major role in particle assembly during which they interplay with elements from the cytoskeleton and endomembrane system. For the acquisition and transmission of the virus from plants by its thrips vector the glycoproteins are indispensable. The tospovirus genome additionally encodes two non-structural proteins, NSM and NSS, that are involved in viral spread (NSM) respectively counter defense against antiviral RNAi, but also trigger two intracellular innate immunity sensors in plants. During an overviewing presentation the roles of the tospoviral proteins in the "life cycle" of these viruses in plants and thrips will be described and their modes of action discussed compared to/from the perspective of their animal-infecting counterparts.

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Discovery and characterization of dimeric tospoviral S RNA molecules

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Polygonum ringspot virus (PolRSV) is a tospovirus recently reported in Italy. Northern blot analyses of PolRSV infections in *Nicotiana benthamiana* and tomato plants showed that a viral RNA species with nearly twice the length of the Small genomic RNA (S-RNA) accumulated abundantly in the former host, but was not detected in the latter. Additional hybridization assays confirmed that biogenesis of this novel RNA species was common to all PolRSV isolates tested but was isolate-specific for tomato spotted wilt virus (TSWV). Given its size, we hypothesized that the novel RNA species was a dimer molecule and we confirmed this hypothesis by RNA sequencing (RNAseq) analysis and reverse transcription (RT)-PCR of putative predicted dimer junction sites in RNA extracts of *N. benthamiana* challenged with PolRSV isolates Plg6 and Plg13/2. We also confirmed that these molecules are derived from head-to-tail dimers and often contain small deletions of 1 to 4nt at their junction sites. We named these novel molecules imperfect dimer RNAs (IMPD-RNAs). PolRSV IMPD-RNAs systemic accumulation was investigated in a range of host plants and is shown to be restricted to *N. benthamiana* and *Nicotiana occidentalis*. Notably, IMPD-RNAs accumulation was modulated by temperature: it is shown that higher temperatures favour IMPD-RNA accumulation whereas lower temperatures restrict IMPD-RNA accumulation. IMPD-RNA generation in a single passage was tracked and found to occur only in late stages of systemic infection (12 days post-inoculation) in *N. benthamiana*. Differently from all other PolRSV isolates used in this study, Plg13/2 generated more IMPD-RNAs coupled with low amounts of genomic S-RNA and maintained them even at 18 °C, besides having lost the ability to infect tomato plants. RNAseq analysis showed that Plg13/2 actually carries two populations of dimer molecules with extensive deletions in their junction sites. This is the first characterization of S-RNA dimers for the Tospovirus genus and the first report of occurrence of dimers of genomic segments at the whole organism level for the *Bunyaviridae* genus.

Effects of Ourmia melon virus movement protein mutants on virus infection, and their co-localization with plant cytoskeleton and organelles

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Plant viruses in the genus *Ourmiavirus* are peculiar, having particles of unique morphology and a unique combination of phylogenetic affinities for the three proteins encoded by its genomic RNAs: the RNA-dependent RNA polymerase (RdRp) has been recently shown to have close affinity with the RdRp of viruses found in samples from invertebrate species and related to the *Narnaviridae* family, but distinct from the *Narnavirus* genus of yeast viruses; the movement protein (MP) is similar to the MPs of tombusviruses, and the coat protein shows limited similarity to the CP of several plant and animal viruses. The acquisition of the movement protein from a parental plant virus might have represented the critical event for the evolution of a new plant-infecting virus. We generated eleven Ourmia melon virus (OuMv) MP mutants, and studied the effects on symptomatology, movement, subcellular localization, and tubule formation in protoplasts. We identified five OuMV mutants that were impaired for local and systemic infection in *Nicotiana benthamiana* and *Arabidopsis thaliana*, and two mutants showing respectively necrosis and pronounced mosaic symptoms in *N. benthamiana*. GFP fusion constructs of movement-defective MP alleles failed to localize in distinct foci at the cell wall, whereas a GFP fusion with wild-type MP accumulated at the periphery of epidermal cells and mainly co-localized with plasmodesmata. The movement-defective mutants also failed to produce tubular protrusions in protoplasts isolated from infected leaves, suggesting a link between tubule formation and the ability to move. By imaging of GFP:MP fusions and fluorescent markers for cellular cytoskeleton components and organelles, we have investigated the intracellular localization of OuMV MP and the host cell structural remodeling occurring during viral infection.

Turnip mosaic virus P1 protein is a novel interaction partner of the stress granule component G3BP

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Members of the genus *Potyvirus* (family *Potyviridae*) belong to the picorna-like supergroup and represent one of the largest groups of plant-infecting RNA viruses. Their single-stranded RNA genome is <10 kb in size and encodes a large polyprotein comprising (from N- to C-terminus) P1, HCPro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and the coat protein (CP). An additional protein, P3N-PIPO, is originated by a frameshift in the P3 cistron. The P1 protease is responsible for their own release by a cis cleavage at its respective C-termini. Located at the beginning of the polyprotein, P1 was the last potyviral endopeptidase identified; inactivating mutations of its catalytic domain preclude virus viability. In contrast to the two other genome-encoded proteases, P1 relies on a still unidentified host factor for its activation. Computational analysis of P1 potyviral proteins showed its great variability both in length and in amino acid sequence, and its diversification in potyviral species was thus associated with host specialization. Although P1 involvement in the definition of virus host range was highlighted, its specific contribution to potyviral infection is still unclear. Many functions were attributed to P1, such as cell-to-cell movement, systemic spread, and viral genome replication

enhancement; P1 was later shown to strengthen the RNA silencing suppressor activity of HCPro. P1 of turnip mosaic virus (P1TuMV) harbors an FGSF-motif and FGSL-motif at its N-terminal end. The FGSF-motif of AtUBP24 was shown to be essential to interact with G3BP. G3BP is a key factor for stress granules (SGs) formation in the mammalian system and often targeted by viruses. This suggests that also P1TuMV interacts with G3BP to control and regulate plant SGs to optimize cellular conditions for the production of viral proteins. P1TuMV co-localized with the AtG3BPs under stressed conditions and interaction was shown by pulldown and BiFC experiments. Alanine substitution mutants reveal that the N-terminal FGSF-motif and FGSL-motif are necessary to target P1TuMV into stress granules or G3BP interaction, respectively. In the mammalian system, virus interference with G3BP is often a strategy of the virus to inhibit SG formation. Thus, we propose that the interplay between P1TuMV and SGs is an anti-antiviral plant defense mechanism.

Investigations on pseudorecombinants from beet soil-borne mosaic virus and beet necrotic yellow vein virus in *Beta vulgaris* subsp. *vulgaris*

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Beet necrotic yellow vein virus (BNYVV) and beet soil-borne mosaic virus (BSBMV) are both members of the genus *Benyvirus* in the family *Benyviridae*. Although representing closely related species, the symptoms they induce in their natural host sugar beet (*Beta vulgaris* subsp. *vulgaris*), differ considerably. BSBMV-infected sugar beet roots appear asymptomatic, whereas the leaves display light yellow vein banding, mottling or mosaic patterns. In contrast, BNYVV-infected roots display extensive secondary root proliferation and the canopy shows symptoms such as vein yellowing and necrotic spots on the leaf surface. Recently, it has been shown that both viral species allow the formation of viable pseudorecombinants in *Beta macrocarpa* and N. *benthamiana* but nothing is known about their viability in the natural host sugar beet. Therefore, pseudorecombinants generated with full length-clones of RNA1, RNA2 and RNA3 from BSBMV and BNYVV were studied in sugar beet for long-distance movement and symptom expression.

Young sugar beet seedlings were mechanically inoculated with leaf sap obtained from *B. macrocarpa* plants systemically infected with the different pseudorecombinants. Pseudorecombinants with RNA3 of BNYVV or BSBMV replicated and mediated long-distance movement in sugar beet. BSBMV carrying BNYVV-RNA3 caused symptoms similar to BNYVV with vein yellowing and necrotic leaf tissue, whereas the opposite was observed when sugar beet plants were infected with BNYVV carrying BSBMV-RNA3. In contrast, plants infected BNYVV carrying BSBMV-RNA2 displayed symptoms characteristic for BSBMV. This result highlights the importance of RNA3 for symptom expression in both viruses. Other pseudorecombinants with BSBMV RNA1 and BNYVV RNA2 did not mediate long-distance movement in *B. macrocarpa* and therefore could not be tested in sugar beet. Besides symptom development, the expression of other genes by the plant due to infection of BNYVV/BSBMV was also investigated. A set of expansin genes involved in lateral root formation was shown to be induced specifically by BNYVV but not by BSBMV. However, the pseudorecombinant BSBMV carrying BNYVV-RNA3 could induce the same set of expansin genes whereas the opposite was observed when BNYVV carried BSBMV-RNA3. This indicates that RNA3 plays a crucial role in reprogramming the host cell. The results of the present study highlight the exchangeability of genome components between both viral species and help to understand molecular reasons for differences in symptom expression.

Section V

Sequence variation of a full-length clone derived PLRV isolate over several passages

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The complete genome sequence of a Germany PLRV field isolate was determined by overlapping RT-PCR fragments and 5'- and 3'-RACE. The determined sequence was confirmed by a deep sequence approach. The full-length genome sequence was cloned in a binary vector under the control of a 35S promoter and a hammerhead ribozyme sequence. The virus isolate was recovered by *Agrobacterium tumefaciens*-mediated inoculation on three different hosts. The original host (*Solanum tuberosum* L. var. Bamberger Hörnchen) from which the PLRV isolate originated was used and to two experimental hosts (*Nicotiana benthamiana* and *Physalis floridana*). Following confirmation of a successfull infection with PLRV by TAS-ELISA, the virus isolate was transmitted by aphids (*Mycus persicae* spp. *nicotianae*) to the same host species. From each new infected plant the virus transmitted up to four times. The success of each transmission was again confirmed by TAS-ELISA. Samples were taken at each step from all three hosts for deep sequence analysis. The observed sequence variation of the full-length clone derived PLRV isolates on the different hosts was compared to the sequence variation of the natural PLRV isolate. The number and pattern of the single nucleotide polymorphisms (SNP) of the recovered isolates compared to the natural isolate was undistinguishable.

Players and mechanisms in antiviral pattern-triggered immunity in plants

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Viruses cause major economic losses by reducing crop yield and quality worldwide. To identify targets for the development of new antiviral crop protection strategies an in-depth understanding of the mechanisms playing a role during plant-virus interactions is needed. Among the factors determining the outcome of plant-virus interactions are host defense responses restricting infection and viral counter-defense responses promoting infection. The balance between these processes defines the degree of susceptibility and resistance. We recently found that virus infection is restricted by pattern-triggered immunity (PTI) in plants. With the aim to further characterize this novel PTI-mediated immune response and its efficiency to limit the severity of viral infection we address the response network induced during virus infection and search for viral elicitors and their receptors involved in the induction of antiviral PTI.

Ty-1: a resistance gene against geminiviruses

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Tomato yellow leaf curl virus (TYLCV) is a member of the family *Geminiviridae* and belongs to the genus *Begomovirus*. It contains a monopartite circular single-stranded DNA genome that encodes for several viral proteins. TYLCV causes large yield losses of up to 100% in tomato production and ranks 3rd on the list of economically and scientifically most important plant viruses. Since it is difficult to

control the vector, the whitefly *Bemisia tabaci*, breeding for resistance against TYLCV is used to combat the viral disease. We have recently cloned the resistance gene *Ty-1*, which codes for an RNA-dependent RNA polymerase (RDR), and demonstrated that this gene enhances the antiviral RNA interference (RNAi) response. In comparison to susceptible tomato Moneymaker (MM), *Ty-1* bearing tomato lines produced relatively higher amounts of TYLCV-specific small interfering RNAs (siRNA). Furthermore, the methylation of cytosines in the viral DNA genome was higher compared to viral DNA collected from susceptible tomato MM. This altogether implicated that *Ty-1* confers resistance by enhancing transcriptional gene silencing (TGS). This was not only observed for TYLCV but also the bipartite begomovirus tomato sever rugose virus (ToSRV). While most dominant resistance genes are specific for one pathogen/virus only and are being triggered by an effector protein, the *Ty-1*-mediated enhanced TGS thus seems more generic. We therefore hypothesized that *Ty-1* is likely to protect against a broad range of geminiviruses, and not only restricts to the begomoviruses. To test this idea, *Ty-1* bearing tomato plants and susceptible MM plants were challenged with beet curly top virus (BCTV), the type species of the *Curtovirus* genus within the family *Geminiviridae*. Whereas tomato MM plants showed severe symptoms upon infection with BCTV, Ty-1 bearing tomato plants were symptomless. In addition, viral titers were lower compared to MM plants, indicating resistance by *Ty-1* indeed is more generic towards a wide range of geminiviruses. In contrast to most dominant resistance genes, *Ty-1* thus is a unique resistance gene that confers protection against a broad spectrum of geminiviruses.

Use and misuse of methyl-cytosine assays in geminivirus research

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It has been proposed that geminivirus multiplication is limited by a host defense pathway mediated by transcriptional gene silencing (TGS) in response to post-transcriptional gene silencing (PTGS). Inversely, geminiviral proteins have been investigated that could suppress TGS. However, geminiviruses can evade TGS-mediated resistance efficiently by various modes of replication (1). Scrutinizing the level of TGS may, therefore, be a key to explain their virulence. TGS in general operates on chromatin, with modifications of histones leading to methylation of cytosines (mC) in DNA. The ratio of mCs may, hence, been taken as an indicator for TGS. To this aim, various techniques have been employed, including the use of mC-specific antibodies, methylation-sensitive and methylation-dependent restriction enzymes as well as bisulfite sequencing of PCR and RCA products. The pitfalls of the individual assays will be discussed. Moreover and most importantly, a fundamental bias of the assays will be emphasized which is caused by the different composition and different levels of replicative intermediates. Consequently, a meaningful quantitative determination of mCs is only possible, if the different geminiviral DNA conformations are measured separately (2-4). The current best practice in this context is a combination of methylation-dependent restriction enzymes digestion and rolling circle-mediated amplification of the products followed by high-throughput sequencing to identify mCs unequivocally (5).

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Controlled assembly of 2- and 3-dimensional virus crystals

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Plant viruses are interesting building blocks in nanobiotechnology because of their small size, their simple icosahedric or rod-shaped structure and the possibility to extend the capsid by genetic modification with e.g. different amino acid residues.

In nanotechnology „top-down" fabrication processes reach their physical and technical limits. Therefore, "bottom-up" approaches using small building blocks like plant viruses which are able to built larger elements via self assembly gain increasing interest.

We could already show that the extension of the tomato bushy stung virus (TBSV) capsid with 4xHis respectively 6xAsp improves self-assembly of the modified viruses (1) what could be explained by electrostatic forces. We now investigated the influence of electrostatics in detail. We therefore monitored the assembly of differently modified TBSV particles (extension with 4xArg, 4xAsp6xHis and 6xAsp) in solutions with different pHs on silicon. The differently modified TBSV particles possess different isoeletric points and therefore display different charges at different pH values. We could show that the coverage of the surface area of the substrate with viral crystals could be improved by favorable electrostatic conditions thus allowing the controlled adjustment of the substrate coverage.

In a next step we constructed 3-dimensional structures. For this purpose we exploited the chemical selectivity of the different engineered side chains. A first homogenous layer was built by 4xAsp6xHis-TBSV on silicon which served as a base to tether nickel-nitrolotriacetic acid (Ni-NTA) via a nickel-His interaction. The Ni-NTA is itself tethered to a nano-gold particle (5 nm diameter), therefore a third layer can be constructed via the interaction of engineered gold-binding peptides of the virus capsid and the gold particles.

This now enables to exploit the self assemly of plant viral particles to systematically construct systems which are structured in all three dimensions.

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Tobacco mosaic virus-derived nucleoprotein domains as building blocks for porous bio-functional materials

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Tobacco mosaic virus (TMV) derivatives offer promising perspectives as biotemplate building blocks for nanotechnological applications. We aim to generate a novel type of porous bio-functional material by assembling functionalized TMV-like particles with control over their spatial arrangement by the use of DNA branching elements.

TMV is an exceptionally stable plant virus consisting of a single-stranded RNA helically encapsulated by a defined number of identical coat protein (CP) subunits, predetermining the tube-shaped geometry of 300 nm x 18 nm and a hollow channel of 4 nm. Novel nanostructures can be designed and assembled *in vitro* by combining RNA constructs containing the TMV origin of assembly (OAs)

with CPs, which can also be modified genetically and chemically to allow selective coupling of functional molecules.

By combining chemical and enzymatic ligation methods with procedures guiding the self-assembly of TMV, we have produced novel nucleoprotein structures based on DNA-terminated branching elements, RNA and TMV protein. To arrange TMV like particles (TLPs) into tetrahedral nucleoprotein structures in a spatially controlled manner, branched DNA-containing molecules that enable the formation of a three-dimensional structure were used as core elements. They were coupled to DNA linkers by chemical ligation [1]. These DNA-extended branching elements were then bound to RNA-based TLPs using splint-mediated enzymatic ligation. Two types of nucleoprotein nanostructures with different lengths of functionalizable protein domains were generated: One by ligating OAS-containing RNA scaffolds to the DNA linkers which allowed the growth of short proteinaceous domains, and the other by ligating the protruding RNA of partially assembled TLPs [2] to the DNA linkers, thereby interconnecting longer nucleoprotein domains in a tetrahedral orientation. This novel strategy for producing branched nucleoprotein building blocks combines organic synthesis for building the DNA branching elements with nucleic acid-based structuring and the “toolbox” of TLP generation. The RNA-guided self-assembly of stiff TLPs together with the rigid DNA-terminated branching elements as tetrahedral core molecules is expected to provide the possibility of creating 3D nucleoprotein lattices with adjustable pore size and selectively addressable protein domains in the long run. Such products may give rise to novel mesoporous functional materials for various applications.

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Abstracts Poster Presentations

Dispersal of plant viruses in irrigation water prevented by electrolytic disinfection of nutrient solution

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Closed irrigation systems conserve resources and minimize production costs. Several sources of water can be used for irrigating crops. Some of them pose a high risk to disseminate plant pathogens such as zoosporic organisms, fungi, bacteria and viruses. A considerable number of pathogens is of significant concern when introduced directly from crops in cultivated fields, greenhouses or natural vegetation surrounding the fields as those are stable, difficult to combat and cause economic losses. Among these pathogens, plant viruses are of particular interest because they can´t be cured. Therefore, effective sanitation methods are required to minimize their dispersal. Currently, the grower can utilize different physical or chemical water treatments (Hong et al. 2014). Beside cost effectiveness and ecological concerns none is suitable to inactivate the multitude of relevant plant pathogens, in particular viruses.

We determined and evaluated the potential of a new sensor-based disinfection procedure to inactivate viral plant pathogens in hydroponic systems in greenhouse tomato production. An electrolytic disinfector (newtec Umwelttechnik GmbH, Germany), especially developed for disinfection of irrigation water in greenhouses was used.

The efficacy of the disinfectant, a low concentrated potassium hypochlorite (KClO), was first tested *in vitro.* Dose-effect relations were calculated for different fungal, bacterial and viral plant pathogens. As expected, contact time and dose required to eradicate pathogens varies with pathogen species and life stage. Subsequently trials under practical conditions were initiated focusing on the potential of the disinfection procedure to prevent the spread of plant viruses by recirculating nutrient solution. The disinfectant injected once weekly into the nutrient solution at 0.2 or 0.5 mg free chlorine/l nutrient solution for 60 or 30 minutes by a sensor, prevented the dispersal of pepino mosaic virus in tomato crops (Bandte et al., 2016). Sanitation of nutrient solution by electrolytically generated KClO ensured that virus particles released from infected plants did not accumulate and form an infectious reservoir. Results of the *in vitro* test and trials under greenhouse conditions are presented and evaluated.

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Electronmicroscopical observations of doubly infected *Hortensia*-leaves with Hydrangea ringspot virus and eggplant mottled dwarf virus

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In 2014 a *Hortensia* plant with very unusual virus symptoms was sent from the local plant protection service for virus diagnosis. Symptoms included strong veinal yellowing, leaf rugosity and flecking as well as flower breaking. Electron microscopical investigation revealed the presence of two different

virus types: one being filamentous (genus *Potexvirus*) and a second with bacilliform complex morphology (family *Rhabdoviridae*). The filamentous virus was identified as *Hydrangea ringspot virus* (HdRSV) by ELISA and the second was identified by DSMZ (W. Menzel) as an isolate of *Eggplant mottled dwarf virus* (EMDV) by sequencing. EMDV was never before reported to infect *Hydrangeas*.

Sections of symptomatic parts of infected leaves showed doubly infected palisade and spongy mesophyll cells with large cytoplasmic inclusions, typical for potexvirus-infections as well as bacilliform particles in the perinuclear spaces. Rhabdovirus particles were always surrounded by parts of outer nuclear membranes. Cytoplasmic areas with intermingled virus particles could not be observed. Chloroplasts were degraded and showed a gradient of cytopathological changes such as circular membraneous structures and many plastoglobuli outside the chloroplasts, often appearing in the cytoplasmatic virus factories of HdRSV. Necrotic cells, however could not be observed at that state of infection.

Split GFP as a tool to study viral infection: Cauliflower mosaic virus

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The split GFP technique is based on the spontaneous auto-assembling of two non-fluorescent GFP fragments, GFP^{1-10} (residues 1-214, the detector) and GFP^{11} (residues 214-233, the tag), to reconstitute a fluorescent GFP (Cabantous et al. 2005). Its primary use is tracking proteins *in vivo* when for various reasons the whole GFP molecule can not be fused to the protein of interest. We used here the split GFP technique to contribute to the characterisation of molecular and cellular details of *cauliflower mosaic virus* (CaMV) infection.

GFP^{11} was fused to the N-termini of proteins P2 or P6 of CaMV strain B-JI. We chose the virus factory matrix protein P6, because it is the first viral protein to be translated during infection and thus suited to study early infection events, and P2 because it is the aphid transmission factor, and transmission a focus of our research. Infectious plasmids were used for mechanical inoculation of transgenic *Arabidopsis thaliana* Col0 plants expressing GFP^{1-10} constitutively with the 35S promoter (Sakalis et al., 2014). We obtained infected plants showing typical symptoms and displaying fluorescent virus factories (CaMV-P6-GFP^{11}) or transmission bodies (CaMV-P2-GFP^{11}). We are currently analyzing the viruses for their suitability as a research tool to study virus factories and transmission bodies by life fluorescence and to identify interaction partners with the GFP-Trap® technology.

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Analysis of the complete genome sequence of Euphorbia ringspot virus, an atypical species in the genus *Potyvirus*

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The complete genome sequence of an isolate of Euphorbia ringspot virus (EuRSV) was determined by deep sequencing and rapid amplification of cDNA ends (RACE) RT-PCR. It has an RNA genome of 10,154 nucleotides in size, excluding the poly(A) tail, and encodes a polyprotein of 3265 amino acids. Phylogenetic analysis from this study supports the earlier taxonomic assignment to the genus *Potyvirus*; however, a gene encoding the HAM1h protein, inserted between NIb and CP of the EuRSV

genome, which was previously only observed for cassava brown streak virus and Ugandan cassava brown streak virus of the genus *Ipomovirus*, is an unusual feature of this potyvirus, which otherwise has typical potyvirus genome features.

Strategies for the implementation of a certification program for virus-tested plant material in Colombia

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Countries in the global south are major exporters of agricultural products, and in certain places no tools for controlling pathogens exist. Plant viruses severely affect Colombian crops, and studies indicate that economic losses caused by phytosanitary problems could be avoided with a standard procedure for preventive management. Three important exports from Colombia have been chosen as model plants for experimentation: ornamental rose (*Rosa sp*.), cape gooseberry (*Physalis peruviana*), and purple passion fruit (*Passiflora edulis* Sims). A test system for routine diagnosis is being developed for these cultivars based on an inventory of known and novel viruses detected in large and small representative farms in 2016-17 by serological and molecular tools. Next Generation Sequencing (NGS) and bioinformatics will enable the discovery of new viruses and allow the observation of an increasing diversity of variants for known viruses and the frequent existence of a complex of different viruses. Reliable and practical diagnostic tools will be developed for the most important viruses leading to a national agricultural certification program that will be established in a common project between German and Colombian universities, the Colombian Agricultural Institute (ICA), the Colombian Corporation of Agricultural Investigation (CORPOICA), and the International Center for Tropical Agriculture (CIAT). The competitiveness of Colombian agriculture in international markets depends on the use of healthy domestic plant material and therefore, virus-free certification can improve quantity and quality of yields and contribute to better trade policy decision-making.

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Labeling of beet mosaic virus (BtMV) with green and red fluorescent proteins and analysis of distribution *in Nicotiana benthamiana*

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Beet mosaic virus (BtMV) is a typical member of the economically important genus *Potyvirus*, family *Potyviridae*. BtMV is worldwide distributed, especially in temperate regions causing a mosaic disease on beet crops. The virus was first reported in 1950s in *Beta vulgaris* L. from Germany. The host range of BtMV includes many plants of the families *Solanaceae*, *Chenopodiaceae* and *Poaceae*. It is a non-persistent aphid-transmitted virus, especially by *Myzus persicae* (Dusi and Peters, 1999).

Potyviruses have a single stranded positive-sense RNA containing a poly (A) tail at its 3`-terminus and a genome linked protein (VPg) at its 5`-terminus (Rajamäki et al., 2004). The genome is translated into a big polyprotein of 340-368 KDa, which is subsequently processed by three viral proteases into the functional proteins (Ivanov et al., 2014).

The aim of this study was the labeling of a BtMV infectious full-length clone either with the monomeric red fluorescent protein (mRFP) or the green fluorescent protein (GFP) to get a better understanding of the virus movement and distribution in infected plants. We used Gibson Assembly as a one step cloning method (Gibson et al., 2009) to introduce the fluorescent marker genes (mrfp, gfp) between the cistrons of NIb and CP. The reporter genes (mrfp, gfp) were flanked by NIa protease cleavage sites to ensure proper release form the polyprotein after translation. Agroinfection of *Nicotiana benthamiana* with pBtMVmrfp or pBtMVgfp resulted after 15-20 days in systemic infection and intensive fluorescence. In addition, the existence of the recombinant virus in systemically infected plant parts was verified by RT-PCR. The labeled viruses BtMVmrfp and BtMVgfp offer opportunities for future studies of virus movement and distribution in Beta vulgaris.

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Cell death triggering and effector recognition by the single dominant *Sw-5* and *Tsw* resistance genes

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Tospoviruses are unique members of the arthopod-borne *Bunyaviridae*, comprising the only genus whose members infect plants rather than animals. Tomato spotted wilt virus (TSWV), the type species of the tospoviruses, is one of the most important plant viruses worldwide with a large host range of more than 80 different plant families. Currently, two single dominant resistance (R) genes are available for commercial resistance breeding. The first, *Sw*-5 from *Solanum lycopersicon*, confers

resistance to TSWV and a few additional tospoviruses. The second, *Tsw*, from *Capsicum chinense* confers resistance to TSWV only. Although their mode of action is still largely unknown R genes, besides the actual resistance response, trigger a concomitant hypersensitive response (HR). This is based on a programmed cell death and leads to the formation of necrotic spots that prevents further spread of the pathogen from the primary site of infection.

Recently, the cell-to-cell movement protein NSm of TSWV has been identified as the avirulence (Avr) determinant of *Sw-5b*-mediated resistance. *Sw-5* belongs to the class of SD-CC-NB-LRR (*Solanaceae* domain-coiled coil-nucleotide- binding-leucine-rich repeat, SD-CNL) resistance genes. Further analysis and dissection of three *Sw-5* homologs have indicated the importance of the NB domain in HR triggering and the LRR domain in Avr/effector recognition. *Tsw* is also a CNL type of resistance gene and is triggered by the second non-structural protein of TSWV, NSs, the protein that also suppresses antiviral RNAi. For triggering of *Tsw*-mediated HR, the N terminal domain of NSs is most important. Like with *Sw-5*, the resistance mediated by *Tsw* also has been broken and analyses of resistance breaking TSWV isolates have shown that their NSs protein is compromised in the ability to suppress local RNAi suppression, but are still able to suppress systemic RNA silencing.

First report of mint vein banding-associated virus infecting *Mentha x gracilis* in Germany

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Mints are used in traditional medicine for thousands of years and dried or fresh leaves are the source of mint flavor for dishes and teas. More recently, mints have also established as a perennial bedding plants. In August 2016, symptomatic *Mentha* x *gracilis* (common name: ginger mint) plants were offered in a supermarket in Braunschweig, being produced in a nursery in Papenburg. The plants showed vein banding/clearing symptoms on many leaves. Mechanical transmission of a putative virus as causal agent to herbaceous indicator hosts was not successful. dsRNA extraction of symptomatic leaves resulted in a high molecular weight dsRNA which was significantly larger than 10 kbp, indicating the presence of a clostero- or endornavirus. Random RT-PCR, cloning and sequencing resulted in viral sequences showing the highest nt sequence identities to mint vein banding-associated virus (MVBaV), an unassigned species of the family *Closteroviridae*. One sequence was located in the polymerase gene having 80% nt/88% aa sequence identity to MVBaV. The second sequence covered partially ORF4 (putative minor CP) and the entire ORF5 encoded CP, to which it showed 87% nt/95% aa sequence identity. The sequence identity values are above the species demarcation threshold of 75% for aa sequences of relevant genes (polymerase, HSP70, CP), identifying this virus as a deviating isolate of MVBaV. This virus species was discovered in a mint survey in the United States (USA) by Tzanetakis et al. (2005). Our report is the first for MVBaV occurring outside the USA. Based on the polymerase gene sequence obtained in this study, a primer pair was designed to specifically test six individually sampled symptomatic plants of the same mint propagation batch as well as the plant originally used for dsRNA extraction. Amplicons of the expected size were obtained for all samples. Subsequent direct sequencing revealed that all were almost identical to the sequences originally obtained (>99.5% nt identity). The MVBaV isolate is available at the DSMZ plant virus collection under accession no. W16-112.

Symptom expression and virus accumulation in screen house *Nicotiana* hosts co-infected with cassava brown streak viruses

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Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), family *Potyviridae*, genus *Ipomovirus* present variability in patterns of symptoms expression on cassava and host plants. Both viruses are emerging as serious cassava production problems in eastern Africa. The viruses have been found together in cassava plants of fields infected by CBSD, indicating that infection by one virus sp. does not prevent infection by a second. In order to elucidate the effects of co-infection on CBSD symptom expression and virus accumulation, we inoculated *Nicotiana* (*N*.) *benthamiana*, *N. rustica*, *N. glutinosa* 24A and *N. tabacum* Samsun "nn" with either CBSV or UCBSV alone or both viruses together under greenhouse conditions. Symptom development assessment revealed that double infection resulted in severe necrosis of leaves in *N. benthamiana* and *N. glutinosa* compared to single infections, whereas mild vein clearing and leaf curling were observed in both singly and co-infected *N. rustica*. However, *N. tabacuum* Samsun "nn" did not express any CBSD symptom either in single or mixed infection. Plants were tested by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), and results indicated host-specific differences in accumulation by CBSV and UCBSV and changes of accumulation patterns during co-infection compared with single infection. In *N. benthamiana* and *N. glutinosa* 24A, CBSV titers increased during co-infection compared with levels in single infection, while UCBSV titers decreased. However, in *N. rustica*, titers of both CBSV and UCBSV increased during mixed infection compared with single infection, although to different degrees. The results illustrate that CBSD epidemiology is impacted by interactions between viruses and efficiency of accumulation in host plants.

Key words:

Cassava, epidemiology, host-specific differences, qRT-PCR, titers

Tobacco mosaic virus with defined discriminable coat protein domains: Manipulating RNA-directed self-assembly

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The well-known tobacco mosaic virus (TMV) with its highly defined structure is extensively employed as a promising tool for nanobiotechnology applications. Tobamoviral *in vitro* self-assembly has been studied over many decades, and manipulating the mechanism offers unique prospects for designing new nucleoprotein bio-templates.

We have developed a DNA-based "stop-and-go" strategy to control the RNA-directed self-assembly of TMV for the fabrication of TMV-like particles with highly defined subdomains. The growth of nucleoprotein tubes from viral coat protein (CP) subunits could be blocked site-specifically by a stopper DNA oligomer hybridized to the RNA downstream (3') of the origin of assembly (OAs) sequence. The conditions established for blocking 3' of the OAs were not suitable to stably stall the assembly at two upstream (5') sites tested, which correlates with previous findings on distinct

molecular mechanisms of the 3'- and 5'-directed assembly of TMV nanotubes. The growth of 3'-DNA-arrested particles could be restarted efficiently by displacement of the stopper via a toehold segment included in the stopper sequence, by means of a release ('fuel') DNA oligomer.

Purification in-between the serial assembly stages and the use of a second distinct CP variant finally enabled the production of nanotubes with two discriminable, selectively addressable and highly defined domains. The “stop-and-go” strategy thus might pave the way towards production of bio-templates with two or even more adjacent protein domains of tightly pre-defined lengths and different functionalities.

Transmission of tomato chlorotic dwarf viroid assisted by potato leafroll luteovirus by *Myzus persicae*

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Tomato chlorotic dwarf viroid (TCDVd), first discovered in Canada in 1999, has a wide host range, including many high-valued solanaceous crops. In the meantime it has been detected in many different locations worldwide, raising potential risk of spreading the disease in wide areas. In this study, transmissibility of TCDVd via aphid vector was investigated by exposing PLRV plus TCDVd carrying *M. persicae* to tomato plants under different temperature conditions. The results showed that the efficiency of TCDVd transmission was higher at 5 weeks after aphid inoculation at 26-28°C than at 20-22°C, with 5 infected out of 20 test plants (25%) compared to only 1 out of 20 test plants (5%), respectively. Moreover, the efficiency of TCDVd transmission by aphids from mix-infected plants after discharge into tomato host plants and non-host plants (cabbage) was 5 out of 15 inoculated plants and 2 out of 10 inoculated tomatoes, respectively. This finding indicates that TCDVd is transmitted within, or at least attached to PLRV capsids in a persistent way by *M. persicae*. No distinguishable symptoms between a combined PLRV/TCDVd infection and a single TCDVd infection on tomato developed during the first five weeks after inoculation. However, 6-8 weeks after inoculation, the symptom development of doubly infected tomato plants differed from that of singly TCDVd-infected plants, as new sprouts developed on doubly infected plants.

Complete sequence determination and construction of an infectious full-length clone of a celery mosaic virus isolate from Quedlinburg (Germany)

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One of the first descriptions of celery mosaic virus (CeMV) was done in the year 1935 in California, USA by Severin and Freitag (Severin und Freitag 1935 in Xu et al. 2011). The virus has a worldwide distribution and is responsible for large economic losses in many countries. There are for example reports from England (Pemberton und Frost 1974), Australia (Alberts et al. 1989 in Latham und Jones 2003), the Netherlands (Bos et al. 1989), Venezuela (Fernández et al. 2006), Poland (Paduch-Cichal und Sala-Rejczak 2010) and Tehran (Khoshkhatti et al. 2011). In most cases infected plants show severe symptoms like stunting, mosaic, vein-clearing and rolling of the leaves. CeMV belongs to the genus *Potyvirus* in the Family *Potyviridae*. As it is typical for this genus, CeMV is transmissible by aphids in a non-persistent manner and forms flexuous, rod-shaped particles (Severin et al. 1938). The genome consists of a positive orientated single-stranded RNA closely related to a*pium virus Y* and *carrot virus Y*. To date there is one complete sequence of an isolate from California (Xu et al. 2011; NC_015393.1) and seven partial sequences comprising a part of the nuclear inclusion protein b (NIb) and the coat protein of isolates from Germany (AJ271087), Venezuela (DQ211986.1) the Netherlands

(AF203531) and Australia (AF203532.1, AF203533.1, AF203534.1, AF203535.1) available. In this study we report the complete sequence of a new celery mosaic virus isolate from Quedlinburg (DSMZ PV-1003) and the construction of an infectious full-length clone along with the successful infiltration of *Apium graveolens* with *Rhizobium radiobacter*.

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Complete sequence and construction of an infectious full-length clone of a Panicum mosaic virus isolate from Aschersleben (Germany)

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Panicum mosaic virus (PMV) is a member of the family *Tombusviridae* in the genus *Panicovirus*. In 1957 Sill and Pickett discovered PMV in the switchgrass *Panicum virgatum* in Kansas (Sill Jr und Pickett 1957 in Turina et al. 1998). The St. Augustine decline strain (PMV-SAD) causes severe symptoms in *Stenotaphrum secundatum* (St. Augustinegrass) and it was found in Arkansas in the 1980s (Dale 1982). Another disease in *Eremochloa ophiuroides* (centipedegrass) is also triggered by PMV infection and occurred in several states in the USA (Haygood 1992). In many cases an additional satellite virus without any serological relation to PMV was detected (Buzen 1984). The host-range is limited to monocotyledons. The PMV particles are non-enveloped, spherical icosahedrons and their genome consist of a positive orientated single-stranded RNA of approximately 4.3 kb in length. There are five open reading frames (ORFs) which are expressed with different strategies as for example production of subgenomic RNAs and read-through of stop-codons. In 1998 Turina et al. were able to construct a T7 promotor based infectious full-length clone of the PMV-Kansas strain. They showed that an infection of pearl millet plants with *in vitro* transcripts was successful (Turina et al. 1998).

In this work we present a new complete genomic sequence of a PMV isolate collected in Aschersleben, Germany with 86 % identity to the Kansas strain of PMV (BLASTn). Additionally a 35S-

promotor based full-length clone was established, which is able to initiate systemic infections in *Eleusine coracana* (finger millet) and *Setaria italica* (red foxtail-millet). In the electron microscope, PMV particles were detected with and without decoration.

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A novel virus is associated with the ringspot disease in common oak (*Quercus robur* L.)

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Exploring the virome of deciduous tree species becomes more and more prominent and sheds light into the complex world of pathogens in woody plants. High-throughput sequencing technology (NGS) is a powerful tool to discover previously unidentified plant viruses. Applying such an approach to a diseased common oak (*Quercus robur*) from a seed production stand in Fellinghausen, Germany led to the discovery of such a new virus. Sequence analyses revealed closest relationship to emaraviruses, a yet unassigned genus of viruses with a segmented single stranded RNA genome. Each genome segment encodes a single open reading frame in negative orientation; RNA1 consists the replicase, RNA2 a glycoprotein precursor, RNA3 the viral nucleocapsid protein, and RNA4 the putative movement protein of approximately 42 kDa (Mielke-Ehret and Mühlbach 2012). Some members of the genus contain up to four additional genomic RNAs encoding additional proteins of unknown function. The affected oak tree expressed chlorotic ringspots and mottle of leaves since several years. Specific RT-PCRs were established targeting the different identified genome segments of the putative novel emaravirus. They were applied to study the association of the virus with observed symptoms and to confirm the four putative viral RNAs in diseased oaks. Leaves from oak trees (n = 163) were sampled from sites in different European countries including a seed collection stand, park and forest trees. Virus detection was closely correlated with common oaks exhibiting characteristic chlorotic ringspot symptoms while it was neither detectable in leaf material collected from trees without virus-like symptoms nor in *Quercus spp*. showing regular chlorotic patterns or partial chloroses of leaves. Showing a frequent infection of oaks in different locations indicates that it is widespread in oak populations in Germany, southern Sweden and Norway.

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Strawberry latent ringspot virus in lily is seed transmitted and localised in the embryo

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Background and Objectives

Strawberry latent ringspot virus (SLRSV, family *Secoviridae*, not assigned to a genus) has a very broad host range in both dicotyledons and monocotyledons. One of its hosts is lily (*Lilium spp*.) in which

SLRSV does not cause symptoms. However, SLRSV causes economic losses in lily as infected bulbs cannot be exported to certain countries due to phytosanitary legislations.

Early work on SLRSV in vegetable crops revealed the nematodes *Xiphinema diversicaudatum* and *X. coxi* as vectors (Lister, 1964; Murant, 1974). Also seed transmission was reported in the crops raspberry, celery and *Mentha*, and in weeds such as henbit dead-nettle (*Lamium amplexicaule*), chickweed (*Stellaria media*) and goosefoot (*Chenopodium quinoa*) (Murant, 1974).

The objective of our research was to determine whether SLRSV is seed-transmitted in cultivated lily.

Materials and Methods

Experiments were set-up with two SLRSV-infected lily cultivars which are known as 'garden lilies' and are normally propagated by seed. Seed pods were harvested from SLRSV-infected plants, and from a non-infected control plant, dried and kept at 4°C for several months in order to break dormancy. Individual seeds were tested in DAS-ELISA and RT-PCR for the presence of SLRSV. Seeds were also sown in sterilised soil for growing-out tests. In order to localise the virus in the seeds, seeds were dissected. Seed coat, endosperm and embryo were tested for SLRSV separately with RT-PCR.

Results

In a number of individual seeds SLRSV was detected using DAS-ELISA. These results were confirmed by RT-PCR using specific primers for the SLRSV isolate present in the research material. In the sowing-out test, up to 30% of the young seedlings turned out to be infected. Localisation of the virus within the seed proved that SLRSV is present in the embryo.

Conclusions

From our experiments it can be concluded that SLRSV is seed transmitted in garden lilly. The virus could be detected within the embryo of the infected seed, and therefore disinfection methods cannot be applied. To what extent this seed transmission contributes to the epidemiology of SLRSV in garden lily remains to be established.

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Q-bank for sharing data and information on plant virus and viroid isolates in collections

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The availability and accessibility of well characterised reference isolates of plant viruses and viroids is crucial for research and diagnostic laboratories. To ensure the availability of isolates and reference materials, it is of utmost importance to collaborate at an international level. Seeing the general tendency of decreasing budgets and loss of experienced staff, future efforts should focus on sharing data as well as expertise on reference collections. Q-bank, the comprehensive databases on plant pests and diseases, offers an excellent platform to share data on plant virus collections (http://www.q-bank.eu/Virus). It includes biological, serological and molecular characteristics of available virus and viroid isolates and indicates the collection from which they can be obtained. In addition, the database provides easy access to overviews of regulated species, various test protocols and a sequence BLAST tool to assist diagnostic laboratories in species identification. Although most laboratories endorse the need for reliable reference isolates or materials that fulfil basic quality standards, they do not have the means to establish a 'certified reference collection' under official ISO

standards like ISO Guide 34. Q-bank offers the opportunity to share data of characterised isolates that are physically available for public use. Moreover, curators ensure that quality standards are met and conservation is safeguarded. To further strengthen the Q-bank database and collections, the EUPHRESCO project VirusCollect aims to extend the network of reference collections for regulated and other important viruses and viroids at the European level.

Effects of pepino mosaic virus and cucumber green mottle mosaic virus on drought tolerance in *Nicotiana benthamiana*

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Background and Objectives

Viruses are often seen as pathogens which cause disease and limit the growth of the host. However, it may be time to rethink that paradigm. Earlier it was observed that a virus infection can induce drought tolerance in a plant and increase its survival (Xu et al., 2008). We wanted to know if a virus infection of a symptomatic (pepino mosaic virus, PepMV) and a non-symptomatic virus (cucumber green mottle mosaic virus, CGMMV) can induce drought tolerance in *Nicotiana benthamiana*, and possibly affect its drought survival.

Materials and Methods

N. benthamiana plants were inoculated with CGMMV or a mild tomato strain of PepMV. Negative controls were inoculated with water. Once systemic infections were confirmed by DAS-ELISA, watering was halted for two weeks. Wilting symptoms were scored visually and the daily weight loss of the pot was measured to calculate the transpiration rate per plant. Whole plant thermal pictures were taken to analyse the leaf temperature as an indication of the aperture of stomata. Proline contents were determined from systemically infected leaves 7 and 14 days after the on-set of water starvation, and used as an indicator of water stress.

Results

Drought symptoms were delayed in PepMV-infected plants compared to CGMMV- and non-infected plants. PepMV infection caused only very mild symptoms and PepMV infected plants showed a significantly lower transpiration rate in comparison to non-infected plants. The CGMMV-infected plants did not show symptoms and the transpiration rate was similar as non-infected plants. Proline contents were not significantly different between the treatments. Upon extended drought PepMV-infected plants showed had a much higher survival rate in comparison to non-infected plants.

Conclusions

PepMV, but not CGMMV, induced an improved drought tolerance in *N. benthamiana*. PepMV infection caused a delay in the appearance of drought symptoms and a clear extension of the period in which the plants could recover from and survive extended drought (‘tipping point’). Results, including thermal pictures, indicate that the extended drought tolerance conferred by PepMV is likely based on a lower rate of transpiration and a more efficient use of water

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Application of next generation sequencing for the study and diagnosis of plant virus diseases in agriculture

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Through their holistic approach NGS technologies have the ability to detect and characterize the complete virome (virus, viroid and any virus-like agent) present in any plant sample and potentially even in vectors or various substrates. NGS technologies have evolved rapidly with significant improvements both for laboratory and bioinformatics processes and NGS can be now be translated into practical solutions for virus control in plant production.

The recently started EU-funded COST action FA1407 (DIVAS: Deep Investigation of Virus Associated Sequences. See also www.cost.eu/COST_Actions/fa/Actions/FA 1407) brings together a multidisciplinary and multi-actor consortium to ensure cost-effective research and build up a strong Pan-European knowledge-base network for better control of established, emerging and exotic viral plant diseases. The action focusses on four main Work Packages (WPs): 1) Comparison and validation of NGS-based protocol for virus diagnostic in different matrices, 2) Etiology of graft-transmissible diseases and biological impact of newly discovered agents, 3) Taxonomy and viral population genetics, and 4) Socio-economic and regulatory impact and dissemination.

The objectives of the Action will be achieved through A) Developing a collaborative multidisciplinary community of researchers through thematic workshops, B) Establishing a joint platform and concrete channels for harmonizing and continuously updating NGS protocols for plant virus diagnostics, including laboratory and bioinformatics workflows, C) Organising meetings and workshops focused on the distinct but inter-related topics for the working groups of the proposed Action, and D) Running Technical and Scientific Training Schools and Short Term Scientific Missions (STSMs) in NGS for plant virus diagnostic and in the consequence of NGS for plant-virus interaction frameworks by the leading institutes of the Action.

Studies on the identification of a silencing suppressor of celery latent virus

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Plant pathogenic viruses are both initiators and targets of various plant defence mechanisms. Especially RNA silencing plays a fundamental role in antiviral defence. However, to counter host defence plant viruses are able to produce proteins which suppress RNA silencing by interfering with various parts of this multi-step mechanism (Voinnet et al. 1999). Knowing that viral suppressors of RNA silencing (VSR) have been identified from nearly almost all plant virus genera (Pumplin et al. 2013), we investigated this activity for a putative corresponding CeLV protein.

CeLV forms flexible, filamentous rods and has a single-stranded positive-sense RNA-genome consisting of 11504 nucleotides. So far the RNA-genome and polyprotein sequence of CeLV revealed

only a few similarities and motifs compared to members of the family *Potyviridae*. Therefore, CeLV may represent a new genus of the family, which has not yet been classified. In addition, no suppressor protein could be identified from sequence comparisons.

To investigate the suppressor activity, partial clones of the CeLV sequence were integrated into a binary vector and tested in a transient silencing-suppression assay. The test system is based on the local expression of *gfp* together with a potential suppressor gene via *Rhizobium radiobacter* transfer in the *gfp*-transgenic *Nicotiana benthamiana* line 16c (Varrelmann et al. 2007). By virtue of the stabile expression of the *gfp* gene, the expression system enables the visual detection of the activity of a VSR. HC-Pro of *plum pox virus* (Genus *Potyvirus*) was used as a positive control for a VSR during the transient silencing-suppression assays. The detection of a putative VSR activity for a particular region of CeLV will give a first indication for the presence of a novel suppressor element.

Our findings indicate the presence of a protein with a suppressor activity in the N-terminal region of the CeLV sequence. However, associated protease cleavage sites for the suppressor protein of CeLV were not determined so far. Therefore, the exact processing and localization of the VSR still has to be determined.

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Characterizing cassava resistance against cassava brown streak viruses

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Cassava brown streak disease (CBSD) is the most serious disease threat to cassava *(Manihot esculenta* Crantz*)* in Africa. The disease is prevalent wherever cassava is grown in East Africa spreading towards the west into DR Congo. The two virus species *cassava brown streak virus* (CBSV) and *Uganda cassava brown streak* virus (UCBSV) most often occur together in the field and while cassava cultivars show a differential response to infection with the viruses - from very severe root symptoms and decline to mild symptoms on leaves and stems only – the African cultivars so far studied are susceptible to CBSD viruses and show tolerance at most with highly resistant cultivars not yet identified.

A drawback of virus screening under field conditions is the dependency on natural virus infection pressure and the time set of inoculation which hampers a clear cut verdict on a virus susceptibility/ resistance status. For CBSD, it is yet to be proven that a correlation between virus concentration, replication and virus movement and the resistance status of a plant exist as the virus concentration fluctuates considerably within a plant making it difficult to use this parameter for comparison. The aim of this is study is to establish a sensitive and reproducible RT-qPCR indexing tool for CBSD viruses, to assess virus replication and movement and, to compare virus accumulation in infected cassava. RT-qPCR is currently used to quantify CBSV and UCBSV accumulation in cassava cultivars studied at the Plant Virus laboratories to identify and characterize new sources of CBSD resistance.

Feasibility of cowpea chlorotic mottle virus-like particles in nanobiotechnology: potential VLP scaffold for epitope-based vaccines

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Within the last decade virus-like particles (VLPs) have increasingly received attention from scientists for their use as a carrier of (peptide) molecules or as scaffold to present epitopes for use in subunit vaccines. To test the feasibility of cowpea chlorotic mottle virus (CCMV) particles as a scaffold for epitope presentation and identify sites for epitope fusion or insertion that would not interfere with virus-like-particle formation, chimeric CCMV coat protein (CP) gene constructs were engineered, followed by expression in *E. coli* and assessment of VLP formation. Various constructs were made encoding a 6x-His-tag, or selected epitopes from influenza A virus [IAV] (M2e, HA) or foot and mouth disease virus [FMDV] (VP1 and 2C). While His-tag or epitope insertions in the exposed loop structures did abrogate VLP formation, high yields of VLPs were obtained with all terminal His-tag fusions or when various epitopes (13- 27 aa) from influenza A (HA, M2e) and foot and mouth disease virus (VP1 and 2C) were fused to the N-terminal or C-terminal ends of CCMV CP, or fused to a N-terminal 24 amino acid (aa) deletion mutant (NΔ24) of the CP protein. So far, epitopes up to 27 aa have been successfully fused without disrupting VLP formation. VLPs derived from CCMV CP still encapsulated RNA, while those from CCMV CP-chimera containing a negatively charged N-terminal domain had lost this ability. The results indicate that CCMV VLPs can be successfully exploited as scaffold for epitope fusions up to 31 aa at the N- and C-terminus, and at a N-terminal 24 amino acid (aa) deletion mutant (NΔ24-CP) of the CP protein.

Novel insight in the interaction between REP, PCNA and SCE1, key factors in geminivirus replication

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Whitefly-transmitted geminiviruses are one of the most serious threats to economically important cultivation worldwide. To generate novel sources of resistance against geminiviruses, detailed insight in the infection process is of critical importance because with this knowledge novel resistance strategies that are based on altered functions of susceptibility genes can be designed. Potential susceptibility genes for geminivirus infection are those genes that encode proteins essential for virus DNA replication.

Over the past few years, studies have established that several host proteins associate with REP, such as PCNA (proliferating cell nuclear antigen), the processivity factor for host DNA polymerase-δ, and the E2 SUMO-conjugating enzyme SCE1. Even though REP doesn't alter the global sumoylation level of the infected plant cells, we demonstrated that REP is able to modify *in planta* the sumoylation state of at least one important specific target, PCNA. Moreover, we are interested in characterizing the interaction between REP and SCE1: the REP-SCE1 complex occurs in nuclear structures, named nuclear bodies, which change localization and distribution in different light conditions. We tested several nuclear bodies markers in order to determine the biological function of these structures and understand the effect of light/dark on body formation. We also identified, by using a series of SCE1 mutants, the sites on SCE1 protein that are essential for the interaction with REP. SCE1 is highly conserved at the protein sequence level across the plant kingdom and biochemically active SCE1 variants that fail to interact with REP might potentially provide broad durable resistance to geminiviruses.

Looking for elicitors and receptors of cauliflower mosaic virus (CaMV) transmission activation

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Cauliflower mosaic virus (CaMV) is, like many other plant viruses, transmitted by aphids and using the non-circulative transmission mode: when the insects feed on infected leaves, virus particles from infected cells attach rapidly to their stylets and are transmitted to a new host when the aphids change plants. Mandatory for CaMV transmission, the viral helper protein P2 mediates as a molecular linker binding of the virus particles to the aphid stylets. P2 is available in infected plant cells in a specific structure that is formed beforehand during CaMV infection. This structure is specialized for transmission and named the transmission body (TB). When puncturing an infected leaf cell, the aphid triggers an ultra-rapid viral response, necessary for virus acquisition and called transmission activation: tubulin flows massively into the TB, followed by its disruption. As a consequence, P2 is redistributed onto cortical microtubules, together with virus particles (that are simultaneously set free from intracellular storage sites) and forms the so-called mixed networks (MNs). The MNs are the predominant structure from which virus is acquired by aphids; inhibiting their formation reduces drastically transmission rates. We want to identify elicitors that trigger the TB response. For this, we screen different compounds for their capacity to induce MNs in CaMV-infected protoplasts and correlate it with aphid transmission rates obtained using these protoplasts. Preliminary results confer a role to chitin, which is an important compound of the aphids' exoskeleton and to *Myzus persicae* saliva effector proteins MP1 and MP2. In parallel, an *Arabidopsis thaliana* mutant screen has been initiated to dissect the genetic and molecular basis of signal perception and signal transduction pathways involved in the TB response. The latest results will be presented.

A proteomics approach in finding host factors essential for tospovirus replication

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Tospoviruses are the plant infecting members of the negative RNA virus family *Bunyaviridae*. Tomato spotted wilt virus (TSWV) and other tospoviruses are among the most devastating plant viruses worldwide. Dominant resistance genes in crops, like *Sw*5 and *Tsw*, and in wild varieties are scarce. Tospoviruses evolve quickly and have, therefore, already broken the few known dominant resistance genes used in plant breeding. As means to contain topspovirus infections is to eradicate the insects that transmits these viruses, thrips, by spraying insecticides. Application of insecticides is not a sustainable solution. To generate novel genetic sources of resistance against tospoviruses, detailed knowledge on the infection process is needed. Host factors that are required for tospovirus replication are a potential source for resistance breeding. Such host factors are potentially generic for all tospoviruses.

Our aim is to identify host proteins that are essential for TSWV replication. We plan to find host factors by means of a proteomics approach based on the transient expression of tagged nucleocapsid protein in *N. benthamiana*, followed by immunoprecipitation and mass spectrometry analyses of the co-immunoprecipitated protein complexes. Upon identification of such a host factor a more in-depth characterization will be performed to obtain detailed knowledge on the role of the gene product in the host and in viral proliferation.

Viruses associated with diseased broad-leaved tree species in Europe

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The identification of previously unknown viruses as well as the determination of mixed infections with several different plant viruses is a prerequisite to determine the impact of viruses on long-living forest trees as well as woody species dominating the urban green space (Büttner et al. 2013). High-throughput sequencing technologies enable the characterization of virus communities affecting important diseased broad-leaved tree species.

The virome of several deciduous tree species exhibiting virus-suspicious symptoms such as chlorotic ringspots, veinbanding and necroses of leaves associated with dieback of twigs and parts of the canopy were determined by High-throughput sequencing (NGS, RNA-Seq, Illumina). Analyses of NGS-generated sequence contigs identified plant viruses known to affect investigated tree species such as cherry leaf roll virus (CLRV), elm mottle virus (EMoV) and European mountain ash ringspot-associated virus (EMARaV). Further, nearly complete genomes of novel plant viruses could be identified in diseased tree species. Thus, NGS contributed to the discovery of previously unknown viruses belonging to the genera *Badna-*, *Carla-*, and *Emaravirus*, respectively, infecting important tree species of European forests and urban stands. Virus-incidence were confirmed by virus-specific RT-PCR in diseased trees of investigated species. Results provide first insights into geographical distribution, impact of known and newly identified viruses as well as the occurrence of mixed infections in ash, aspen, birch, elm, mountain ash, maple, and oak.

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Phosphorylation of the begomovirus movement protein affects host range, symptom development, and viral DNA accumulation

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The DNA-B component of bipartite begomoviruses (family *Geminiviridae*) encodes for a nuclear shuttle protein (NSP) and a movement protein (MP), which enable systemic spread within host plants and affect pathogenicity. The MPs mediate multiple functions during intra- and intercellular trafficking, such as binding of viral nucleoprotein complexes, targeting to and modification of plasmodesmata and release of the cargo after cell-to-cell transfer is completed.

A phosphorylation of Abutilon mosaic virus (AbMV) MP was shown for bacteria-, yeast- and *Nicotiana benthamiana*-derived protein [1, 2]. Mass spectrometry analyses of yeast-expressed MP identified three phosphorylation sites (Thr-221, Ser-223 and Ser-250) located in the C-terminal oligomerization domain [1, 3]. To assess their functional relevance for the viral life cycle within plants, several point mutations were introduced into the MP gene of AbMV DNA-B, which lead to an exchange of Thr-221, Ser-223, and Ser-250, either singly or in various combinations, with either an uncharged alanine or a phosphorylation-mimicking aspartate residue [1]. When co-inoculated with a

wild-type DNA-A, all mutated DNA-B variants give raise to a systemic infection in *N. benthamiana*. However, some mutations in MP abolished an AbMV-infection in other plant species of the families *Solanaceae* and *Malvaceae*. In systemically infected plants, symptoms and/or viral DNA accumulation were significantly altered for several of the tested DNA-Bs encoding MP mutants. The identification of three phosphorylation sites in AbMV MP, which have an impact on host range, symptom development, and/or viral DNA accumulation, indicates a regulation of the diverse MP functions by plant-derived posttranslational modification and underscores their importance for geminivirus/host plant interaction.

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Full genome characterization of tospoviruses by next generation sequencing

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Background and Objectives

The genus *Tospovirus* currently contains 11 officially recognized species (ICTV, 2015) however, more tentative species have been described. For most tospoviruses no specific antisera are available and only partial sequence data have been described. This hampers reliable detection of these agronomical important viruses. To facilitate the development of diagnostics and ensure the future availability of reference isolates the full RNA genomes of 10 different tospoviruses were determined, their sequences and biological data included in Q-bank and the individual isolates included in the physical plant virus collection at Wageningen UR.

Materials and Methods

Isolates of the different tospoviruses were obtained from plant virus collections from the Dutch NPPO and Wageningen UR and inoculated on indicator plants. Total RNA extracts were used for Illumina RiboZero 125 base paired-ends library preparations with individual MIDs and subsequently run in batch on a HiSeq 2500. After MID splitting individual datasets were fed into custom designed workflows within CLC Genomics Workbench (Qiagen, Denmark). These workflows comprised 'De novo' and reference-assemblies with and without substraction of plant-related reads. Resulting contigs were analysed by BlastN and BlastX against the NCBI database to identify tospovirus related sequences. Primer walking and 5'- and 3'-RACE and Sanger sequencing was used to determine the complete genomes.

Results

Most assemblies of the individual tospovirus datasets resulted in near full length RNA segments (L, M and S). A few RNAs were split in two contigs most likely by poor assembly of the hairpin regions. Primer walking and conventional Sanger sequencing resulted in linking contigs enabling assemblies of full length RNA sequences. Full sequences and additional information on the tospoviruses will be made publically available through Q-bank (www.q-bank.eu/virus).

Conclusions

NGS proofed to be a quick and relatively easy method to determine the complete sequences of a significant number of tospoviruses simultaneously. These sequences can now be used for the development of diagnostics and the identification of viral sequences obtained from plant samples through the virus ID function in Q-bank/virus.

References:

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Direct identification of sugar beet soil-borne viruses in soil samples

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Protocols for sensitive RT-PCR detection of sugar beet soil-borne viruses directly in soil samples were developed and optimized to diminish the time and costs required for determining the virus infestation of fields. The protocols utilize an appropriate isolation method for total RNA from soil, suitable enzymes and specific primer combinations, based on the genomes of three viruses: beet necrotic yellow vein virus (BNYVV), beet soil-borne virus (BSBV), beet virus Q (BVQ) and their vector *Polymyxa betae* (*P. betae*). The latter can serve as an internal amplification control. Different combinations of the most appropriate master mixes and primers designed were tested in both standard RT-PCR and qualitative real-time RT-PCR for amplification specificity, applicability and reproducibility. One- and Two-Step formats of RT-PCR were performed and compared to each other. The reliability of the approach was analyzed using numerous soil samples contaminated with the above mentioned pathogens. In all cases two parallel isolations of total RNA from roots of sugar beet plants grown in these soils were tested separately for the occurring viruses to confirm the conformity of results of specific virus amplification in two independent identification assays.

Tobacco mosaic virus as an additive in hydrogels

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Hydrogels are versatile materials for a variety of applications. They can be used for example as adsorbers, supports for biosensors and biocatalysts or as scaffolds for tissue engineering.

Tobacco mosaic virus (TMV) is a stiff, rod-like plant virus which has been investigated as template or building block for nano-scaled hybrid materials since many years. Especially its defined structure of 18 x 300 nm presenting more than 2000 identical coat proteins and the ability for *in vitro* assembly have made it such an interesting bio-scaffold. We have combined poly(ethylene glycol) diacrylate (PEG-DA, Mn 700) based hydrogels with a thiol-groups presenting TMV mutant, TMV_Cys. The covalent coupling of TMV to PEG-DA adds focal strengthening points into the gel matrix and gives the opportunity to incorporate further functions such as enzymes. We investigated the influence of covalently coupled TMV_Cys and encapsulated wildtype TMV on the mechanical properties of the PEG-DA/TMV hydrogels. While the swelling behaviour remained the same as in hydrogels without TMV, the covalent coupling lead to an enhanced storage modulus. For structural characterization the gels were embedded in epoxy resin and ultrathin sections were investigated by transmission electron microscopy.

Using a PEG-DA/Poloxamer/TMV hydrogel solution printable hydrogels could be obtained. Here about 50 % of the thiol-groups on TMV_Cys were used to add a maleimide biotin linker to the virus

which then bound streptavidin-coupled alkaline phosphatase. These alkaline phosphatase containing hydrogels were then incubated in a calcium-glycerol phosphate solution to induce mineralization. The mineralized hydrogels were characterized with regard to their mechanical properties and their mineral content. There was a clear dependence of the mineral content on virus concentration. This could make TMV-containing hydrogels interesting scaffolds for the cultivation and differentiation of cartilage or bone tissue.

Molecular characterization of a new 'rule-breaking' tobacco rattle virus RNA2 and its stepwise degradation and finally loss in potato plants grown in a nematode-free environment

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Tobacco rattle virus (TRV) has a bipartite genome. In almost all TRV RNA2s studied so far the coat protein (CP) gene occupies the 5'proximal position. In full length RNA2s, the CP gene may or may not be followed by a small gene for an 8 kDa peptide and further downstream by the so called 2b and 2c protein genes. With some strains only the 2b protein is required for the transmission of the virus by a certain nematode, but with others all virus-encoded proteins, including the CP are needed for nematode transmission. So far only two TRV RNA2s have been described in which the CP gene is preceded on the 5'side by one or several other genes. Ashfaq et al. (2011) have analyzed a spinach isolate of TRV RNA 2 in which the cp gene is preceded on the 5'side by three partially overlapping ORFs encoding proteins of ca. 7, 30 and 21 kDa with unknown functions. In a TRV RNA2 found by Yin et al (2015) in potatoes the CP gene is preceded by a gene for an unrelated ca. 12 kDa peptide. We have recently detected in potatoes a TRV RNA2 in which the CP gene is preceded on the 5'side by a gene for a 35 kDa protein unrelated to those described previously for a TRV RNA2. A NCBI Blast search did not reveal any significant similarities with other nucleotide sequences, on the amino acid sequence level, however, some similarities were found with a membrane protein of *Mycoplasma leachii*. Downstream of the CP gene this RNA contains a 1311 nt gene for a 50 kDa protein distantly related to the 2b proteins which in other TRV strains are needed for nematode transmission. During the development of plantlets grown from sprouting tuber cuttings planted in soil free of nematodes already after 5 days 80 of the 1311 nts of this 2b-related gene were lost in the newly formed roots. After 33 days the complete 2b gene and a 3' portion of the CP gene were deleted in roots, leaves and stolons, while the 35kDa protein gene upstream of the CP gene was still unchanged stressing its importance for the virus. After 50 days only TRV RNA1, but no TRV RNA2 was detected in any parts of the plants including the newly formed secondary tubers.