16th TRIENNIAL MEETING OF THE VIROLOGY SECTION OF THE EUROPEAN ASSOCIATION OF POTATO RESEARCH

&

8th ANNUAL MEETING OF PVYWIDE ORGANIZATION

May 31st – June 3rd 2016
Ljubljana, Slovenia

eapr
EUROPEAN ASSOCIATION FOR POTATO RESEARCH

PVY wide ORGANIZATION

It is our pleasure to welcome you at the **16th triennial meeting of the Virology Section of the European Association of Potato Research (EAPR)**, which is combined with the **8th annual meeting of PVYwide organization**.

The meeting is held from **May 31st till June 3rd 2016** in Ljubljana, Slovenia.

The programme consists of plenary talks, oral presentations, poster sessions and scientific excursion. The meeting covers different aspects of virus research in potatoes, ranging from diagnosis and detection to plant-virus interactions.

Welcome to Ljubljana!

Adrian Fox,
on behalf of the Virology Section of EAPR

Christophe Lacomme,
on behalf of PVYwide organization

Maruša Pompe-Novak,
on behalf of Organizing committee

COMMITTEES

COMMITTEES

Scientific committee:

- Adrian Fox
- Christophe Lacomme
- Annelien Roenhorst
- Jan Kreuze
- Laurent Glais
- Brice Dupuis
- Dirk Bellstedt
- Maja Ravnikar
- Maruša Pompe-Novak

Organizing committee:

- Maruša Pompe-Novak
- Maja Ravnikar
- Ana Mihevc
- Polona Kogovšek
- Nataša Mehle
- David Dobnik

NATIONAL INSTITUTE OF BIOLOGY

- Peter Dolničar
- Irena Mavrič-Pleško

Kmetijski inštitut Slovenije
Agricultural Institute of Slovenia

Program at glance

Program at glance

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PROGRAM

Tuesday, 31st of May

12:00 - 14:00	Registration
13:00 - 14:00	Lunch

Chair: Maruša Pompe Novak

14:00 - 14:20	Opening

Session 1: Diversity and evolution
Chair: Maruša Pompe Novak

14:20 - 15:20	Invited speaker	Marilyn J. Roossinck	Spillover/spillback of agricultural viruses into wildland plants
15:20 - 15:40	Oral presentation 1	Dirk Bellstedt	Why are some PVY recombinant strains replacing the older non-recombinant strains?
15:40 - 16:00	Oral presentation 2	Denis Kutnjak	Discovering potato virus Y within-plant population structure changes with deep sequencing of small RNAs

16:00 - 16:30	Coffee and Posters 1 - 3

Session 1: Diversity and evolution
Chair: Dirk Bellstedt

16:30 - 16:50	Oral presentation 3	Jerzy Syller	The effects of within-host interactions between isolates of Potato virus Y on virus accumulation in some solanaceous plants
16:50 - 17:10	Oral presentation 4	Maruša Pompe Novak	The influence of the potato circadian rhythm on the disease development after infection with PVY
17:10 - 17:30	Oral presentation 5	Renate Koenig	Characterization of several groups of Tobacco rattle virus (TRV) RNA1s and RNA2s and recognition of different TRV RNA1/RNA2 pairings in various corky ringspot-ffected potato-growing areas in Germany
17:30 - 17:50	Oral presentation 6	Renate Koenig	Detection of a variably sized internal poly A tract ('IPAT') in a new type of Tobacco Rattle Virus (TRV) RNA2

Session 5: Diagnostic and detection methods
Chair: Dirk Bellstedt

17:50 - 18:10	Oral presentation 35	Victor Gaba	The detection of Potato virus Y in seed potato lots in Israel by a TaqMan Real Time PCR technique
18:10 - 18:30	Oral presentation 39	Colin Jeffries	The United Kingdom Potato Quarantine Unit (UKPQU): an accredited off-shore potato quarantine station for New Zealand and an approved source of high health material for Australia

18:30 - 20:30	Guided tour around Ljubljana
20:30 - 22:00	Welcome dinner

Wednesday, 1st of June

Session 2: Plant - virus interactions
Chair: Christophe Lacomme

8:30 - 9:30	Invited speaker	Kristina Gruden	Modellling of potato immune signaling response
9:30 - 9:50	Oral presentation 7	Aleš Sedlar	Differential gene expression patterns in PVYNTN infected potato tubers
9:50 - 10:10	Oral presentation 8	Polona Kogovšek	Primary metabolism, phenylpropanoids and antioxidant pathways are regulated in potato as a response to Potato virus Y infection
10:10 - 10:30	Oral presentation 9	Zhimin Yin	Host miRNA response in leaves of PVY infected tobacco plants
10:30 - 10:50	Oral presentation 10	Maja Križnik	Identification and characterization of small RNAs involved in tolerance response of potato (Solanum tuberosum L.) to Potato virus Y (PVYNTN)
10:50 - 11:10	Oral presentation 11	Špela Baebler	Enhancing understanding of potato – virus interactions using GoMapMan

11:10 - 11:40	Coffee and Posters 4 - 11

Session 2: Plant - virus interactions

PROGRAM

Chair: Jan Kreuze

11:40 - 12:00	Oral presentation 12	Elena Voronkova	Novel DNA locus associated with PVY resistance in potato is mapped to chromosome V
12:00 - 12:20	Oral presentation 13	Douglas Pyott	Engineering of CRISPR/Cas9-mediated virus resistance in transgene-free Arabidopsis plants
12:20 - 12:40	Oral presentation 14	Anna Coll	GenoCAD plant grammar, a Computer-Aided Design software tool for plant expression vectors

Session 3: Transmission and control
Chair: Jan Kreuze

12:40 - 13:00	Oral presentation 15		
13:00 - 13:20	Oral presentation 16	Christophe Lacomme	Genetic Diversity of PVY isolates in Potato Crops: Impact of Strain Competition and Ability to Overcome Host Resistance Mechanisms
13:20 - 13:40	Oral presentation 17	Pankaj Kumar	Recombinant strains of Potato virus Y overcome mature plant resistance in Solanum tuberosum L.
13:40 - 14:00	Oral presentation 18	Adrian Fox	The transmission of PVY and PVA in the UK

14:00 - 15:00	Lunch

Session 3: Transmission and control
Chair: Annelien Roenhorst

15:00 - 15:20	Oral presentation 19	Brice Dupuis	The influence of aphids flights on the transmission of the potato virus Y (PVY) in the field
15:20 - 15:40	Oral presentation 20	Jean-Louis Rolot	Fighting strategies against PVY dissemination in a potato seed multiplication field: comparison of several methods using insecticides (systemic and pyrethroïds) and paraffinic mineral oils
15:40 - 16:00	Oral presentation 21	A. Mityushkin	Minimizing of sources and vectors of virus infections on seed potatoes in the Central region of Russia
16:00 - 16:20	Oral presentation 22	Uta Priegnitz	Virus control in Ugandan seed potato tubers over multiple field generations by applying different seed technologies
16:20 - 16:40	Oral presentation 23	José Alberto Caram De Souza Dias	Potato virus Y (PVY): Attempts toward bio-immune monitoring system for potato and tobacco production of propagating material
16:40 - 17:00	Oral presentation 24	Jan Kreuze	A process based epidemiological model for the Potato yellow vein virus – Trialurodes vaporariorum (greenhouse whitefly) - potato pathosystem

17:00 - 17:30	Coffee and Posters 12 - 16

17:30 - 20:00	Tour to Postojna cave
20:00 - 22:00	Dinner

Thursday, 2nd of June

Session 4: Epidemiology
Chair: Brice Dupuis

8:30 - 8:50	Oral presentation 25	Stewart Gray	Evolving disease dynamics of the Potato virus Y complex affecting the U. S. potato crop: A group effort between selection pressures and farming practices
8:50 - 9:10	Oral presentation 26	Zofia Wojcik	Trends in PVY population changes in Poland
9:10 - 9:30	Oral presentation 27	Piret Peterson	Viruses in Estonian seed potatoes; Strains of Potato Virus Y
9:30 - 9:50	Oral presentation 28	Asmaa Youssef	Serological, Molecular and Biological Characterization of Potato Virus Y Isolates from Potato in Sweden
9:50 - 10:10	Oral presentation 29	Antonia dos Reis Figueira	Occurrence of Potato virus Y strains in Brazil and quantification in single and mixed infections by qRT-PCR
10:10 - 10:30	Oral presentation 30	Cigdem Ulubas Serce	Survey and detection of Potato virus Y (PVY) and its strains from the Nigde province of Turkey

10:30 - 11:00	Coffee and Posters 17 - 18

Session 4: Epidemiology

PROGRAM

Chair: Laurent Glais

11:00 - 11:20	Oral presentation 31	Hakan Carpar	Determination of PVY and AMV infection in weeds in potato fields in Hatay-Turkey
11:20 - 11:40	Oral presentation 32	Üftade Güner	Screening of Potato Virus Y (PVY) Strains in Potato, Weeds and Aphid Samples in Bolu Province (Turkey)
11:40 - 12:00	Oral presentation 33	Gulsen Sertkaya	Status of Natural Alfalfa mosaic virus (AMV) Infection in Potatoes in Hatay-Turkey

12:00 - 14:00	Visit of National Institute of Biology
14:00 - 14:30	Lunch at NIB
14:30 - 18:00	Visit of experimental fields at Jablje

18:00 - 20:00	Tour to Bled lake
20:00 - 22:00	Congress dinner

Friday, 3rd of June

Chair: Christophe Lacomme

8:30 - 9:30	PVYwide meeting

Session 5: Diagnostic and detection methods
Chair: Maja Ravnikar

9:30 - 9:50	Main sponsor	Adam Bemis	Ultrasensitive Rare Sequence Detection with Droplet DigitalTM PCR
9:50 - 10:10	Oral presentation 34	Denise Altenbach	Potato DNA/RNA rapid extraction suitable for molecular test systems such as qPCR and PCR macroarray
10:10 - 10:30	Oral presentation 36	Olivier Schumpp	High throughput qPCR detection of virus for certification of seed potatoes

10:30 - 11:00	Coffee and Posters 19 - 24

Session 5: Diagnostic and detection methods
Chair: Adrian Fox

11:00 - 11:20	Oral presentation 37	Pruthvi Kalyandurg	Sequencing and Molecular Characterization of Peruvian Potato mop-top virus full-length cDNAs Reveals Two Genotypes and Evidence for Recombination
11:20 - 11:40	Oral presentation 38	Carolyn Nisbet	A proficiency test for potato infecting phytoplasmas
11:40 - 12:00	Oral presentation 40	Maja Ravnikar	New technologies for diagnostics and epidemiology of viruses

Chair: Adrian Fox

12:00 - 12:40	EAPR Virology Business meeting
12:40 - 13:00	Closing

ABSTRACTS

Spillover/spillback of agricultural viruses into wildland plants

Marilyn J. Roossinck[1,2], Prasenjit Saha[2] and Anthony H. Stobbe[1]

[1] *Center for Infectious Disease Dynamics, Penn State Univ., University Park, PA*
[2] *The Samuel Roberts Noble Foundation, Ardmore, OK*

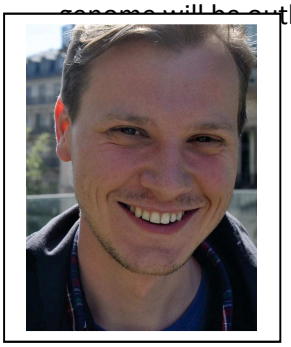
We have been analyzing plant viruses in a biodiversity hotspot in northwestern Costa Rica with about 10,000 species of native plants. We find many viruses that are distantly related to known viruses, an equal or greater number of completely unknown viruses, and a few known viruses. In particular we have found a massive invasion of a plant pathogenic virus, Zucchini yellow mosaic virus, that appears to be from spillover events from adjacent agricultural areas. The virus does not cause any detectable disease in native plants, which may be dead-end hosts. There is some evidence of spill-back into crops, however these viruses are attenuated in the disease symptoms that they induce.
A common assumption is that emerging viruses invade humans and their domesticated plants and animals from wild relatives; however, little has been done to look at how "domestic" viruses affect wildlife. These studies suggest that wild plants may be equipped to tolerate viruses acquired from domestic hosts, and perhaps change them to less virulent forms. In addition, there is little evidence that viruses evolve towards increased virulence. Implications for agriculture will be discussed.

Why are some PVY recombinant strains replacing the older non-recombinant strains?

Dirk U. Bellstedt and J. Christiaan Visser

Department of Biochemistry, University of Stellenbosch, South Africa

Since the first reports of the PVY^{NTN} and PVY^{NW} strains in 1984 and 1990 respectively, there has been a steady replacement of the older non-recombinant strains i.e. PVY^{C}, PVY^{O} and PVY^{N} in most regions of the world. Our analysis of whole genome sequences of South African isolates shows a similar pattern. Recombination must hold selective advantages for these strains in that recombination has enhanced their pathogenicity, most probably through a combination of characters from the original parent strains. Using parsimonious deductions a complex pattern of which regions are required for the recombinants to show enhanced pathogenicity can be derived. Furthermore an analysis of which proteins these regions code for reveals which viral proteins apparently give these selective advantages. Specifically it appears that the HCPro and the coat protein play important roles. Recent research has shown that the HCPro protein possesses a number of functions which apparently enables these recombinants to replace the older strains. In this presentation, the regions required by recombinants for increased pathogenicity, and the functions of the proteins in the recombined genome will be outlined and discussed.



Discovering potato virus Y within-plant population structure changes with deep sequencing of small RNAs

Denis Kutnjak[1], Matevž Rupar[1], Ion Gutierrez-Aguirre [1], Tomaž Curk[2], Jan Kreuze[3], Maja Ravnikar[1]

[1]*National Institute of Biology, Ljubljana, Slovenia*
[2]*University of Ljubljana, Faculty of Computer and Information Science, Ljubljana, Slovenia*
[3]*International Potato Center (CIP), Lima, Peru*

RNA viruses exist within a host as a population or swarm of mutant sequences. The composition of this swarm is an important characteristic of the virus, since it represents a reservoir of genetic variants, which can be subjected to different evolutionary processes. High mutation rate of RNA viruses drive their quick adaptability and evolution rate. Thus, new viruses can emerge as a result of different processes, such as e.g. host shifts (1).
Next generation sequencing technologies enable through investigation of the viral population structure within a host. This allows us to detect relevant mutations in the viral population even before the emergence of new pathogenic viruses or viral strains (2). Before being able to use such a tool to follow the emergence of new viral variants in a viral mutant cloud, efficient sample preparation and bioinformatics pipelines are required.
Deep sequencing of virus derived small interfering RNAs (vsiRNAs) has been used efficiently for the reconstruction of consensus viral genome sequences from insects and plants (3). In our research, we are focused on potato virus Y, an important potato pathogen. Using this virus as a model, we first tested if the variation observed in vsiRNAs reflects the full diversity of viral populations in plants. Using ultra deep Illumina sequencing, the diversity of two coexisting Potato virus Y sequence pools present within a plant was investigated: RNA isolated from viral particles and vsiRNAs; both sequence pools reflect highly similar mutational spectrum (4).
Currently we are employing deep sequencing of small RNAs to track the dynamics of virus adaptation to several potato cultivars. We are interested if the structure of virus population is changed in different potato cultivars and if there are convergent patterns of virus evolution emerging in the same cultivar. To answer this question, we have performed evolution experiment by serially passaging potato virus Y in different potato cultivars. Extensive time-point sampling was performed. Quantitative PCR was used to determine virus titre in different cultivars through time and next generation sequencing was employed to track the fluctuations of different variants frequencies over the course of the experiment. The data will be analysed with the state of the art computational approaches to discover the loci under selection and compare the genetic diversity of potato virus Y populations in different cultivars.
References:
1. Domingo et al. 2012. Microbiol. Mol. Biol. Rev. 76, 159–216; 2. Kreuze et al. 2009. Virology 388, 1–
2015. J. Virol. 89, 4761–4769; 4. Stapleford et al. 2014. Cell Host Microbe 15, 706–

The effects of within-host interactions between isolates of *Potato virus Y* on virus accumulation in some solanaceous plants

Anna Grupa, Jerzy Syller

Plant Breeding and Acclimatization Institute – National Research Institute, Laboratory of Phytopathology, Centre Mlochow, 05-831, Mlochow, Poland

Mixed infections of viruses are quite common in cultivated and wild-living plants. They are classified as super-infection, when different viruses enter the host cell(s) at different times (sequential infections), or co-infection, when two or more viruses enter the cell(s) simultaneously. The potential consequences of a mixed infection for the viral and host fitness depend on the type of interactions between the viral counterparts. Viruses belonging to different genera and species either interact in a synergistic manner or have no mutual influence. A synergistic interaction has a beneficial effect on one or both of the viral partners, manifested by an enhanced replication of the beneficiary virus(es) and more severe disease symptoms than those induced by either virus alone. In contrast, mostly antagonistic interactions occur between closely related viruses. Typically, a previous infection with one virus prevents or interferes with subsequent infection by a homologous second virus, a phenomenon termed super-infection exclusion (SIE), but more widely known as cross-protection when referring to an agricultural practice. When two similar viruses enter the host cell(s) at the same time, the phenomenon of mutual exclusion (ME) can occur.

Despite the fact that both SIE and ME seem to play a crucial role in viral pathogenesis, very little has been known until now about the interactive effects of mixed infections with different isolates of Potato virus Y (PVY) in major hosts. PVY, the type species in the genus Potyvirus (family Potyviridae), is one of the most devastating pathogens of important solanaceous crops. Field populations of the virus consist of numerous isolates, mostly recombinants. Since these isolates generally share the same hosts, the probability that two or more viral variants will tend to infect the same host plant is high. Consequently, antagonistic interactions might be expected to occur among such closely related viral variants. Indeed, the here presented results clearly indicate that PVY isolates inoculated simultaneously or sequentially to potato or tobacco plants interact with each other in an antagonistic way. The studies included eight and subsequently six chosen PVY isolates classified in ordinary (O) or necrotic (N) strain groups, depending on the pathotype/serotype combination: PVY^{O} (O/O), $PVY^{N:O}$ (N/O), and PVY^{NTN} (N/N). The presence of PVY isolates in doubly or singly infected source and/or assay plants was detected using a variety of diagnostic methods, including ELISA, multiplex RT-PCR, duplex quantitative real-time RT-PCR (RT-qPCR), aphid transmission tests, and bioassays. ELISA performed with monoclonal antibodies was successfully used to quantify the isolates in doubly vs singly infected plants. Significant reductions in concentrations of certain PVY isolates following super-

infection or co-infection with other isolates were recorded both in potato and tobacco plants. The extents to which two isolates sequentially inoculated to the host plant were able to accumulate in the host's cells depended primarily on the fitness of these isolates, whereas inoculation sequence definitely played a secondary role.

This work was supported by The National Science Centre in Poland, grant UMO-2421.

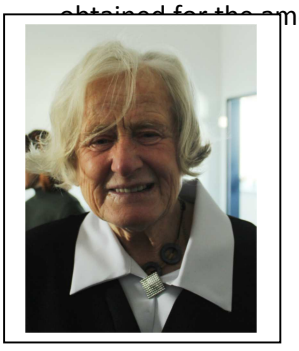
The influence of the potato circadian rhythm on the disease development after infection with PVY

Maruša Pompe-Novak[1], Maja Švigelj[1,2], Kristina Gruden[1]

[1]*National Institute of Biology, Ljubljana, Slovenia*
[2]*University of Nova Gorica, Nova Gorica, Slovenia*

The plant immune response is strictly controlled in order to effectively defend against pathogenic organisms and is regulated by abiotic factors such as light, humidity, circadian rhythm, temperature and others. Circadian rhythms are driven by biological clock of the organism and are a response to 24-hour changes in the physical environment. They are associated with day and night and also function when the plant is deprived of external time indications. Circadian clock is essential for the control of various physiological activities and gene expression. Transcriptional feedback loops play an important role in circadian clock on a molecular level.

We investigated the effect of inoculation with PVY^{NTN} on potato plants of cultivars Désirée and Igor at different times during the day. In the case of infection in different parts of the day the symptoms in certain times after inoculation were differently expressed and symptoms began to express at different times after inoculation. In general, PVY^{NTN} inoculation of potato plants of cultivars Igor and Désirée at 2 p.m. and 8 p.m. in the early days after infection led to the appearance of symptoms on greater proportion of leaves than the infection at 8 a.m. During later course of disease the proportion of leaves with disease symptoms in plants infected at 8 a.m., 2 p.m. and 8 p.m. equalized, 14 days after inoculation it was even higher in plants infected at 8 a.m. Very similar results were also obtained for the amount of viral RNA and viral protein in plant leaves.



Characterization of several groups of Tobacco rattle virus (TRV) RNA1s and RNA2s and recognition of different TRV RNA1/RNA2 pairings in various corky ringspot-infected potato-growing areas in Germany

K. Lindner[1], I. Hilbrich[1] and R. Koenig[2]

[1]*Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, 38104 Braunschweig, Germany*
[2]*c/o Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany*

The complete genome of tobacco rattle virus consists of two RNA species. RNA1 contains the genetic information for two replication-associated proteins, the movement protein and a silencing suppressor protein. Intact RNA2s contains the coat protein (cp) gene and at least two additional genes for the so called 2b and 2c proteins. The 2b proteins and - at least with some strains - also the 2c proteins are necessary for nematode transmission. Additional genes have been found on some further TRV RNA2s. Upon cultivation in systemically infected plants some RNA2 genes, especially those needed for nematode transmission, may be partially or completely deleted. Based on the results of hybridization experiments RNA1 was for a long time believed to be more or less identical in all TRV infections, whereas RNA2s were recognized already very early to represent a great variety of various forms. Based on sequence analyses we have recognized three major groups of TRV RNA1 in potatoes in Germany. The RNA2s which we have found in potatoes in Germany so far fall into two groups (Koenig et al., 2016). The first group shows a similar genome organization as the British PpK20 RNA2 (Visser & Bol, 199). In Germany it comprises two new RNA2s named ByKT-2 and HaW-2. The cps encoded on the PpK20, ByKT and HaW RNA2s show high degrees of amino acid (aa) sequence identities ranging from 90 to 95 %sequence identities. The 2b proteins share only 62 to 68 % aa sequence identity and no appreciable aa identities are found between the 2c proteins. A second group of TRV RNA2s found in potato growing areas in Germany comprises isolates which have a similar genome organization as the British TpO1 RNA2 (MacFarlane et al., 1999). In addition to the cp, 2b and 2c genes they carry a small gene for a 9K peptide. RNA2s closely related to TpO1 RNA2 are widely distributed in various potato growing areas in Germany, but in addition we found another type of TRV RNA2 (CeWF and CeWGH) which encodes a cp sharing 97% aa sequence identity with the TpO1 cp. However, the 2b and 9K proteins encoded on the TpO1 and the CeW RNA2s share only ca. 82% aa sequence identity and no relationships were detected between the 2c proteins. - In different geographic areas closely related TRV RNA2s were found to be associated with distinct TRV RNA1s and, vice versa, closely related RNA1s may support very different RNA2s. The ability of TRV RNA1s to [illegible]d with different TRV RNA2s will enable their transmission by different nematode [illegible]t be especially important with highly pathogenic or resistance-breaking strains.

Detection of a variably sized internal poly A tract ('IPAT') in a new type of Tobacco Rattle Virus (TRV) RNA2

Koenig, R.[1], Ziebell, H.[1], Hilbrich, H.[2] and Lindner, K[2]

[1]*c/o Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany*
[2]*Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, 38104 Braunschweig, Germany*

The nucleotide (nt) sequences of two closely related isolates (CeWF-2 and CeWGH-2) of a new tobacco rattle virus (TRV) RNA2 species were determined. The sequences of their RNA2-specific parts were almost identical and contained four open reading frames (ORF) in a similar arrangement as in the previously described TpO1 isolate (MacFarlane et al., 1999). The gene product of ORF 1 shared 97 % amino acid sequence identity with the coat protein (cp) of TpO1, but no appreciable sequence identity was found between the ORF 4 gene products. Sequencing of PCR products which cover the 3' ends of the RNA2-specific and the 5' ends of the RNA1-related parts revealed that in CeWGH-2 the two parts were separated by seven (A) residues. CeWF-2, however, obviously had much larger internal poly (A) tracts ('IPAT's). The exact number of (A) residues in its PCR products could not be determined, because the sequences became unreadable after about 20 (A)s. In cloned PCR products the number of (A)-residues ranged from 21 to 29. CeWF-2 has thus a variably sized IPAT. Similar IPATs have previously been found also in the RNAs of some other plant viruses. In RNA b of some hordeiviruses they separate the 3' UTR from the 5' coding body which contains the cp gene and the triple gene block coding for the viral transport proteins (Atabekov et al., 1986). In five out of six bromovirus species IPATs of heterogeneous lengths are located in the RNA 3 downstream of the RNA3 protein gene and upstream of the start of the subgenomic cp gene-carrying RNA4 (Karpova et al., 1989). The RNA of Hibiscus latent Singapore virus (HLSV), a tobamovirus, carries an IPAT of variable length ranging from 77 to 96 nts upstream of its 3' UTR (Niu et al. 2015). It is located in the RNA in a region where in other tobamovirus RNAs an upper pseudoknot domain is found. In all these viral genomes the IPATs are essential for efficient virus replication. Ours is the first report of an IPAT occurring also in a tobravirus RNA. Its exact role in a tobravirus RNA is not yet known.

1. Atabekov JG et al. (1986) Hordeiviruses, The Plant Viruses Vol. 2. pp. 397-424; 2. Karpova OV et al. (1989) J Gen Virol 70: 2287-2297, 3. MacFarlane SA et al. (1999). J Gen Virol 80: 273-276;
ang LJ, Tan KCL, Wong SM (2015) Virology 474: 2-64

Efficiency of cryotherapy for elimination of three viruses from potato plants

Dědič Petr[1], Faltus Miloš[2], Horáčková Vendulka[1]

[1]*Potato Research Institute Havlíčkův Brod, CR*
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Continuous maintaining and keeping of cultivars and germplasm in in vitro conditions is nowadys well established technique in potato breeding. In order to maintain virus-free genotypes different procedures must be employed depending on the specified contaminant. Usually the combination of thermotherapy followed by meristem culture and/or chemotherapy. In connection with kryopreservation technique which utilise very low temperature of liquid nitrogen, the promising effect on patogenes erradication was discovered.

In our experiments we compared in similar arrangement the well-established virus elimination procedures as thermotherapy followed by meristem culture and chemotherapy with newly recommended cryotherapy. Used cryogenic protocol followed essentially procedure described by Faltus and Zámečník (2008) with some modifications. Before cryotherapy, the potato explants were acclimated osmotically with sucrose and subsequently air-dehydrated above silica-gel. Thereafter the potato shoot tips were plunged into liquid nitrogen, after 60 minutes the explants were removed and planted on the regeneration medium. Survival and shoot regeneration were evaluated after two and eight weeks of cultivation, respectively. All the therapeutic procedures were compared at 17 similar potato cultivars infected systemically with PLRV, PVY and PVS, respectively, maintained in in vitro cultures.

The presence of viral pathogens was at all the treatments evaluated preliminary in in vitro explants, and subsequently (in the case of negative results) in seedlings from the greenhouse by means of ELISA diagnosis. It was demonstrated that cryotherapy was effective for elimination of PLRV and PVY, but ineffective for elimination of PVS. Average virus elimination by the cryotherapy method was 66% in genotypes infected with PLRV and 65% in genotypes infected with PVY. Unfortunately no virus-free plants were obtained after cryotherapy of genotypes infected with PVS. Compared to all three therapy techniques, it is possible to infer that cryotherapy is very similar to thermotherapy with meristem culture, but not effective for therapy of all viruses e.g. PVS which is very frequent in many potato genotypes. On the contrary, the advantage of cryotherapy may be its less labour and time demand.

Enhanced accumulation of potato mop-top virus in mixed infections with a novel pomovirus suggests synergistic interactions between two viruses belonging to the same genus

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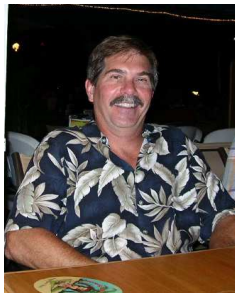
In Colombia, Potato mop-top virus (PMTV, Pomovirus), along with its vector *Spongospora subterranea*, has been reported to infect potato throughout the country. However, no symptoms have been shown to be associated with its presence. Recently, we reported a unique group of isolates from the country. This group appears to possess a 'mosaic' gene, encoding a viral suppressor of RNA silencing, the 8K protein. Comparison of the 'mosaic' gene sequence to the sequences of previously characterised PMTV isolates suggest that the gene arose as a result of recombination between two isolates. Virus variant with a 'mosaic' gene induces more severe symptoms in *Nicotiana benthamiana* (isolate PMTV-CO1) as compared to those induced by a common strain (isolate PMTV-CO3). Yet, another pomo-like virus has been detected and molecularly characterised from the central and southern potato growing regions of Colombia. The virus has provisionally been named Colombian potato soil-borne virus (CPSbV) and the virus co-infects potato plants along with PMTV. CPSbV can also be transmitted by tuber-seed. A noticeable feature of this virus is the lack of the gene for the 8K protein. When CPSbV was co-inoculated with PMTV-CO3 on *N. benthamiana* plants, accumulation of both CPSbV and PMTV-CO3 in inoculated and upper systemically infected leaves was reduced as compared to the single infections with any of these viruses. On the other hand, whereas the accumulation of CPSbV did not chance, when co-inoculated with PMTV-CO1 (a severe PMTV strain), PMTV-CO1 accumulated at much higher levels (up to 88-fold increase in the mixed infection compared to a single PMTV-CO1 infection) as was determined by quantitative RT-PCR. The infected plants had also more severe symptoms when co-infected with PMTV-CO1+CPSbV compared to the PMTV-CO3+CPSbV combination. Overall, these data suggest that the Colombian strains of PMTV, when co-infecting with CPSbV might represent an epidemiological concern if introduced to territories where neither of these viruses are present. The role of the 8K protein in the apparent synergism between PMTV-CO1 and CPSbV is being investigated.



Potato cultivar and virus isolate affect the disease dynamics of mixed-strain infections of *Potato virus Y*

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Infections of more than one strain of Potato virus Y (PVY) are not uncommon in potato grown in the United States. The disease dynamics overtime may influence the virus available for aphids to acquire and spread to other plants, and may affect the number of daughter tubers infected with either or both strains. Two isolates of PVY^{O} (Oz, NY31) and two isolates of PVY^{NTN} (ME4, NY29) were mechanically inoculated in all possible mixtures to three potato cultivars, Pike, Goldrush and CalWhite. Relative virus titer was monitored at various leaf positions over a four week period and then again just prior to harvest to determine how quickly both viruses moved out of the inoculated tissue and if they were detected at similar levels in various positions on the plant over time. Additionally, tubers were harvested from the plants and after suitable dormancy, were planted and the resulted stems tested to determine relative titers of both strains. Immuno-fluorescence confocal microscopy was used to monitor the distribution of both virus strains in leaf tissues and individual cells. PVY infection of CalWhite plants resulted in very mild foliar symptoms regardless of the infecting strain, but all four isolates reached similar titers when inoculated alone. Mixed infections of NY31 / ME4 resulted in both viruses reaching similar titers in all tissues, whereas NY31 and Oz completely dominated NY29. Titer of ME4 was more than twice that of Oz. Virus found in the daughter tubers reflected what was in the leaves of the mother plants. PVYO infection of Goldrush resulted in severe mosaic symptoms; PVY^{NTN} infection induced noticeable mosaic. Both isolates of PVY^{O} were completely dominated by both isolates of PVY^{NTN} in all leaf tissues up to four weeks post inoculation. However by harvest, PVY^{O} titers were similar to PVY^{NTN} except in the Oz / NY29 mixture. Virus found in the daughter tubers reflected the relative virus titers in the leaves at harvest except from plants infected with NY31 / NY29. Although virus titer in the leaves was similar for both strains at harvest, NY31 was found alone in most of the tubers. PVY^{O} infection of Pike usually resulted in a systemic hypersensitive response manifested as vein necrosis and leaf drop. This was true for the Oz isolate, but not NY31, which induced mosaic symptoms as did infection by the two PVY^{NTN} isolates. Both NY29 and ME4 dominated the mixed infections with Oz, whereas relative titers of both NY29 and ME4 were similar to NY31 in the mixed infections. Virus found in daughter tubers reflected relative titers in leaves at harvest, except, similar to Goldrush, NY31 was found alone in tubers from plants infected with both NY31 and NY29. Immuno-fluorescence studies indicate that both PVY^{O} and PVY^{NTN} can co-infect cells in potato and tobacco and mixed infected cells dominate most of the

infected cells in mature leaf tissues. These studies indicate that potato cultivars as well as isolates within a virus strain can vary considerably in infection dynamics when two virus strains are competing. In this study, both viruses were inoculated at the same time. It is likely that staggered inoculation times would have a more profound impact on virus titer and distribution in the plant and thereby influence virus acquisition by aphid vectors and also virus transmitted vertically to daughter tubers.

Modellling of potato immune signalling response

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Infection of a plant by a pathogen initiates a complex interaction between both players involved, leading to changes in the complex signalling network, which result in gene activity changes and reprogramming of the cell metabolism. A systems biology approach was adopted for the purpose of modelling complex biological processes in order to understand the mechanisms and dynamics involved in potato plant defense following the infection with potato virus Y (PVY).

A qualitative model of potato plant defence signalling network (PDS) was constructed (Miljkovic et al., 2012), describing the biosynthesis and signal transduction pathways for three crucial phytohormones involved in plant defence: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The prior knowledge from literature was expanded with information on the viral and plant component interactions, protein-protein interactions and protein-DNA interactions in plant Arabidopsis.

The resulting robust qualitative model offers new insights into the plant-virus interaction by expanding the knowledge on critical components of plant defence signalling, thus producing novel hypotheses to be tested in the wet lab. First efforts of dynamical modelling of the selected ethylene model sub-component were already performed, indicating the importance of protein degradation efficiency to obtain biologically relevant representations of network responsiveness.

Differential gene expression patterns in PVY^{NTN} infected potato tubers

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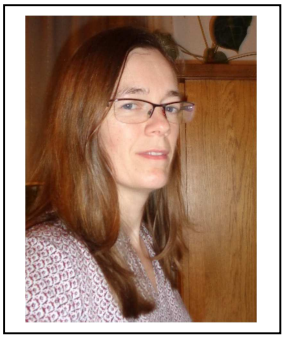
Yield and quality of tubers of susceptible potato cultivars are severely affected by the infection with NTN isolates of *Potato virus Y* (PVY^{NTN}). The frequencies of necrosis on tubers may increase during storage. Virus movement within plant and formation of necrosis on tubers are influenced by the susceptibility of the host plant, virus isolate and/or abiotic factors however the exact mechanisms are not known. The aim of our study was to analyse gene expression patterns characteristic for PVY^{NTN} infected tubers during storage and to evaluate the PVY-host interactions in meta-analysis.
Study was performed on potato tubers of PVY^{NTN} susceptible cv. Igor stored at different storage conditions (1). Gene expression analysis was performed using potato genome 44K POCI microarrays. Differentially expressed genes were determined using R package Bioconductor Limma. Over-represented metabolic pathways were determined using tools MapMan and GSEA (2). Microarray expression data was validated using RT-qPCR. Comparison of infected versus non-infected tubers under different storage conditions revealed differential expression of genes involved in host-virus interaction, including genes associated with biotic stress, pathogenesis-related proteins (proteinase inhibitors), secondary metabolism of phenylpropanoids (lignin biosynthesis) and several signalling, regulatory and defensive (kinase activity regulation, cell wall degradation) functions. The highest quantity of DE genes was found in necrotic tissue. Meta-analysis was used to elucidate organ specific (tubers, leaves) and genotype specific (susceptible, resistant) patterns of gene expression during the PVY^{NTN} infection. Preliminary results of meta-analysis hint on differential patterns of expression responses in tubers and leaves, as well as in pathosystems with different susceptibility. The study is the first comprehensive transcriptomic study analysing gene expression of PVY infected tubers during storage and the results were evaluated together with the results of other studies in meta-analysis.
The study was supported by Slovenian Research Agency (L4-2400-0401), the FP7 Project CropSustaIn (FP7-REGPOT-CT2012-316205) and The World Federation of Scientists (National Scholarship).

(1) Dolničar P, Mavrič Pleško I, Meglič V (2011) Long-Term Cold Storage Suppress the Development of Tuber Necrosis Caused by PVY^{NTN}. Am J Potato Res 88:318-323 (2) Ramšak Ž, Baebler Š, Rotter A, Korbar M, Mozetič I, Usadel B et al (2014) GoMapMan: integration, consolidation and visualization of plant gene annotations within the MapMan ontology. Nucleic Acids Res, 42:D1167:D1175.



Primary metabolism, phenylpropanoids and antioxidant pathways are regulated in potato as a response to *Potato virus Y* infection

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Potato (*Solanum tuberosum*) production is one of the most important agricultural sectors, and it is challenged by various detrimental factors, including virus infections, e.g. *Potato virus Y* (PVY). To control losses in potato production, knowledge about the virus–plant interactions is crucial. Response of potato plants to PVY infection has already been studied on morphological, biochemical and transcriptom levels [Pompe-Novak et al., 2006, Physiological and Molecular Plant Pathology; Baebler et al., 2009, Molecular Plant Pathology; Kogovšek et al., 2010, Plant Pathology; Baebler et al., 2011, Plos One].

To upgrade and deepen the understanding of the potato-PVY interaction, the potato metabolome was recently analysed and linked with changes in the transcriptome in potato leaves inoculated with the mild PVY^{N} and aggressive PVY^{NTN} isolates at different times through disease development [Kogovšek et al., 2016, Plos One.] At the beginning of infection (1^{st} day post-inoculation), virus-infected plants showed an initial decrease in the concentrations of metabolites connected to primary metabolism (sugars, amino-acids, TCA cycle and GABA shunt intermediates), ROS scavangers and phenylpropanoids, relative to the mock-inoculated control plants. A pronounced increase in those metabolites was detected at the start of the strong viral multiplication in infected leaves (6^{th} day post-inoculation). The alterations in these metabolic pathways were also seen at the transcriptome level, as analysed by quantitative PCR.

More rapid onset of accumulation of ROS scavengers was observed in leaves inoculated with PVY^{N} than in those inoculated with PVY^{NTN}. This appears to be related to the lower damage observed for leaves of potato infected with the milder PVY^{N} strain, and at least partially explains the differences between the phenotypes observed.

Host miRNA response in leaves of PVY infected tobacco plants

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Plant microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that regulate gene expression by sequence-specific cleavage or translational repression of target transcripts (Bartel 2004). They play important roles in developmental processes and in plant response to abiotic and biotic stresses (Jones-Rhoades et al 2006, Chen 2010, Khraiwesh et al 2012). Alteration of host miRNAs in virus-infected plants has been reviewed (Yin et al. 2014[1]).

In this work, the expression levels of a set of miRNAs in PVY-infected tobacco were studied by stem-loop real-time RT-qPCR. Two recombinant PVY^{NTN} isolates were used: 12-94 (AJ889866) causing veinal necrosis (VN) in tobacco, and Gr99 (AJ890343) which contains R-400 and D-419 in the HC-Pro cistron unable to induce VN in tobacco. For each isolate, five plants (4-5 leaf stage) of *Nicotiana tabacum* cv. Samsun were mechanically inoculated with the infectious leaf sap. Five mock-inoculated plants were used as controls. Samples from the upper non-inoculated leaves were taken at 3- and 14-days post inoculation (dpi) and stored at -80 °C for RNA extraction. All the plants were kept in a growth chamber under controlled conditions (22 °C, photoperiod 16 h light). Real-time qPCR was performed in LightCycler480 instrument with SYBR method. The relative expression value for each miRNA was normalized to the best reference gene selected based on analysis of 13 tobacco candidate genes.

Of the fourteen tested miRNAs, no changes in their expression levels were observed at 3 dpi. At 14 dpi, nta-miR168a together with its target transcript *AGO1-1*, were highly induced by PVY irrespective of isolates. The levels of six miRNAs were not altered in plants inoculated by 12-94 and Gr99. However, the expression levels of the remaining seven miRNAs were increased 1.5- to 31.5-fold only in the plants infected with 12-94 showing VN. The levels of the same set of miRNAs remained unchanged in Gr99 infected plants which did not exhibit VN. Our results indicated that these seven miRNAs might specifically relate to the VN in tobacco infected with PVY^{NTN} isolate 12-94.

[1]Yin Z, Chrzanowska M, Michalak K, Zimnoch-Guzowska E (2014) Alteration of host-encoded miRNAs in virus-infected plants - experimentally verified. In: Guar RK, Hohn T, Sharma P, eds. *Plant Virus-Host* ...er, Chapter 2, pp17-55.

Identification and characterization of small RNAs involved in tolerance response of potato (*Solanum tuberosum* L.) to *Potato virus Y* (PVY^{NTN})

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Potato (*Solanum tuberosum* L.) is the world's most widely grown tuber crop and the fourth most important food crop, following maize, rice and wheat. Viruses pose a serious agronomic problems, not only because of effects caused by primary infection, but also because the crop is propagated vegetatively so that they are transmitted through the tubers. One of the most widely grown potato cultivars is cultivar Désirée, which is tolerant to infection with PVY^{NTN} allowing replication and systemic spread of the virus, however, expression of disease is reduced and tubers of those plants are not affected. Up to now, studies on Désirée-PVY^{NTN} interaction have focused on the detection of changes in plant transcriptome and proteome, especially related to plant hormonal signaling. Of the hormones, salicylic acid (SA) was found to be the crucial component for inhibition of the multiplication and spread of PVY^{NTN} (Baebler et al., 2011). Recent findings reveal that small RNAs, broadly divided into microRNAs (miRNAs) and small interferning RNAs (siRNA) are important regulators of gene expression and could play crucial roles in defence responses.
Since tolerant response in potato to PVY^{NTN} is not fully understood, we aimed to investigate the role of small RNAs and in parallel evaluate the role of SA in the response using tolerant Désirée and SA deficient NahG-Désirée plants. Small RNASeq analysis enabled us to identify and define the expression profiles of miRNAs. 68 unique known miRNAs were found to be responsive to PVY^{NTN} infection, of which 22 were up-regulated in both genotypes, while the remaining 46 showed contrasting expression pattern between potato genotypes. In addition, almost 200 novel potato miRNAs were identified. Since miRNAs are able to initiate regulatory cascade that involve secondary small interfering phasiRNA, we also searched for phasiRNA loci and identified several PHAS genes, out of which some were shown to be targeted by differentially expressed miRNAs or even with phasiRNAs which reinforce the response. To understand the functions of PVY responsive small RNAs, target transcript were predicted using *in silico* plant target prediction tool and confirmed with degradome sequencing. PVY-responsive small RNAs were found to target several important transcripts involved in regulation of photosynthesis, hormonal signaling, stress response, RNA regulation of transcription etc. Taken together, our findings show the importance of small RNA gene regulation in potato and their contribution to tolerance response to PVY^{NTN}.

2011. PLoS ONE 6, e29009.

Enhancing understanding of potato – virus interactions using GoMapMan

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Understanding the biology of potato-virus interactions using systems biology tools can contribute to precise breeding and development of efficient agricultural practices. However, the approach is hindered by lacking or dispersed pathosystem-specific experimental data, functional annotations and visualization tools.

Recently we have developed GoMapMan (http://www.gomapman.org), an open web-accessible resource for gene functional annotations in the plant sciences. It is based on the MapMan ontology, organized in the form of a hierarchical tree of biological concepts, which describe gene functions.

The main features of GoMapMan are 1) dynamic and interactive gene product annotation through various curation options, 2) consolidation of gene annotations for different plant species through the integration of orthologue group information, 3) traceability of gene ontology changes and annotations, 4) integration of external knowledge about genes from different public resources, and 5) providing gathered information to high-throughput analysis tools via dynamically generated export files. Currently, genes of the model species Arabidopsis and six crop species (potato, tomato, tobacco, cacao tree, beet and rice) are included.

Using GoMapMan, the knowledge on potato-PVY interaction was improved by translating knowledge from the model and other species to potato and by easier interpretation of our experimental data on potato-PVY interaction.

Reference: Ramšak, Ž., Baebler, Š., Rotter, A., Korbar, M., Mozetič, I., Usadel, B., and Gruden, K. 2014. GoMapMan: integration, consolidation and visualization of plant gene annotations within the MapMan ontology. Nucleic Acids Res. 42:D1167–75

Novel DNA locus associated with PVY resistance in potato is mapped to chromosome V

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Earlier we produced diploid hybrids by means of pollination of allotetraploid species *Solanum stoloniferum* by the pollen of male fertile *S. tuberosum* diploid line and selected among them the genotypes with extreme resistance to PVY [Voronkova et al., 2007]. DNA analysis of the hybrids did not reveal the markers of known R-genes (*Rysto* or *Ryf-sto*) mapped to chromosome XII. The aim of the work was to study the genetic control of the PVY resistance introgressed from *S. stoloniferum.* Two hybrid populations segregating for extreme resistance to PVY^{O} or PVY^{N} have been produced by crossing resistant and susceptible interspecific hybrids. The level of resistance of hybrids was determined in their tuber generation by means of ELISA after inoculation of leaves by the sap of infected plants (by PVY^{O} or PVY^{N} strains). Segregation for resistant to susceptible hybrids in both populations corresponded to 1:1.

RAPD PCR with the primer OPA18 has shown that there was polymorphism in 600 bp DNA locus between resistant and susceptible hybrids of the population IGC 08/13.n (infected by strain PVY^{O}). Availability of this locus (OPA18-600) in hybrids correlated with PVY resistance (r_s = 0.68**). The sequence of OPA18-600 and analysis of the results with FASTA and BLAST, as well as the analysis of its recombination with some SSR and TG markers made it possible to map it to chromosome V in the region of 52 cM between TG60 and TG185 (corresponding coefficients of recombination (rf) were 0.35 and 0.0). High level of homology was revealed of 3'region of sequenced part of OPA18-600 (as large as 200 bp, 30-40%) and nucleotide sequences of copper chaperon (CCH or ATX1-proteins of copper transport) of some *Solanum* species (homology 91 - 93%). As well as its entire identity with the sequence of V type peroxidase (NCBI XM 006345938.1) has been shown. All this indicates that sequenced locus is connected with genes of plant defense against different stresses. The sequenced area occupied the part of the locus associated with PVY resistance that was closer to SSR locus STM1031 than to TG185 (rf =0.15 and 0.60, correspondingly). Thus, SSR marker STM1031 273+275 (binary peak) can be used for detection in breeding material of the novel PVY resistance gene introgressed from *S. stoloniferum.* This marker was available exclusively in resistant genotypes.

Engineering of CRISPR/Cas9-mediated virus resistance in transgene-free *Arabidopsis* plants

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A recently developed technology, commonly referred to as CRISPR, has opened up the possibility to edit the genomes of many model and crop plants with high precision and efficiency. To date, there is a paucity of studies which utilise this technology to create plants with improved vigour/marketability. Hence, the mechanisms of CRISPR technology in plants has been widely characterised but its usefulness for agricultural applications is yet to be fully explored. In this research we demonstrate an exciting application of CRISPR technology by editing the genome of the model plant *Arabidopsis thaliana* to create a stable, heritable resistance to certain *Potyviruses*. Importantly, the resistant plants with edited genomes do not carry any transgenes and, as such, should not be restricted by current legislation for transgenic GMOs (genetically modified organisms). We hope that this path-finding research will lead the way for creating virus resistance in important crops.

GenoCAD plant grammar, a Computer-Aided Design software tool for plant expression vectors

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Plant biologists rely on increasingly complex DNA constructs not only for basic research, for functional analysis of genes, but also in applied research to improve crops or express proteins of interest in various plant species. However, the design of complex multigene vectors is still a big challenge. Therefore, there is a need for software tools that guide plant biologists through the design of application-specific expression vectors.

Here a vector design strategy to express genes in plants is formalized and implemented as a grammar in GenoCAD, a Computer-Aided Design software for synthetic biology. It includes a library of plant biological parts organized in structural categories and a set of rules describing how to assemble these parts into large constructs. Rules developed here are organized and divided into three main subsections according to the aim of the final construct: protein localization studies, promoter analysis and protein-protein interaction experiments. The GenoCAD plant grammar guides the user through the design while allowing users to customize vectors according to their needs. Therefore, the plant grammar implemented in GenoCAD will help plant biologists take advantage of methods from [illegible] o design expression vectors supporting their research projects.

Plasma treatment of PVY^{NTN} infected potato plants and plant extracts

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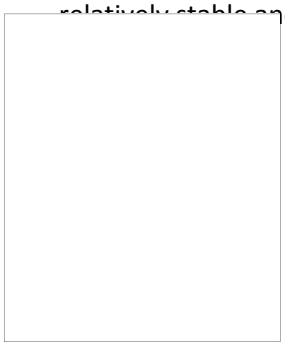
Cultivated potato (*Solanum tuberosum* L.) is, after rice, maize and wheat, the world's fourth most important food crop. Its susceptibility to wide range of pathogens, which diminish its yield, could therefore have a great impact in the food production chain. One of the most important potato pathogens is the *Potato virus Y* (PVY). The necrotic isolates of PVY are still responsible for huge agronomic and economic losses, where PVY^{NTN} is the most devastating.

The aim of our work was to test, whether low pressure gaseous plasma treatment can be used to eliminate PVY from the infected material and to check, if plasma treatment can destroy the virus particles or reduce the infectivity of PVY present in plant extracts. The elimination of PVY^{NTN} from infected plants was performed on plant nodes in tissue culture, using two different cultivars, Igor and Pentland. The infected plant extracts were prepared from infected plantlets of potato cv. Pentland. Test subjects (nodes or plant extracts) were treated in inductively coupled plasma reactor with oxygen plasma in E-mode. Two different plasma powers and two different oxygen flow settings and pressure that were not devastating for the plant material (nodes) were used. Treated explants were grown in tissue cultures and 4 weeks after treatment the amount of virus in plants was quantified on RNA level using one-step real-time PCR. The effect of plasma on PVY^{NTN} and ^{N}-GFP was tested by exposing dry plant extract to the same plasma treatment as for the plant explants. Treated plant extracts were used for inoculation of tobacco plants, which were checked after two weeks for virus presence and quantity.

We have shown that this type of plasma treatment of infected plant explants had no significant effect on virus quantity, most probably due to the fact that plasma treatment affects the surface of the object, but virus is present throughout the plant tissues. Plasma treatment of infected plant extracts did not significantly reduce the infectivity, however it has shown that the virus particles are relatively stable and remain infective despite drying and exposing them to plasma treatment.



The development of tuber necrosis caused by PVY^{NTN} on tubers of different cultivars during long-term cold storage

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The aim of our work was to study the development of tuber necrosis caused by *Potato virus Y*^{NTN} (PVY^{NTN}) during long term cold storage of different potato genotypes. It was shown previously that low storage temperatures (4°C) in the first months after harvest delayed or prevented the development of necrosis in susceptible potato cultivar Igor. In order to test if other susceptible potato genotypes react with similar response, susceptible potato cultivars Igor, Nicola, Hermes and Donald were selected for the experiment. *In vitro* plantlets of selected cultivars infected with Slovenian isolate of PVY^{NTN} (PVY-55/1) and virus-free *in vitro* plantlets were planted in screen-house in 2012. All tubers were visually inspected for the presence of necrosis immediately after harvest in July 2012. Only tubers without necrosis were used in the storage experiment. At harvest time cultivars Hermes and Donald yielded necrosis on majority of the tubers. Therefore only infected and non-infected tubers of Igor and Nicola were used in storage experiment. Tubers were randomly distributed into 7 storage regimes. Control treatment was stored at constant temperature of 4°C. Tubers in other six treatments were stored at 4°C at the beginning of experiment and transferred to 24°C after 1, 2, 5, 10, 21 and 25 weeks. High storage temperatures enhanced the development of tuber necrosis in both cultivars. Prolongation of low temperature storage delayed the development of necrosis and reduced the number of tubers with necrosis in both cultivars. Igor yielded the highest number of tubers with necrosis in the first four treatments. When tubers were stored at low temperature for 21 weeks or more, no necrosis developed after transfer to high temperature in Igor and only one in Nicola. In both other cultivars, Hermes and Donald, necrosis on tubers developed already before harvesting indicating that they should be harvested earlier in order to study their response to long-term cold storage.

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Interaction of StubGAL83 with *Potato virus Y* in the virus sensitive cultivar Désirée

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Potyvirus is the largest genus of plant viruses that cause significant losses in a wide range of crops. Potyviruses are transmitted through aphids, and the helper component proteinase (HC-Pro) of the virus is required for transmission. However, HC-Pro is a multifunctional protein that has self-interactions and interactions with other potyviral and host-plant proteins. We found that HC-Pro of the Potato virus Y O strain (PVY^O) interacts with the StubSNF1 protein kinase complex regulatory subunit StubGAL83 in a yeast two-hybrid assay. StubSNF1 is an SnRK1 family member of plant proteins that regulate carbon metabolism and responses to biotic and abiotic stresses. To determine which amino acids are required for the interaction, we constructed a series of deletion derivatives of both proteins and tested them by yeast two-hybrid assay. This experiment localised the binding site to the 149-253 bp region encoding the KITCPTCAQQYANLPASDLLKILHKHASDGLN motif on the N terminus of HC-Pro, which has been implicated in aphid transmission. When we tested the StubGAL83 deletion derivatives, we detected strong interactions with the fragments encoded by 460-603 bps and 460-561 bps, suggesting that the FIVDGEVRYIPELPCVADETGVVFNLLDVNDNVP motif is a binding domain in StubGAL83 for HC-Pro. This region largely overlaps the C-terminal end of the SNF1 binding KIS (kinase association) domain of GAL83. To understand the functional importance of HC-Pro-StubGAL83 interaction the multiplication of PVY^O was tested in StubGAL83-knockdown plants and found that StubGAL83-repressed plants are less susceptible to virus infection than wild-type plants. Nevertheless, it was also shown that PVY^O infection reduces StubGAL83 mRNA expression. StubGAL83 is a positive regulator of SNF1 kinase that phosphorylates and inactivates at least four important metabolic plant enzymes: 3-hydroxy-3-methylglutaryl-CoA reductase, sucrose phosphate synthase, nitrate reductase, trehalose phosphate synthase, and pyruvate kinase. Binding of HC-Pro to StubGAL83 may reduce SNF1 kinase activity that in turn affects metabolism and leads to decreased StubGAL83 expression.

Prediction of MYC2 transcriptional regulatory network in potato – *Potato virus Y* interaction signalling

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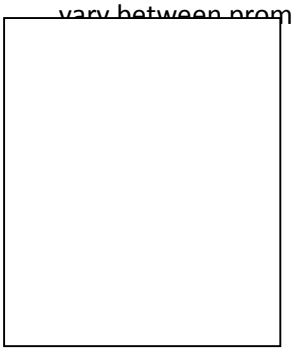
Plant hormones are crucial signalling molecules that coordinate all aspects of plant growth, development, reproduction and defence. Three hormones are especially important for plant immune response. The SA, JA and ET signalling pathways represent the backbone of the defence signalling network, with other hormonal signalling pathways feeding into it. The importance of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) as dominant primary signals in local and systemic induced defence signalling has been well documented. However, the way these signal molecules function in a complex network of interacting pathways is less well understood and the majority of research has been done on model plant species and very little on potato. Our aim is to upgrade our current understanding of the roles of SA, JA and ET in the plant's immune system and crosstalk between defence hormone signalling pathways, with a focus on promoter analysis of chosen genes from defence signalling network.

In order to understand transcriptional network of signalling components in potato following PVY infection we decided to analyse the promoters of several genes that are crucial components in plant defence signalling pathways. One of them is transcription factor MYC2 from jasmonic acid pathway.

Promoters obtained from different potato cultivars were sequenced, compared to the available model genome sequence and analysed with TRANSFAC and PlantCARE. The results showed that promoter sequences of the same gene differ between cultivars. Furthermore, each gene can have different promoter sequences within the same cultivar. Using TRANSFAC and PlantCARE we characterized transcription factor binding sites and established how transcription factor binding sites vary between promoters of the same gene within each cultivar.



Effect of Root Zone Calcium on PVY Infected Potato Plantlets

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The important role of calcium on plant growth and development including cell division and cell elongation is well documented. Plant under biotic and abiotic stress conditions prefers to have sufficient calcium in root zone to prevent damage to cells. The cytosolic calcium concentration is influenced by extra or intra cellular signals such as pathogens. Stimulation of cytosolic calcium activates immune system of the plant by related gene(s). The purpose of the present study was to determine the impact of root zone calcium on the growth and health of PVY infected potato plantlets. For this purpose 4 week of second and third nodes of micropropagated *Potato virus Y* (PVY) infected potato (*Solanum tuberosum* L. cv Innovator) plantlets were grown in sterilized MS media with varying amount of calcium (using $CaCl_2$). The media calcium concentration range was from 0.2 to 440 ppm. After 2-4 weeks old plantlets will be harvested to collect data (number, height ect. of shoot). PVY accumulation will be determined by quantitative real-time RT-PCR analysis. Stem of the plantlets will be cut into pieces to ash at 450^{o}C about 6 hours for determination of calcium concentration.

Key words: Solanum tuberosum , PVY, cytosolic calcium, MS media

Transcriptome-wide mining of potato genes targeted by Potato virus Y-derived viral small interfering RNAs

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Potato virus Y (PVY) is an economically important pathogen of potato. In the US, PVY-O, PVY-N, and PVY-NTN have been endemic and PVY-N:Wi has become more prevalent in the Pacific Northwest. The existence of biologically distinct strains that differ in the symptoms they cause in the same host complicates disease management efforts. The molecular mechanisms governing the differential behavior of potato cultivars to different PVY strains are largely unknown. We previously characterized virus-specific, small interfering (vsi) RNAs for PVY-N, PVY-NTN and PVY-O infecting potato cv. Russet Burbank. The vsiRNAs of the three strains exhibited diversity in absolute number, copy number and hotspots for siRNA. We mined, in silico, the potato genome using strain-specific vsiRNAs. The vsiRNAs exhibited propensity to target host (potato) transcripts resulting in a silencing effect. Differential behavior in targeting host transcriptome was observed and this could partly account for the differential phenotype of PVY strains in potato. Selected in silico vsiRNA-targeted transcripts were validated by RT-qPCR. Further characterization of targeted host transcripts revealed the tendency of the PVY strains to target common set of pathways involved in plant hormone signaling, genetic information processing and plant-pathogen interactions. This suggests a similar mode of defense for vsiRNA-based counter defense among the PVY strains.

Better understanding of potato-PVY interaction using shotgun proteomics

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We are studding potato-PVY (potato virus Y) interaction using systems biology approaches and one of them is label-free shotgun proteomics.
Proteins were extracted with TriZol and digested with Lys-C and Trypsin. Peptides were subjected to LC MS/MS analysis using OrbiTrap mass spectrometer (University of Vienna). Relative quantification of peptides was determined by spectral counting. Proteins were identified by searching the database combining all known potato genes (prepared on National Institute of Biology (Ljubljana) and Jožef Stefan Institute (Ljubljana)).
We investigated differences in potato proteome 4 days after infection with virus PVY on cultivar Desiree using non-transformed Desiree and two different transformants (defective in salicylic acid and jasmonic acid signaling pathway). Approx. 300 proteins were identified. Differentially expressed proteins were mostly included in photosynthesis.
Some of proteins were found interesting on transcriptional level but not identified with shot gun proteomics. To observe some of those proteins we have in plan to use another approach like MRM (multiple reaction monitoring) technique and detection with antibodies.

Analysis of potato (*Solanum tuberosum* L.) response to *Potato virus Y* with systems biology approach identifies novel regulators of plant defence

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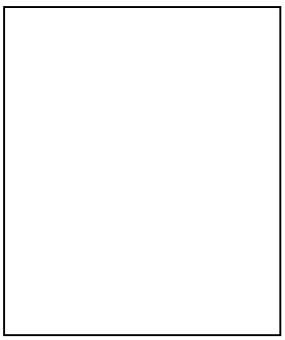
Potato is the world's most widely grown tuber crop. Potato virus Y (PVY) is a major pathogen that causes substantial economic losses in worldwide potato production. The interaction between plant and its pathogen initiates a complex signalling network, resulting in massive changes of the gene activity and extensive reprogramming of the cell metabolism. In this study systems biology approach was used to model complex biological processes and understand mechanisms and dynamic involved in plant defence to viral infection.

We constructed signalling network topology describing the biosynthesis, hormone recognition and signal transduction leading to activation of effector molecules of crucial phytohormones involved in plant defence: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The primary model has been expanded with model with Arabidopsis (*Arabidopsis thaliana*) protein interactors and translated to potato. In parallel with model construction, analysis of dynamic potato response to PVY was evaluated. Dynamics of whole transcriptome changes of cultivar 'Désirée' and NahG-Désirée were analysed in inoculated and systemically infected leaves following 0, 1, 3, 4, 5, 7, 8, 9, and 11 days after infection (dpi). Potato proteome changes on set of viral multiplication were analysed and compared to transcriptional dynamics. Dynamics of physiological changes were evaluated on the level of symptoms development, measuring virus accumulation and spread to uninfected tissue and callose accumulation.

Integration of both, modelling with novel biological data enabled us to identify novel regulators of plant defence against viral pathogens. The role of two kinases and two phosphatases has been evaluated with functional genomics. The results imply that novel players of virus induced-potato response gap the bridge between regulation of abiotic and biotic plant response signalling.



Genetic Diversity of PVY isolates in Potato Crops: Impact of Strain Competition and Ability to Overcome Host Resistance Mechanisms

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Potato virus Y (PVY) is the most important viral pathogen affecting potato crops worldwide. PVY is transmitted non-persistently by non-colonising aphids, resulting in a rapid acquisition and transmission of the virus between plants. PVY exists as a complex of strains that can be distinguished according to their pathogenicity, serology and genome analysis. While virus incidence is low in Scottish seed potato crops, PVY has become the most prevalent virus. A drift in the PVY population structure from PVY_O, PVY_C to PVY_N to recombinant PVY_{NTN} strains (N-Tuber Necrosis) that can cause Potato Tuber Necrotic Ringspot Disease (PTNRD) is currently being observed worldwide. We studied the molecular nature and epidemiology of PVY_N isolates to study which genetic and/or environmental factors are driving their prevalence. A survey of the biological and molecular diversity of PVY field isolates indicated that while a wide range of variants can be identified, the vast majority belong predominantly to the European (EU)-NTN ($PVY_{EU\text{-}NTN}$) molecular group and have the ability to overcome *Nc*, *Ny* and *Nz* PVY resistance genes in potato. Field trials transmission studies were undertaken using plants infected with each of the three main molecular groups identified in seed potato crops (PVY_O, $PVY_{EU\text{-}NTN}$, $PVY_{NA\text{-}NTN}$). Bait plants were monitored on a weekly basis to assess aphid transmission rate and at post-harvest to assess PVY incidence in tuber progeny over a 3 year period. The results showed that $PVY_{EU\text{-}NTN}$ was more efficiently transmitted to the bait plants than $PVY_{NA\text{-}NTN}$ and PVY_O. In addition, post-harvest assessment of PVY incidence in tubers showed a higher proportion potato plants infected with $PVY_{EU\text{-}NTN}$. Furthermore, PVY incidence in plants infected at different times after emergence, suggest that $PVY_{EU\text{-}NTN}$ has the ability to infect older plants in comparison to PVY_O. Altogether our results suggest that $PVY_{EU\text{-}NTN}$ out-compete other PVY strains and overcome mature plant resistance mechanisms, potentially explaining $PVY_{EU\text{-}NTN}$ prevalence in field conditions.

Recombinant Strains of *Potato Virus Y* Overcome mature plant resistance in *Solanum tuberosum* L.

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BACKGROUND AND OBJECTIVES

Potato virus Y (PVY) is vectored in a non-persistent manner by aphids and vector control measures such as the application of insecticides have limited effect on virus spread. Moreover, different strains of PVY (including ordinary, necrotic and recombinants such as NTN and N-Wi) are increasing in incidence, making PVY the most economically important virus in many potato production systems worldwide. PVY can be controlled by natural resistance genes but these are lacking in many commercial cultivars. Mature plant resistance (MPR) is a poorly understood phenomenon where plants become more resistant to infection as they age. MPR could be a useful component for virus control, however, a better understanding of the mechanism MPR is needed to allow it to be effectively incorporated in epidemiological models for integrated pest management.

MATERIALS AND METHODS

Infection of PVY strains (PVY^{O}, PVY^{N}, PVY^{NTN} and $PVY^{N\text{-}Wi}$) was studied in four cultivars of potato: Maris Piper, Atlantic, Desiree and Shepody, at four stages of development (6 leaf, stolon, tuber bulking and flowering). PVY movement into phloem and solute transport from source leaves to sink tissues were monitored using a GFP-tagged PVY clone and a phloem mobile fluorescent probe carboxyfluoresceine diacetate (CFDA).

RESULTS

PVY infection of non-inoculated leaf tissues was significantly affected by PVY strain, host genotype and plant growth stage. MPR was effective against PVY^{O} and resistance in all cvs increased with developmental stage. So that when inoculated at flowering, PVY^{O} was not detected in the upper non-inoculated leaves and did not spread to progeny tubers. MPR was less apparent in cvs inoculated with the other strains. Foliar infection decreased markedly at the flowering stage for all cultivars except Shepody (which did not display MPR) and $PVY^{N\text{-}Wi}$ and PVY^{N} were not readily detectable in foliar tissue of most plants, however, PVY^{NTN} was detected in upper leaves of approx. 25% plants. Despite decreased foliar infections, PVY^{NTN} and $PVY^{N\text{-}Wi}$ were detected in all progeny tubers of plants inoculated at the flowering stage. CFDA translocation studies showed unloading was not detected in developing leaves at the top of the stem and there was a reduction in solute unloading in tubers at flowering stage. However, GFP-tagged PVY^{N} was detected in phloem tissue of inoculated leaves at all

growth stages. Therefore, PVY is not prevented from entering the phloem in inoculated leaves during MPR.

CONCLUSIONS

These results show that MPR can be a useful measure to control PVY^O and PVY^N in some cvs but recombinant PVY strains, despite decreased foliar infection, were able to infect progeny tubers at all developmental stages. Studies are ongoing to investigate the mechanism of resistance and understand the difference in pathogenicity of recombinant virus strains but change in solute transport and inhibition of phloem entry does not appear to be associated with MPR.

Transmission of *Potato virus Y* and *Potato virus A* in the UK

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The aphid transmitted viruses *Potato virus Y* (PVY) and *Potato virus A* (PVA) are the viruses most commonly detected in UK seed potatoes crops. The transmission efficiency for each aphid species is used to calculate a potential transmission risk and is expressed as a Relative Efficiency Factor (REF). These REF's have not previously been calculated for UK strains of viruses or aphid clones. Using a previously published method REF's have been calculated for the aphid species and viruses commonly occurring in UK potatoes. The efficiency of transmission of *Myzus persicae* is nominally set to a REF of 1 and REFs for other species are calculated relative to that figure. These data represent the first set of REF's calculated for PVA transmission, including new PVA vector records for five aphid species. We further analysed the data to compare transmission rates of PVY and PVA using a binomial (logit) generalised mixed model to take into account the potential influence of variation in virus titre between leaves. This approach resulted in the finding that there is little variation between the efficiency of transmission between clones of each aphid species or between strains within a virus species.

The influence of aphids flights on the transmission of the potato virus Y (PVY) in the field

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PVY is transmitted in a non-persistent manner by various aphid species. The efficiency of the transmission of the virus is generally expressed as a relative efficiency factor (REF) using the green peach aphid (*Myzus persicae*) as a reference. *Myzus persicae* is considered as the most efficient PVY vector. These REFs are calculated using data of transmission trials performed in the laboratory in which individuals carrying the virus are put into contact with a healthy plant known as a host plant of the virus. A recent study relating the flight activities of aphid species in Switzerland to the prevalence of PVY in the national seed potato production showed that the abundance of *Myzus persicae* was not correlated with the incidence of the virus. That study also showed that the counts of leaf curl plum aphid (*Brachycaudus helichrysi*) was a good predictor variable for PVY prevalence. It is surprising to observe that the aphid species with the highest REF has no or little influence on the spread of PVY in the field. To clarify this further, a 14 years field trial was setup in Switzerland to study the influence of aphid flights on PVY spread. Tubers of the susceptible cultivar Bintje were planted in two locations 200 km apart from each other. Aphids were captured with a 'Rothamsted suction-trap' during the entire growing season in both locations and the species were identified. Each year, 100 potato tubers were analysed by ELISA to determine PVY incidence. A simple linear regression analysis was used to assess the relationship between the total captures of each aphid specie the first 7 weeks after emergence with the percentage of PVY infected tubers after harvest. Our results revealed that the capture data of *Brachycaudus helichrysi* as well as the capture data of aphids of the genus *Aphis* were the best predictors of PVY incidence in the field. In contrast, the captures of *Myzus persicae* revealed to be a poor predictor of the PVY incidence in the field, since the coefficient of determination was lower than the mean of the other species. These results are consistent with previous findings and highlight the limits of PVY transmission trials performed in the laboratory to predict the risk of PVY spread in the field. REF values need to be complemented with data of vector behaviour in the field to get a more complete understanding of the relative importance of different aphid species as vectors

Fighting strategies against PVY dissemination in a potato seed multiplication field: comparison of several methods using insecticides (systemic and pyrethroïds) and paraffinic mineral oils.

ROLOT Jean-Louis

Walloon Agricultural Research Centre, Life Sciences Department, Breeding and Biodiversity Unit, Bâtiment Haute Belgique, B-6800 Libramont

6 protection schemes were tested in a trial conducted in 2015 in Libramont in order to advise the potato seed growers association in Wallonia (Belgium). The measured variables were: aphids populations activity by counting apterous and alate aphids on leaves and by trapping alate aphids in a yellow water pan trap, PVY spread in the foliage, PVY presence in tuber samples taken after foliage destruction. Efficiency of each method compared to a non-treated control will be discussed.

Minimizing of sources and vectors of virus infections on seed potatoes in the Central region of Russia

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On the base of knowledge of the means and characteristics of transmission and distribution of viral pathogens as well as migration of their vectors, in Russia recommended to maintain initial stock and subsequent field generations of seed potato at a distance of 500 m and 100 m, respectively, from potential sources of infestation. For establishing specially designated seed potato production areas, certification of agricultural lands is of great importance. This certification implies detection surveys for plant pests, diseases and weeds and subsequent issuance of field phytosanitary passports, which are one of the requirements for certification of companies and individuals producing (growing), processing, packing and marketing seed potatoes of the higher grades.

The guidelines for designating virus-free seed potato production areas are followed by the Korenevo Agricultural Center LLC follows. Its farmlands are located in the center of Meshchera Lowlands and are surrounded by naturally occurring forest stands. Moreover, micro-isolation is additionally used, i.e. winter wheat is grown along the edges of the seed potato field and along the access passages across the field.

The Korenevo Agricultural Center LLC uses in vitro material for further production of seed potatoes of high quality. Microplants of 20-25 potato varieties tested and found free from viruses, viroids and bacteria using immunoassay and PCR are propagated during winter and spring by cuttings until the necessary amount is obtained on the agar (solid) substrate in accordance with the conventional technique.

In protected cultivation of minitubers (cold frames of various types), phytosanitary requirements and agricultural practices to exclude new pathogens are strictly followed.

When the first field generation is grown from minitubers and subsequent field generations are cultivated in pre-basic nurseries, seed potato crops are efficiently protected from new viral infestations. The ELISA results for tubers sampled at a nursery for super-elite seed potatoes provide strong evidence that utilizing mineral and plant-based oils in combination with ½ insecticide dosage is of prime importance. This mitigates the risk of new infestations of plants by plant pathogenic viruses vectored by migrating aphid species and limits the potential of viruses to infest the new crop; hence standard requirements are observed.

During the growing season, plants in field nurseries are visually examined three times and diseased plants and tubers are screened and removed from the field. The quality of the first field generation

and super-elite potato crops is determined by using ELISA. 200 tubers in every variety are sampled for postharvest testing for viral infections. The lot meets the standard requirements if the number of positive tubers in a sample doesn't exceed 5%; for PVX, PVS, PVM the tolerance is 4,5%, and for PVY – 0,5%.

Taking into account the current international practices, we have established a special phytosanitary and agricultural production regime in the designated pre-basic seed production area. This regime enables to observe physical isolation standards in the production of respective seed potato classes, to conduct surveys for major pests and their vectors and to supervise unauthorized use of uncertified seed potatoes by the local population.

Virus control in Ugandan seed potato tubers over multiple field generations by applying different seed technologies

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Potato (*Solanum tuberosum*) is an important crop for poverty reduction in rural south-western Uganda because it is both a source of food and a source of income. The national mean potato yield is about 7 t/ha, which is low compared with an estimated potential yield of 25 t/ha. The major yield constraining factor is the overall poor seed potato quality. In Uganda there is no formal seed certification scheme for potato, therefore most farmers obtain seed from the informal sector, in which seed-borne virus diseases accumulate over generations. Positive selection, pegging healthy looking plants during flowering to potentially serve as seed for the next season, is a tool to reverse degeneration thus sustainably improving the quality of seed potatoes. Positive selection, in comparison to farmers' selection where the seed is chosen from the bulk of the harvest, was shown to increase yield after one season, but has not been tested across multiple seasons. This study aims at improving agricultural productivity by evaluating and understanding improved seed potato production technologies and their feasibility at local scale. Therefore, field trials were carried out spanning four seasons (from 2013 until 2015) to provide insight in virus accumulation and yield formation. Effects of positive selection were tested using three varieties (Victoria, Katchpot1 and Rwangume) from a total of four sources (farm saved seed tubers, seed tubers from the local market, quality declared seed tubers from a seed grower, and 3G seed tubers from a research station) laid out as a split-plot design in three locations. After emergence, leaf samples were taken and virus incidence (PVS, PVM, PVX, PVY, PVA, PLRV) was detected through DAS-ELISA and Luminex xMAP technology. Observations in the field were made on tuber yield per plot, tuber numbers, ground cover per plot, stem number per plant, and bacterial wilt incidence per plot. The results showed yields were variable, ranging from 0.7% to 24.5% (+4.8% on average) across seasons when positive selection was applied compared with selection from bulk. A very high incidence of PVS and PVM was encountered from the beginning of the trials. PVX and PLRV accumulated over the seasons. PVY and PVA accumulated to a lesser extent. Positive selection lessened the virus incidence compared to farmers' selection. However, positive selection has limitations when a high virus pressure is present [illegible]heless, degeneration of potato seed tubers can be reduced or delayed over time.

Potato virus Y (PVY): Attempts toward a bio-immune monitoring system for potato and tobacco production of propagating material

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Work supported byCNPq-PDTEI Project No. 320276/2012-8.
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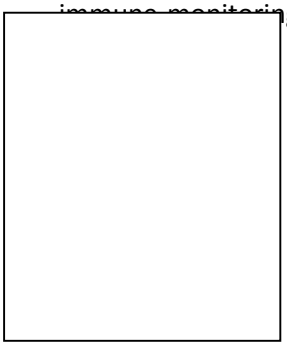
In Brazil, potato and tobacco are neighboring crops in most regions in the southern states and are infected with the same major viruses, such as PVY, with mutants and recombinants strains of PVY^O and/or PVY^N groups (Lacroix, C. et al. 2011, Plant Pathol, 60**:**1048–1054; de Ávila, AC et al. 2009. Hort. Bras. 27:490; Galvino-Costa et al. 2012**,** Plant Pathol 61:388–398**;** Sawasaki, HE et al. 2009. Potato Res 52:379-392). Primary symptoms of PVY^N strains infecting potato or tobacco may be not recognized or misidentified by rougers. Over the past 5 years, we have been evaluating a bio-immune monitoring system for use by small farmers to aid the early detection of PVY and virus management in seed-potato and tobacco crops.

Methodology For bio-monitoring, *D. metel* (*Dm*) and *D. stramonium* (*Ds*) are planted as virus-free sentinel plants (flowers removed throughout the season). These serve as a PVY indicator and discriminator respectively. *Dm* is highly susceptible/sensitive to PVY infection, taking usually 10-15 days to show distinct vein clearing and waving of top leaves (Hahn & Monroe, 1970. Phytopathology 60: 1183-5), while *Ds* is immune to most PVY strains. The sentinel plants (4-5 fully expanded leaves) are transplanted from the greenhouse to the crop/field borders, in 4–6 groups of 5-10 plants of each *Datura* spp. In potato, transplanting takes place at emergence and in tobacco at the seedling stage. Immune-monitoring is based on kits of ten (8-PVY tests and negative (ck) and positive controls) IC-PCR microtubes (mtb) of 200ul w/cap punched into styrofoam (5 x 2 x 3 cm). Mtbs are pre-coated with PVY IgG (SASA/UK or DSMZ/DE), filled with 50 ul buffer and sent to growers with plastic bags. Growers crush leaf samples in the plastic bag and put 2-3 drops of sap (20-30 ul) into a mtb. Kits are returned to the laboratory where sap+buffer is removed, mtb washed, conjugate added, incubated and washed, and finally substrate added. Mtbs are read as for an ELISA plate (visual+optical: PVY positive= A_{405nm}>2 x ck). Results are e-mailed within 1-2 days.

Results - Bio-monitoring - For *Dm* in potato and tobacco, visual symptoms of PVY indicated a potential risk of PVY infection to these crops. Similarly for *Ds*, visual symptoms of other viruses: PLRV, TSWV, ToCV and *Begomoviruses* (ToSRV, ToYVSV causing potato deforming yellow mosaic symptoms, Jeffries, C.J. 1998. Bulletin 19.FAO/IPGRI, 177p), confirmed by ELISA and/or PCR, indicated a potential risk of crop infection by these viruses. In tobacco, symptoms in *Ds* but not in *Dm* indicated TSV. Conversely lack of symptoms in *Dm* and *Ds* indicated no spread of these viruses, from outside the crops being monitored.

-**Immune-monitoring -** For 3 tobacco and 7 potato fields, PVY was ELISA-mtb detected in 75-100% of samples and confirmed PVY^{N} (chlorotic and vein necrotic symptoms) by mechanical transmission to *N. tabacum* cv Burley-21, or when daughter-tubers were grown on and leaves tested by ELISA or PCR.

Conclusion: Bio-monitoring assists the monitoring of virus spread into potato and tobacco crops and immune-monitoring using the prepared kits gives reliable results.



A process based epidemiological model for the *Potato yellow vein virus – Trialurodes vaporariorum* (greenhouse whitefly) - potato pathosystem

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Virus epidemics cause major crop losses throughout the world. Minimizing these losses in an ecological and economically sustainable way could make a major contribution to maintaining the world's food supply. Sustainable control methods would be particularly effective if supported by predictive epidemiological models that can efficiently and accurately forecast disease spread and enable taking appropriate decision about the pre-emptive deployment of different control measures. Whereas several different modelling approaches have been described for insect pests, specific models for predicting impacts of climate change on virus epidemics have to date not been developed.

Potato yields in Latin America are affected numerous viral pathogens, among which *Potato vein yellow virus* (PYVV) can reduce potato yields by 30% to 50%. PYVV is semi-persistently transmitted by the whitefly *Trialeurodes vaporariorum* (Westwood). It has sporadically caused problems on potato crops in Northern South America for over 60 years but has during the last 20 years started spreading from Venezuela and Colombia southwards along the Andes, where it has currently reached central Peru, while at the same time increasing in prevalence in Colombia. The reasons for its spread are not well understood, but are thought to be the result of increased see movement and climate change, causing a range expansion of its vector. An insect life cycle model based on phenology was previously developed for the insect vector *T. vaporariorum* which through Geographic Information System (GIS) could predict well the current distribution of the pest and was also able to make predictions regarding future areas at risk of pest expansion based on various climate scenarios. Controlled laboratory experiments for virus transmission under different temperatures showed a clear temperature dependant transmission rate of the virus by the vector with a narrow range for efficient transmission. Non-linear equations including stochastic functions were developed for temperature-dependent virus transmission by the insect vector and validated by transmissions under fluctuating natural temperatures in a screenhouse. The transmission function could be integrated into the existing life cycle model of *T. vaporariorum*, thereby generating a fully climate responsive model for virus spread and transmission. GIS maps produced by the model reflect very accurately the current occurrence of the virus but also predict new areas at high risk of invasion that could be targeted for [illegible] model could also be used with seasonal weather data to make recommendations [illegible] control to reduce crop losses.

Investigation on tuber transmission of phytoplasmas by symptomatic potato plants

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There are a few studies on tuber transmission of phytoplasma diseases and there is not any in Turkey. In 2014-2105, studies were conducted for presence of phytoplasma diseases in potato tubers in Hatay, Turkey. Leaf and shoot samples were collected from the suspected plants which were exhibited typical symptoms of yellowing, purple top, aerial tubers, smaller and upward-rolling of leaves. Tuber samples (18 tubers from symptomatic plants) were also taken during the harvest period (June 2015). These tubers were stored in dark at 4°C for 3 months and hair sprouts were observed on some infected tubers. Symptomatic and asymptomatic tubers were planted in pots filled with moist soil for obtaining potato plants in an insect-free screen-house. Some tubers did not support any shoot by the rate of 78%, germinated plants were very weak and died in a couple of weeks after planting (22%). Additional molecular studies have been in progress on samples from the symptomatic plants. Although the plants germinated from infected tubers can survive in a short time in the field, this period would be long enough to spread the phytoplasmas by insect vectors to other host plants in places like Hatay, Turkey where the field conditions are suitable. Use of healthy production material is also important in terms of the spread of phytoplasma diseases in natural conditions (potato fields or other crops).

Different genetic determinants of PVY should be involved in the elicitation of *Ny* mediated hypersensitive resistance toward PVY^O and/or PVY^N in potato

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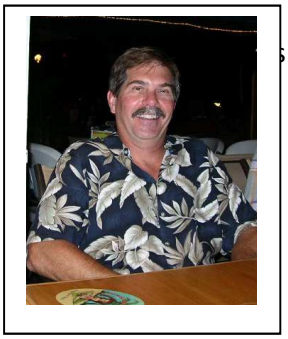
Potato virus Y (PVY), the type-member of the genus *Potyvirus* is the major virus pathogen infecting potato crop. Two main types of resistance to PVY are described in potato: the extreme resistance (ER), which avoids the replication and the spread of all strains of PVY in the plant, and the hypersensitive reaction (HR) which is a PVY-strain specific resistance characterized by the expression of necrotic local lesions (NLL) in inoculated leaves associated or not with systemic infection. The HR genes Ny_{tbr} and *Nc* confer resistance to PVY ordinary strain (PVY^O) isolates and to PVY^C respectively. In addition, *Ny-1* and *Ny-2* genes confer HR resistance to both PVY^O and PVY^N isolates, while *Nz* gene induces HR towards PVY^Z isolates. Main of these HR genes have been mapped to different potato chromosomes, but the specific interactions between all these HR genes with PVY^O and/or PVY^N genome are not well known.

The objective of our study was to improve our knowledge about the HR/PVY interactions and to identify which region(s) or amino acid(s) of PVY genome are involved in the HR-mediated resistance. Five potato genotypes corresponding to Désirée (Ny_{tbr}), Bintje (susceptible to PVY, no *Ny* genes) and Béa, G300, Ger94-8 (susceptible to PVY, no data on available *Ny* gene(s)), were mechanically inoculated with PVY^N605 and PVY^O139 referent isolates and with a collection of $PVY^{N/O}$ chimeras. Aggressiveness and virulence were monitored using observations of inoculated and apical leaves, and serological (ELISA) detection assays, respectively. PVY^O139 and PVY^N605 inoculations of genotype G300 were associated to HR reaction, suggesting the presence in this potato genotype of resistance gene(s) similar to *Ny-1* or *Ny-2*. The cultivars Désirée, Béa and Ger94-8 expressed similar HR to PVY^O139 inoculations, suggesting the presence of Ny_{tbr} gene in the genetic background of Béa and Ger94-8. However, some $PVY^{N/O}$ chimeras were able to induce HR reaction in Béa cultivar but not in Ger94-8, and conversely. These results showed that different resistance processes are challenged by the virus in the tested genotypes. Moreover, using the range of $PVY^{N/O}$ chimera as inoculums, we showed that PVY genomic regions involved in HR were specific to the potato cultivar. In addition, our data indicated that NLL and systemic movement of PVY involve different regions of the viral genome. of knowledge on HR/PVY interactions resulting from this study will help future s for PVY resistance in potato.



Aphid transmission of multiple strains of *Potato virus Y* acquired either sequentially or from mixed infections

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Aphid transmission may be a contributing factor to a recent shift in the predominant strains of *Potato virus Y* (PVY) affecting the U.S. potato crop. Surveys of seed potato fields over the past decade indicate the ordinary strain, PVY^{O}, is being replaced by recombinant strains and that a majority of seed fields harbor multiple PVY strains. Previous studies have suggested that the recombinant strains may be more efficiently transmitted and that the virus strains may differ in their ability to bind to or be released from the aphid acrostyle. Our objectives were to compare transmission efficiency when aphids were allowed either sequential acquisition access to two PVY strains or acquisition from plants infected with two strains. Individual green peach aphids (*Myzus persicae*, Sulzer) were allowed a 2-3 min acquisition access period on potato leaves infected with different combinations of the three strains, or a 2-3 min acquisition access period on a leaf infected with PVY^{O}, PVY^{NWi} or PVY^{NTN}, followed by another 2-3 min acquisition access period on a second potato leaf infected with a different PVY strain. Single aphids were then transferred to healthy potato seedlings for a 24 hr inoculation access period. All possible combinations of the three strains were tested. Strain-specific infection of the recipient plants was determined by DAS-ELISA and RT-PCR 28 days post-inoculation. When aphids acquired PVY^{NTN} and PVY^{O} in either sequence, PVY^{NTN} was transmitted to a majority of the plants. PVY^{NWi} acquisition either prior to or after acquisition access on PVY^{O} or PVY^{NTN}, led to the transmission of PVY^{NWi} to more than 80% of the plants. The data suggest that PVY^{NWi} and PVY^{NTN} may preferentially bind to the aphid acrostyle over PVY^{O} or they may be preferentially released during inoculation. Interestingly, when aphids acquired two strains of PVY from mixed infected plants, PVY^{O} was found to be the infecting virus in a majority of the recipient plants regardless of whether PVY^{NTN} or PVY^{NWi} was the other strain in the mixed infection. Preferential transmission of PVY^{NTN} or PVY^{NWi} was dependent upon the isolate of PVY^{NTN} found in the mixed infection. The contribution of aphid transmission to the shift in predominant PVY strains affecting the potato crop is uncertain, but it does not appear to be a major factor. It is unknown if differential transmission of PVY strains in these experiments was due to aphid-virus interactions in the stylet or due to virus strain competition in the plant following aphid inoculation.

Genetic Diversity among Tobacco rattle virus Populations in the USA

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Tobacco rattle virus (TRV) (Genus: *Tobravirus*) is becoming increasingly important in many potato-growing areas of the world including the USA. The goal of this project is to understand the genetic diversity of TRV isolates from several states in the US. TRV isolates from Colorado, Idaho, Minnesota, and North Dakota were characterized at the molecular level. The genome was cloned and sequenced and compared to known TRV isolates from the US and other parts of the world. Oligonucleotides were designed and used to amplify the conserved region in RNA-1 and complete RNA-2. Amplicons were cloned into pGEM-T easy vector and sequenced. Phylogenetic trees were constructed using MEGA 5. Nearly complete RNA-2 was amplified by using the primer combination 158 and TR1. RNA-2 from TRV isolates from CO, ID, MN, and ND were found to be 3537, 3606, 3024 and 2928 nucleotides long, respectively. The sequences obtained from all the four isolates had the complete coat protein (CP) and 2b protein. Only two isolates (from CO and ID) had the ORF for the complete 2c protein, while MN and ND isolates had truncated 2c protein. The isolates from MN and ND could be nematode non-transmissible as they encode for a truncated 2c protein. Phylogenetic analysis based on a comparison of nucleotide and amino acid sequences of CP and 2b proteins with TRV isolates reported from other parts of the world showed that the North American isolates cluster as a distinct group along with the Asian isolates, while the European isolates formed a separate cluster. Significant homologies among the American isolates were observed, while European isolates were found to be more diverse. Knowledge gained would be useful in understanding the ecology, and epidemiology of TRV and will facilitate in improved virus detection tools and technologies that could be applied to reduce the virus incidence and its impact.

Dodder (*Cuscuta* sp.) as a Host and Vector of *Potato virus* Y (PVY) in Potato Fields in Hatay-TURKEY

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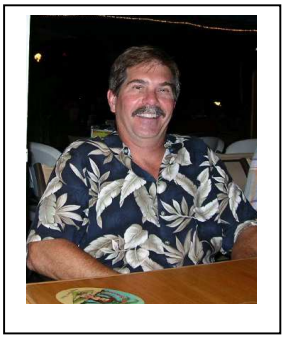
The population of dodder (*Cuscuta* sp.) has been known significantly increasing in recent years and this parasitic plant has been one of the most common species in the potato growing areas in Hatay province of Turkey. *Solanum tuberosum* (potato) plants especially growing in Reyhanlı district has been seriously attacked by dodder in the middle of the vegetation period (since April) in field conditions. The parasitic weed, *Cuscuta* sp. was determined to cause potato plants to die in the region. Besides direct damage on potato plants, it was identified as an important host of *Potato virus Y* (PVY) and its effective vector, green peach aphid *Myzus persicae* (Sulzer). Beside the severe PVY symptoms observed in potato plants infested with dodder, dodder plants with mild symptoms of thinning and pale yellowish discoloration of the vines were also found on virus infected potato plants. It is thought that these were not diagnostic symptoms for virus infections in dodder plants. Suspected dodder samples taken from potato plants were found to be infected with PVY at a high rate (46.2%) by DAS-ELISA (Double antibody sandwich-enzyme linked immunosorbent assay). PVY was able to transmit to test plants, tobacco (*Nicotiana tabacum* L.) by growing vine parts of PVY infected dodder samples on the healthy seedlings. Mosaic symptom was observed in four of the five test plants in an insect proof controlled temperature room. Our results indicated that dodder could also have a role for spreading of PVY among plants including potato and weed species in potato fields under natural conditions. *M. persicae* has been found to feed and reproduce on dodder in potato fields during spring period in 2014. Detection of other main aphid transmissible viruses in dodder is still in progress. This study is the first to report feeding and reproducing of aphids on dodder under the natural conditions in Hatay-Turkey.



Evolving disease dynamics of the *Potato virus Y* complex affecting the U. S. potato crop: A group effort between selection pressures and farming practices

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Potato virus Y (PVY) is the major disease affecting the United States seed potato crop. In the past decade we have documented a rapid shift in the PVY strains; the ordinary strain, PVY^{O}, was most common prior to 2004, but now the recombinant strains are emerging. Our overall objective is to identify the factors contributing to this emergence of recombinant strains. Surveys from 2004-06 found PVY^{NTN}, the strain associated with potato tuber necrotic ringspot disease (PTNRD), was present at low levels only in one state. PVY^{NO} was increasing in all regions with the closely related PVY^{NWi} being a small percentage of the recombinant population. Since 2009, PVY^{O} has continued to decline; PVY^{NTN} is increasing in incidence and distribution, but remains a minority component of the total PVY population. The recombinant $PVY^{NO/NWi}$ population now dominates throughout the U.S., but PVY^{NWi} has nearly displaced PVY^{NO}. Other recombinant strains and genome variants have been detected, but their incidence and distribution changes over years and geographic regions. In general, the recombinant strains induce milder foliar symptoms in most widely grown North American cultivars. This has challenged seed certification programs that rely on symptoms to assess the health of the crop. PVY is the main reason for seed lots failing to be certified. On-farm roguing operations are also less effective at removing diseased plants and this contributes to increased PVY inoculum in the seed potato crop. A lack of coordination between breeding and pathology programs has resulted in the release and widespread acceptance of numerous PVY tolerant potato cultivars that express limited or transient foliar symptoms; a further a challenge to seed certification. Climate change is expanding growing seasons and pushing aphid flights later in the season especially in northern U.S. production areas. This contributes to a reduction in foliar symptoms due to late season infection and there is a greater overwintering of tubers left in the field resulting in a higher number of volunteer potatoes emerging in the spring. All of these factors are contributing to more virus inoculum in the potato crop and a selection for recombinant strains/isolates that induce milder foliar symptoms, but have a propensity to induce PTNRD. We have evaluated most of the major North American cultivars and only a handful are susceptible to PTNRD. We also found that while PTNRD is induced by most PVY^{NTN}

isolates, PTNRD is associated with isolates from nearly all strains of PVY. Interestingly, most North American cultivars express one or more *Ny* resistance genes manifested as a foliar hypersensitive-like response to infection by PVY^{O} and some PVY^{NTN} isolates. These reactions often lead to plant death or severe impairment of tuber production. Additionally, aphids do not efficiently transmit virus from these plants. The unintended *Ny* gene deployment is contributing to the disappearance of PVY^{O} and maintenance of PVY^{NTN} at low levels, but also to the selection of $PVY^{NO/NWi}$ strains. Many factors are driving the transformation of PVY populations and PVY epidemiology in the U.S. potato crop, but most can be traced back to short comings in the science of developing better potatoes and in the ing the potato crop.

Trends in PVY population changes in Poland

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Occurrence of PVY in potato was inspected in over 11700 tubers, representing 64 cultivars, collected from 26 locations of Poland from 2008 to 2013 by ELISA using monoclonal cocktail antibody (for all strain types), PVY^{N}-specific (Bioreba) and PVY^{O}-specific antibodies (SASA). Selected PVY isolates were further characterized by the biological tests according to Singh et al. (2008), and multiplex RT-PCR for strain typing. Potato cv. Nicola was used for the assessment of PTNRD.

Of the tested tubers, ca. 3700 ones (32%) were PVY positive confirmed by ELISA in growing-on tests, and they were used for PVY population study. PVY^{NTN} was the predominant form of PVY infecting potato and it composed 62% of the population in 2008. However, from 2009 to 2012, PVY^{N-Wi} had become predominant reaching up to 63-88% of the population. Very low percent of PVY isolates induced vein clearing (VCl) in tobacco (0.3-8%), and two isolates were confirmed as PVY^{O} by triplex RT-PCR according to Rigotti and Gugerli (2007). From 2 to 20% of PVY isolates reacted positively to both PVY^{N}- and PVY^{O}-specific antibodies, indicating mixed infection. Similar changing trends in PVY structure were observed in Młochów (central Poland) potato fields using tobacco bait plants as bioassay.

Twenty-nine PVY isolates representative for the Polish PVY population structure were tested biologically in differential potato cultivars King Edward (*Nc:ny:nz*), Desiree (*nc:Ny:nz*), Pentland Ivory (*Nc:Ny:Nz*), and tobacco (cv. Samsun) by mechanical inoculation under greenhouse conditions for three years. All the 29 isolates were able to cause systemic infection in all the three potato differentials as confirmed by ELISA at 4-6 weeks post inoculation. Twenty-three isolates caused vein necrosis (VN) in tobacco indicative of PVY^{N} strain, among which 15 were PVY^{NTN} and 8 were PVY^{N-Wi} based on multiplex RT-PCR. All the PVY^{NTN} isolates and one PVY^{N-Wi} isolate induced PTNRD in cv. Nicola. Six isolates induced VCl in tobacco including the two PVY^{O} isolates. One PVY^{O} isolate induced necrotic lesions in local inoculated leaves and necroses, VN, deformation and mosaic in upper non-inoculated leaves in cvs. Desiree and Pentland Ivory; but systemic spreading of PVY which confirmed by ELISA suggested overcoming *Ny*, *Nc* and *Nz*. This may arouse discussion about the criteria to assess hypersensitive resistance (HR) response and to distinguish PVY^{O} and PVY^{E} in such biological

Viruses in Estonian seed potatoes; Strains of *Potato Virus Y*

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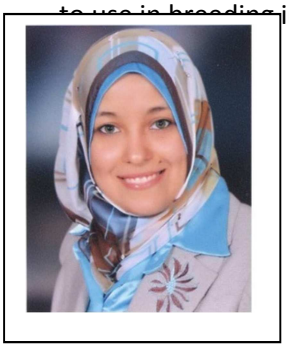
The potato (*Solanum tuberosum*) is one of the most important agronomic crops worldwide, and so it is in Estonia. Potato crop can be infected by different viruses. To control the spread of viruses, it is essential to keep vector population low during growing season by chemical control, to use certified virus free seed potato and cultivate potato varieties with higher resistance to viruses.
In this study, a general overview of Estonian seed potato situation in the years 2005 to 2013 is given. Susceptibility to PVY (predominant virus in local seed potato) was analysed in potato cultivars grown in Estonia. As PVY has many different strains, a survey from 2011 to 2013 was conducted to identify PVY strains spread in Estonia and to analyse the resistance of the most popular potato varieties on strain basis.
For regular seed potato monitoring, ELISA test was used with Bioreba monoclonal antibodies recognizing all common strains of PVY. For the PVY strain survey, more than 700 isolates were collected from annual survey PVY positive samples and tested with ELISA and RT-PCR. Different antibodies were used to distinguish between PVY^{N} and $PVY^{O/C}$ serotypes; and RT-PCR was used to identify PVY strains. The ability of different PVY strains to induce vein necrosis in tobacco was also observed.
The results show that the most common potato virus in Estonian seed potato is PVY. Recombinant strains PVY^{NTN} and PVY^{Wilga} form approximately 70% of Estonian PVY population and the survey reviled that population structure is shifting to favour PVY^{Wilga} strain. Approximately 15% of tested isolates gave controversial results with used methods and this shows the high variability of PVY, hence further investigation is needed. Different potato varieties differ in susceptibility towards PVY and its strains. Estonian survey results show that some potato varieties have higher resistance to PVY^{NTN} and some to PVY^{Wilga}. We also identified varieties with extreme resistance. Based on current data, recommendations to growers and breeders can be made to choose varieties best to grow and to use in breeding in Estonia.



Serological, Molecular and Biological Characterization of *Potato Virus Y* Isolates from Potato in Sweden

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Potato virus Y (PVY) is the most important and widespread virus infecting potato (*Solanum tuberosum*). Recently, new recombinant genotypes of PVY have appeared and their incidence is increasing throughout the world. In Sweden, the non-recombinant PVY^{O} strain used to be most common, but it now seems to have been replaced by the recombinant genotypes, as has been reported for many European countries. To study the present genetic diversity of PVY in Swedish potato, 50 symptomatic potato samples from different parts of Sweden were selected for characterization of the infecting PVY genotypes using serology, multiplex-RT-PCR, sequence analyses and inoculation tests. In DAS-ELISA, one sample was positive only for serotype O, 31 samples for only serotype N and 15 samples for both serotypes. None of the samples were positive for serotype C. Three samples did not react with any of the employed antibodies, but tested positive in the subsequent RT-PCR analyses. To identify specific PVY genotypes, multiplex-RT-PCR was used with diagnostic patterns for known genotypes. The identities of the amplification products were verified by sequencing. Among 41 Swedish samples analysed by RT-PCR, PVY^{NTN} was the most common genotype with 18 samples having a single infection by PVY^{NTN} and 17 samples having mixed infection by PVY^{NTN} and one or several other PVY genotypes. The RT-PCR analyses also indicated frequent infections by PVY^{N}-W and other recombinants, most often in mixed infection with PVY^{NTN}. The previously dominant PVY^{O} strain was detected in 12 of the samples in mixed infections. In inoculation tests, extracts of all 16 tested samples were found to induce vein and stem necrosis on tobacco (*Nicotiana tabacum*), which is indicative of the N pathotype of PVY, including PVY^{NTN}. Leaf/tuber extracts of 12 samples primarily identified to contain the PVY^{O} strain were inoculated into potato cv. Désirée (Ny_{tbr}*:nc:nz:Nd*) and screened for hypersensitive response (HR). Unexpectedly, no HR was observed in cv. Désirée for extract from any of these samples. These findings indicate the absence of PVY^{O} and PVY^{D} strains from the tested samples and that the PVY^{O} patterns obtained in the multiplex-RT-PCR were generated by other related genotypes. Extended sequence analyses are currently carried out to identify in more detail the exact PVY genotypes in selected samples. It can be concluded that PVY^{O} in Sweden mainly has been replaced by recombinant genotypes, such as PVY^{NTN}

Occurrence of *Potato virus Y* strains in Brazil and quantification in single and mixed infections by qRT-PCR

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Potato virus Y (PVY) is the main virus economically relevant considering Brazilian potato crops, due to its ability to spread in the field by aphid vectors and its high genomic variability. This variability might be related to the occurrence of mixed infections of potato plants with several virus strains, which could generate recombinant virus isolates, better adapted to environmental conditions, with diverse serological and biological properties. In this study, 165 potato samples infected with PVY, coming from different locations of Minas Gerais, Brazil, were collected and analysed by DAS and TAS-ELISA, multiplex RT-PCR and qRT-PCR, in order to determine the occurrence and concentration of PVY strains in single and mixed infections. For qRT-PCR primers and probes were designed to target strain-specific recombinant points. All samples were positive in DAS-ELISA. However, when analysed by TAS-ELISA, using the monoclonal antibodies for serotype O (MAb2 (Agdia) and SASA O) and for serotype N (IF5 (Agdia) and SASA-N), 46.7% of the analysed samples were positive for serotype O; 32.7% were positive for serotype N; 12.1% reacted with both groups of antibodies, indicating mixed infection, and 8.5% did not react with any of them. In the multiplex RT-PCR all samples were positive: 44.2% showed the typical pattern of PCR bands for $PVY^{N:O/Wi}$, 37% showed the pattern of PVY^{NTN} , and 18,8% showed mixed infection with those two strains. In qRT-PCR, 47,9% of the samples that were positive in RT-PCR for $PVY^{N:O/Wi}$ also showed the presence of PVYNTN and 82% of the positive samples for PVY^{NTN} were also infected with $PVY^{N:O/Wi}$. Based on the results of qRT-PCR, which as expected showed a higher sensitivity for the detection of PVY strains, more than 70% of the 165 samples infected with PVY had mixed infection. When the samples with mixed and single infections were analysed by qRT-PCR, there was a tendency of PVY^{NTN} strain to present a higher concentration in the plant. Our results showed a prevalence of $PVY^{N:O/Wi}$ and PVY^{NTN} in the sampled fields. The occurrence of mixed infections in most samples analysed highlights the possibility of increasing the genomic recombination events, explaining the high variability of PVY isolates that has been detected in Brazilian potato crops.

Survey and detection of *Potato virus Y* (PVY) and its strains from the Nigde province of Turkey

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Potato virus Y (PVY)**,** family *potyviridae* is a destructive plant virus affecting potato production in Turkey. In order to investigate the PVY infection rate and strain identification, field surveys were carried out in different potato growing regions of Nigde province during 2015. A total of 138 samples were collected from potato plants showing virus symptoms. All samples were individually tested by DAS-ELISA for the presence of PVY. It was determined that 45% (63 samples) of the tested samples were found infected. For the strain identification, multiplex immunocapture reverse-transcription polymerase chain reaction (IC-RT-PCR) assay was performed on positive samples (Chikh Ali et al., 2013). The results obtained from multiplex-IC-RT-PCR revealed that the most prevalent strain was $PVY^{NTN(A)}$ (33.33 %), followed by $PVY^{N\text{-}Wi}$ (23.80 %), $PVY^{NTN\ (A)}$+ $^{N\text{-}Wi}$ (9.52 %), $PVY^{NTN\ (B)}$ (4.76 %), PVY^{E} (3.17 %) and $PVY^{N:O}$ (1.58 %). Furthermore, 19.04 % (12 samples) exhibited unidentified strain profiles. These results showed that the analysed potato samples were severely infected by PVY and may also harbour strains having discrepancy. Further studies have been under investigation for atypical strains.

Chikh-Ali, M., Gray, S. M. and Karasev, A. V. (2013). An improved multiplex IC-RT-PCR assay distinguishes nine strains of Potato virus Y. Plant Disease, 97(10), 1370-1374.

Determination of PVY and AMV infection in weeds in potato fields in Hatay-Turkey

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PVY and AMV infections detected in 3 weed species in potato fields in Hatay province of Turkey between 2013-2015. Leaf and shoot samples were collected from symptomatic plants of *Amaranthus albus* L., *Amaranthus retroflexus* L., *Chenopodium* spp., *Circium arvense*, *Convolvulus arvensis*, *Cuscuta* sp., *Datura stramonium*, *Orobanche ramosa* L., *Physalis angulata*, *Solanum nigrum*, *Sorgum halapense* and *Xanthium* sp. Leaves of *Physalis angulata*, *Solanum nigrum* and *Cuscuta* sp. samples were with virus symptoms. There was not any other symptom of virus infection on *Cuscuta* sp. apart from mild discoloration and slimming. On the other hand, striking yellow blotchs or mosaics and smaller leaves and stunting of the plants on the infected *Physalis angulata*, blistering and smaller leaves on the infected plants of *Solanum nigrum* were observed. Plant samples were tested by biological assays as well as serological methods, DAS-ELISA: Double antibody sandwich-enzyme linked immunosorbent assay) to presence of AMV and PVY. Test plants (*Nicotiana glutinosa*, *N. tabacum*) inoculated by PVY exhibited mosaic, mottling with leaf crinkle symptoms in insect-proof and climated room. Infection rates of PVY were %53.8 (7/13), %100 (2/2), % 46.2 (6/13), %80 (4/5), and %68,7 (11/16) for *Physalis angulata*, *Solanum nigrum, Cuscuta* sp., *Nicotiana glutinosa* and *N. tabacum*, respectively. Further tests on the leaves of *Physalis angulata* plants showing yellow mosaic symptoms were revealed AMV (*Alfalfa mosaic virus*) infection with a rate of 2/13. This study is the first to report PVY on *Cuscuta* sp. and AMV on *Physalis angulata* in Turkey.

Keywords: AMV, *Cuscuta* sp, PVY, weeds, virus.

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Screening of *Potato Virus Y* (PVY) Strains in Potato, Weeds and Aphid Samples in Bolu Province (Turkey)

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Potato (*Solanum tuberosum* L.) is one of the most important agricultural crops of Turkey and the world. *Potato virus Y* (PVY) is the most common and destructive virus found in the most potato production area in the worldwide. It is the type species of the genus *Potyvirus*, family Potyviridae with a single-stranded positive-sense genomic RNA. In the last two years, several strains of PVY have been differentiated and reported by our group in commercial potato cultivars grown in different geographical regions of Turkey. In the present study, we report the presence of several PVY strains, on potato, weed and aphid species, in which a total of 23 potato fields were sampled. In the survey activities performed in 2015 in Bolu province (Turkey), a total of 137 potato leaves, 14 weeds and 23 aphid samples were collected and tested against PVY strains. For the detection of PVY strains, potato and weed samples were tested by serological (with strain specific antisera, AGDIA kits) and molecular (Multiplex-RT-PCR) methods, while for aphid samples the only Multiplex-RT-PCR method was implemented. In molecular tests, the PVY infections were differentiated by strain specific primers. In serological tests of potato leaves, 66 were found infected by PVY^{O+C}, 16 were found infected by PVY^{N}, and 11 were found infected as mixed infection by PVY^{N} and PVY^{O+C}. In molecular tests of potato leaves, out of 35 tested potato leaf samples, 22 were found infected by $PVY^{N:O}$ strain, 3 were found infected by PVY^{NTN} strain, and 7 were found infected as mixed infection with PVY^{NTN} and $PVY^{N:O}$ strains. In serological tests of weed samples, 4 were found infected by PVY^{N} and one was found infected by $PVY^{N \text{ and } O+C}$. In molecular tests of weed samples, out of 14 samples, 3 were found infected by PVY^{NTN} strain and a unique sample was found infected by $PVY^{N:O}$ strain. In molecular tests of aphid samples, out of 23 tested individuals, 2 were found infected by PVY^{O+NTN} strain as mixed manner in *Myzus persicae* Sulz. In this study, we report the presence of PVY infections in surveyed potato fields and NTN strains. This is the first report of the presence of these strains in tested aphid samples in Bolu province (Turkey).

Status of Natural *Alfalfa mosaic virus* (AMV) Infection in Potatoes in Hatay-Turkey

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The status of the main viruses in potato fields in the province of Hatay-Turkey has been investigated under different projects since 2003. *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV) were determined to be the most common viruses by the high rates of infections in all varieties grown in the region according to the studies for nearly ten years. *Alfalfa mosaic virus* (AMV) infection was firstly detected in a potato seed tuber (cv. Agria) obtained from Reyhanlı district of Hatay in 2010 by enzyme-linked immunosorbent assay (DAS-ELISA). However, naturally AMV infected plant was not determined in the field conditions in the same vegetation period in Hatay-Turkey. The plant sap was extracted from potato plant germinated from an AMV infected seed tuber in an insect-proof controlled temperature room with 25^{o}C±2 and 16h light conditions, and then mechanically inoculated to the test plants (*Chenopodium amaranticolor* Cost and Reyn, *C. quinoa*, *Vigna unguiculata*, *Nicotiana tabacum, N. benthamiana, Capsicum annuum, Cucumis sativus*) to confirm the results of DAS-ELISA. Four seedlings of each test plant were inoculated and observed for symptom development for six weeks. Only 1 tuber sample out of 272 potato seeds was found to be infected by AMV (0.36%) in Hatay in 2010. Since that year, symptomatic potato plants have been observed especially in Reyhanlı and Kırıkhan districts of Hatay and began to increase in potatoes grown in the region. Leaf distortion, mosaics, bright yellow blotching or mottling and patterns and then severe chlorosis symptoms were observed on suspected potato plants related to natural AMV infections in field conditions. AMV was found in potato samples by the rates of 0.0%, 5.4% and 4.6% in 2013, 2014 and 2015, respectively.

Although the virus has occurred at low rates, AMV infections have increased in potatoes in Hatay year by year. The seed tubers coming from other regions to Hatay potato growing areas is thought to be the main source of the spread of the virus, and result in high variation in infection rates in consecutive years. The aphid species such as *Aphis fabae*, *Aphis solanella*, *Lipaphis erysimi*, *Myzus* (*Nectorosiphon*) *persicae*, *Macrosiphum euphorbiae,* and *Rhopalosiphum* sp. have also been determined in potato fields which can be playing a key role in the spread of uncommon potato viruses such as AMV to new areas.

Preliminary studies regarding the *Potato virus Y* status in seed potatoes in Romania (for several cultivars)

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The cultivar's selection is essential, being one of the most important critical component in the PVY control. Potato varieties which demonstrate some resistance to PVY can be managed effectively in most regions and maintain their certification status. However, knowledge regarding each cultivar's reaction to PVY and the various strains of PVY (especially the necrotic one) are critical. This preliminary studies investigating the status of the *Potato virus Y* (PVY) during three years (2013, 2014 and 2015), in five main seed potato growing areas of Romania, revealed large differences in PVY incidence. The samples tested were from the following cultivars: Christian, Roclas (romanian cv.), Riviera, Carrera, Hermes and Red Lady. The tests largely confirmed the predominance of the PVY^{N} group. So, serological investigations showed that 40.7% of the PVY positive samples in 2013, 50.9% of the PVY positive samples in 2014 and 70.4% in 2015, viruses belonging to the PVY^{N} group were found. Within this group, the prevalence of recombinant PVY^{NTN} (for the cv. Hermes and Carrera) and PVY^{Wilga} (for the other cultivars) respectively was confirmed by the tests.

Key words: seed potato, potato virus Y, necrotic strains.

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Global Analysis of the Migration Pattern and Evolutionary Lineages of *Potato virus Y*

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Using the complete genomic sequences of 149 Potato virus V (PVY) isolates reported worldwide, we studied the migration and evolutionary pattern of PVY using Bayesian phylogenetic approaches to assess the influence of host (potato) and geography on both virus evolution and spread. To estimate the evolutionary rate and timescale of PVY genomes, Bayesian phylogenetic analyses in BEAST v2.1.3 was performed. The published sampling time of the PVY isolates or sequences reported were used for calibrations of the molecular clock. We used Bayes factors to determine the best-fitting molecular-clock model and coalescent prior for the tree topology and node times. Both strict and relaxed (uncorrelated exponential and uncorrelated lognormal) molecular clocks, respectively were compared and to infer the demographic history we compared five demographic models - constant population size, expansion growth, exponential growth, logistic growth, and the Bayesian skyline plot.

Recombination has been a hallmark of PVY evolution, and some strains had a different evolutionary history than others. Some strains had accumulated a higher number of recombination events compared to others. Molecular clock analyses showed that the viral genes evolved at rates between 1.44 and 2.39×10^{-4} substitutions/site/year, similar to those with an RNA genome. The haplotype network of PVY isolates (149 samples with 116 haplotypes) suggested that PVY haplotypes could be divided into clearly two major groups. One was composed of N and NTN, while the other was of haplotypes from O and O5. Two smaller groups having haplotypes belonging to N:O and Wi/Wilga strains were also observed. All haplotype groups have accumulated their own unique set of mutations. The unique strains isolated from non-potato hosts, such as C, MN and NC57, were found to evolve separately with unknown median vectors i.e., missing haplotypes. The network was found to be spread out rather than star-shaped indicating that there were multiple recombination events. All the haplotype groups had such structures and thus might have accumulated unique mutations in each genomic region.

Bayesian phylogenetic analysis of the origin and global spread of PVY showed strong Bayes factor (BF) support for CP that the virus had spread not only intra-continental in Europe but also outside to different parts of the world. The origin of majority of PVY recombinants can be traced to Europe from where they were likely re-introduced with returning Spanish explorers. PVY probably spread from a single population from Europe to other parts of the world with subsequent frequent gene flow [illegible]ons in Europe and the USA.

Ultrasensitive Rare Sequence Detection with Droplet Digital™ PCR

Adam Bemis, Global Application Specialist

Bio-Rad Laboratories

Just as PCR and qPCR have revolutionized the field of genetics over the past few decades, Droplet Digital PCR offers another advance that can provide novel insights into a wide array of genomics disciplines. With more than 400 publications to date, Droplet Digital PCR has been far-reaching in these fields, from genome editing detection to high copy number resolution in plant genetics. Digital PCR provides unrivalled precision with respect to qPCR applications.
Partitioning of the sample into thousands of discrete droplets provides many other advantages including improved detection of rare targets through massive reductions in effective background, as well as increased PCR inhibitor tolerance. The flexibility to measure multiple targets independently provides additional improvements for many applications including viral load, microbial quantification and identification, copy number status, and pathogen detection.

Potato DNA/RNA rapid extraction suitable for molecular test systems such as qPCR and PCR macroarray

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The Potato DNA/RNA rapid extraction set was developed for fast and reliable nucleic acid extraction from potato tissue (leaf, dormant tuber peel, sprouts). The novel extraction system was validated for PCR based downstream applications such as qPCR and PCR macroarray. Because of low virus titer in dormant potato tubers molecular test methods are required for the detection of viruses from this material. Results will be presented showing duplex detection of PVY and PLRV by qPCR. Furthermore, the PCR macroarray, a novel diagnostic tool, allowing multiplex detection of 7 potato viruses and one viroid (PVA, PVM, PVS, PVX, PVY, PLRV, PMTV, PSTVd) from dormant tuber, will be shown. Large scale validation of the different test systems, including the Potato DNA/RNA rapid extraction, was done with tubers issued from the Swiss potato seed certification program (in collaboration with the Swiss Federal Agronomic Station Agroscope).

The detection of *Potato virus Y* in seed potato lots in Israel by a TaqMan Real Time PCR technique

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Potato (*Solanum tuberosum*) is the largest crop in Israel. Production is based on the import of seed tubers from Europe for the spring planting. Imported tubers are generally free from virus infection. The most important virus infecting potato is *Potato virus Y* (PVY), which can cause severe damage to marketable yields. In Israel, tubers from the spring harvest are stored over the summer for planting in the autumn/winter. Therefore it is important to be able to determine the infection rate of seed tuber lots prior to storage. A popular technique to measure tuber infection is to grow plants from tubers and measure virus in the leaves by ELISA (the "Growing-On test"), which takes at least 6 weeks to give results. There is a need for a faster test, such as Taqman Real Time PCR (qPCR), for direct analysis of viral infection of tubers at harvest. To use qPCR as a diagnostic tool it is necessary to test if the techniques give comparable results on batches of field-grown potatoes. Such a test was performed on potential seed tuber lots of 14 different cultivars for three successive spring harvests. Although the two techniques do not generally agree closely, the results show that the qPCR technique can distinguish well between seed lots with a low PVY infection rate (<5%; suitable for seed), and those unsuitable for seed. Therefore, qPCR is an appropriate technique for the determination of the PVY infection rate of seed tuber lots in Israel.

High throughput qPCR detection of virus for certification of seed potatoes

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Delivery of virus free seed potatoes is the most efficient strategy to protect potato crops from various diseases that alters the productivity. In Switzerland, certification of seed potatoes and the control of the virus infections in seed lots is a regulated process adopted in the late fifties. It has been based on immuno-detection of viruses for the last 30 years. Virus detection through ELISA requires to break the dormancy of the tubers to increase virus load above the detection limit of the ELISA. To do so, tubers are treated with gibberellic acid or gaseous compounds dangerous to human health.

To bypass this time-consuming step and to avoid the use of toxic gases, Agroscope developed a high throughput diagnostic pipeline based on qPCR to monitor the potato viral diseases of the national seed potato production (around 1000 ha). Virus detection by qPCR is highly sensitive and is applicable on dormant tubers for the detection of a large range of viruses. To keep with the timeframe (6 weeks) and to guarantee affordable costs for the seed potato sector, innovative methods were developed for all the steps of the analytical process including the grinding, the RNA extraction, the qPCR and the interpretation of the results.

The analytical chain have been validated over 3 certification campaigns and will replace the ELISA in 2016. Pool size of the samples was investigated to ensure the reliability of the results and to enable a cost-effective certification scheme of seed potato tubers according to Swiss regulations. The most prevalent potato viruses in Switzerland, namely PVY and PLRV, are detected from these pooled samples with a multiplex qPCR. RNA extracts of pooled samples are further gathered in groups and then into a final “super group” with all the samples gathered together. This design enables the detection of rare and non-regulated viruses for marginal additional costs. In parallel, a routine procedure using NGS technologies has been developed. It includes a dedicated bioinformatics pipeline for the quality control of seed potato certification scheme.

Sequencing and Molecular Characterization of Peruvian Potato mop-top virus full-length cDNAs Reveals Two Genotypes and Evidence for Recombination

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Complete sequences were obtained for 10 Peruvian Potato mop top virus isolates collected from 15 fields belonging to three different regions in Andes region of Peru. Previously, molecular characterization of PMTV isolates from different European countries and U.S.A. showed little variability among their genomes. However, sequencing analysis of isolates from Peru revealed significant variability among few ORFs in the genome. Sequence analysis of read through domain of coat protein CP-RT, which is involved in long distance movement of virus revealed that the isolates can be divided into two genotypes, '**A**' (aggressive) type and '**S**' ('suave', Spanish word for soft) type. The alignment also showed an evidence for recombination in between the two genotypes. Comparison through phylogenetic trees showed that all European isolates and few Peruvian isolates belong to 'A' group, while most of Peruvian isolates belong to 'S' type. Data analysis also revealed an evidence for positive selection in few codons of 8K protein, which was previously shown to be the virulence factor of the PMTV. The isolates of A and S types also showed phenotypic variations when inoculated in *Nicotiana benthamiana*.

A proficiency test for potato infecting phytoplasmas

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As part of the EUPHRESCO PHYLIB project (Epidemiology and diagnosis of potato phytoplasmas and *Candidatus* Liberibacter solanacearum and their contribution to risk management - http://www.euphresco.net/media/project_slides/phylib_2.pdf) a proficiency test (PT) for phytoplasmas was undertaken to evaluate assays currently used by laboratories. Five PHYLIB partners requested to take part. Prior to the preparing the PT, the stability of samples: healthy potato leaf DNA spiked with BLTVA ('*Ca* Phytoplasma trifolli') from infected potato plants and '*Ca* phytoplasma asteris' plasmid, at high, medium and low dilutions was checked when stored at 4C, room temperature and 30°C for up to 15 days using real-time PCR (Christensen *et al* 2004, Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging, (*Molecular Plant-Microbe Interactions* 17:1175-1184) and nested PCR (R16f2/r2 primers nested with fu5/ru3). The samples were relatively stable at all temperatures and over the specified time period. PT samples: *Ca* Phytoplasma asteris' plasmid (10-1, 10-2, 10-3); '*Ca* Phytoplasma solani' plasmid (10-2, 10-3, 10-4);'*Ca* Phytoplasma trifolli' plasmid (1:20, 1:50); BLTVA from infected glasshouse grown plants (Neat, 10-1,10-2); *Paenibacillus* sp.; *Brevundimonas* sp.; *Agrobacterium tumefaciens* (as potentially cross reacting bacteria); healthy potato leaf DNA) were despatched, 4 September 2014, to be stored in a fridge on arrival and tested within two weeks. A healthy and BLTVA infected microplant was also sent for testing. Four of the five laboratories returned their results. Only one laboratory obtained the expected results, with all their assays. Lab 4 included a real-time method (Hodgetts *et al* 2009, Panel of 23S rRNA gene-based real-time PCR assays for improved universal and group-specific detection of phytoplasmas.- *Applied and Environmental Microbiology* 75: 2945-2950) which does not detect the plasmid insert used.

The United Kingdom Potato Quarantine Unit (UKPQU): an accredited off-shore potato quarantine station for New Zealand and an approved source of high health material for Australia

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The UKPQU was accredited as an offshore potato quarantine station for NZ in November 2007 and the requirements are specified in MAF Import Health Standard (IHS) for import of nursery stock into NZ (https://mpi.govt.nz/importing/plants/nursery-stock/requirements/), that includes *Solanum tuberosum*. This standard continues to be modified and in 2013 *Papaya mosaic virus* (PapMV) and *Pepino mosaic virus* (PepMV) (both genus *Potexvirus*) and the bacterium *Dickeya solani* were added to the list of regulated pests that must be tested. *Cherry leafroll virus* (CLRV) and *Pepper chat fruit viroid* (PCFVd) were added to the list of viruses that infect potato experimentally. This did not affect the testing programme since the UKPQU already had tests in place that would detect these pests: PapMV and PepMV by bioassay and RT-PCR (Potexvirus assay); *D. solan*i by plating onto pectate medium followed by PCR; CLRV by bioassay and PCFVd by a Pospiviroid digoxigenin cRNA probe. The testing programme agreed between SASA and MAF exceeds that specified in the IHS and includes testing for potato pests already present in NZ and not listed in the IHS e.g. PLRV, PVA, PVM, PVS and PVX*, Ralstonia solanacearum* and '*Ca.* Liberibacter solanacearum'. This additional level of testing means that microplants from the UKPQU can be used as nuclear stock material for direct entry to the potato certification scheme in NZ. The UKPQU is currently New Zealand's only accredited offshore plant quarantine facility for *Solanum tuberosum* (see http://mpi.govt.nz/news-and-resources/resources/registers-and-lists/offshore/).

In 2009, an application was made to the AUS Quarantine and Inspection Service to become an approved source of high health potato material and after a technical audit and a review of policy on the importation of potato (*Solanum tuberosum*) propagative material into AUS, this was approved, 27 August 2013 (http://www.agriculture.gov.au/biosecurity/risk-analysis/reviews/final-plant/review_potato/final-potato). The major difference to the testing programme for NZ was the addition of TNV to the list of regulated pests for testing. Further discussions were held to finalise the testing programme and the PCR primers to be used for TNV detection. The agreement with AUS requires that plants are grown for visual inspection for a minimum of three months under post-entry quarantine in AUS, but no further testing is done. Currently the UKPQU is AUS only accredited offshore laboratory for supply of high health potato material. The first material was dispatched to AUS, 21 August 2014. The material is imported into AUS under condition PC6756. The testing for AUS and NZ has now been harmonised into a single testing programme so that potato material passing [illegible]mme can be sent to either country. This has a financial advantage for exporters [illegible]ngle quarantine testing charge rather than quarantine charge for each country.

New technologies for diagnostics and epidemiology of viruses

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The use of new research and diagnostic tools like digital PCR, loop mediated amplification assays (LAMP) and next generation sequencing enable us to cope with new problematics such as emerging viruses that result in new epidemics, new or previously overlooked transmission routes such as water and new emerging vectors. In this talk we will give few practical examples on how to fill the before-mentioned gaps using new molecular tools by focusing on two important pathogens: *Pepino mosaic virus* (PepMV) and Potato spindle tuber viroid (PSTVd).

PepMV and PSTVd are both quarantine pathogens that pose a serious threat to tomato and potato production due to seed transmission and mechanical transmission, coupled with their long-term stability outside plant host. In the recent years, especially seeds and water contaminations are becoming more important and it is being difficult to detect and confirm the presence of the pathogens in such matrices without efficient concentration methods. We have developed procedures that improve the detection of PepMV and PSTVd in three different matrixes: water, seeds and plant tissue. For efficient concentration of PepMV from water samples we have optimized a procedure using convective interaction media (CIM) monolithic columns, which enabled concentration by three orders of magnitude. For concentration of PSTVd from seed extract we adapted an easy-to-use and efficient method based on RNA binding to positively charged anion-exchange resin beads that provide up to 100-fold more sensitive detection in comparison with standard procedures. This thus allows confirmation of real-time reverse-transcription PCR (RT-qPCR) results with sequencing of RT-PCR products in samples with low viroid levels. In addition, we have also optimized RT-LAMP assays for detection of PSTVd and PepMV. RT-LAMP assays were adapted both for laboratory and direct in field testing requirements, allowing rapid detection of these tomato pathogens in crude leaf homogenate in up to 30 min.

The procedures of concentration and detection were shown to be efficient and fill the gaps in diagnostics of PepMV and PSTVd, especially when present at low concentration in difficult matrices like water and seeds.

Detection of PSTVd originating from ornamental plants by qRT-PCR a Luminex xTAG technology

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In the course of PSTVd surveys aimed at ornamental plants from the family *Solanaceae*, carried out in CR since 2007 to 2009, the occurrence of PSTVd was proved in *Solanum jasminoides, Burgmansia* spp., *Petunia x hybrida* and *Solanum muricatum*. After positive qRT-PCR detection, selected isolates were mechanically inoculated on virus-free potato plants cvs. Vendula, Verne and Vlasta and maintained in isolated greenhouse. Harvested tubers displayed symptoms typical to PSTVd infection, whereas leaves were largely symptomless. Original plants of *S. jasminoides*, *S. muricatum* and *Burgmansi*a spp., as well as potato plantlets infected with PSTVd isolates originating from above mentioned ornamental plants, and maintained in *in vito* conditions, and reference potato PSTVd isolates were used for comparative laboratory detection.
Three molecular detection methods were used:

1) qRT-PCR – SYBR green
2) qRT-PCR – Taq Man
3) Luminex xTAG (combined with house-keeping gene Nad 5).

All the PSTVd isolates were reliably detected by both variants of qRT-PCR as well as by Luminex xTAG technology, utilizing commercial kit (Primediagnostics).
The novel method – Luminex x TAG seems to be suitable as the alternative procedure for PSTVd detection with comparable sensitivity and specificity to the recommended ones.

Assessing Potato virus Y (PVY) infections in seed potato certification

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To enter the marketplace, in the EU and many other countries worldwide, seed potatoes require certification. The majority of the European national and international seed potato regulations currently base their evaluation of virus tolerances on the combined virus incidence and symptom severity. So far, all severe viruses were commonly seen as the origin of severe virus diseases, and mild viruses usually the cause of mild diseases.

However, research is challenging this equation especially regarding the most economically important virus, *Potato virus Y* (PVY) considered a severe virus. Research into PVY-infected potato leaf samples from Bavarian potato seed certification ascertained that the "classical" O and the N strains, largely responsible for severe virus diseases, accounted for less than 1% of the infections present. Ca. 99% of the PVY infections were composed of the new recombinant strains PVYNTN and PVYNW with the ratio between these recombinants differing considerably.

One explanation for that seem to be variations in susceptibility of particular cultivars to certain PVY strains. Thus, the ratio of the recombinants may relate to which cultivars are preferentially grown in different potato growing areas or countries.

Greenhouse tests showed that PVYNTN and PVYNW were associated with both severe and mild disease symptoms. The corresponding field tests presented considerable differences in symptom severity. Also, no correlation could be determined between virus concentration and symptom severity.

These new findings will become part of the worldwide discussion on eliminating the criterion virus symptom severity from potato seed certification. The discussion was initiated in the wake of amendments to international seed potato regulations.

On the EU level, these new findings have been taken into account when revising the annexes of the Directive 2002/56/EG. The tolerances for seed potatoes: In the growing crop (field inspection) for seed potato production and for virus incidence assessment of the direct progeny, the tolerances for plants with virus symptoms no longer differentiate between mild and severe symptoms. On the international level, the UNECE Working Party on Agricultural Quality Standards, Specialized Section on Seed Potatoes, has followed suit and also removed the differentiation between severe and mild virus symptoms from the UNECE STANDARD S-1 concerning the marketing and commercial quality [illegible]TATOES.

One-step RT-ddPCR for quantification of *Potato virus Y*

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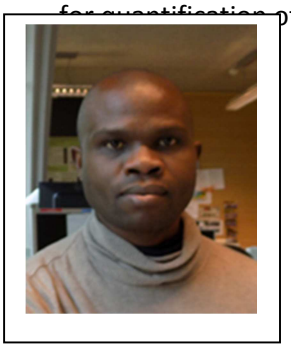
In most cases, testing for plant viruses provides, or can be interpreted as, qualitative data; i.e., a negative or positive response. However, the quantitative property of assay can be useful to monitor virus kinetics, such as the progress of an infection, and variations of virus titer through the season and in different plant tissues. Also, quantification is important in screening plants for resistance against viruses, or to estimate the number of copies carried by the vectors. Droplet digital PCR (ddPCR) is an emerging nucleic-acid detection method that provides absolute quantification of target sequences and it has been already shown to be useful for quantification of different pathogens, including plant viruses (Rački et al. Plant Methods (2014) 10:42). An important advantage of ddPCR is that no calibration curves are needed for quantification of the viruses, compared to real time PCR (qPCR), which is at present the method of choice for virus quantification. In ddPCR, the reaction mixture is distributed across a large number of droplet partitions containing zero, one or more copies of the target nucleic acid. After end-point PCR amplification, each partition is examined and defined as positive or negative. The absolute number of target nucleic-acid molecules contained in the original sample before partitioning can be calculated directly from the ratio of the positive to total partitions, using binomial Poisson statistics. ddPCR has been shown to be less affected by inhibitory substances and more sensitive for rare targets when compared to qPCR. We will present the first results of development of *Potato virus* Y (PVY) specific one-step reverse transcription ddPCR (RT-ddPCR). The developed RT-ddPCR will have the potential to be used for quantification and quality control of RNA based on in-house reference materials typically used in diagnostics laboratories and for quantification of PVY in different studies.



Sample collection methods for *Potato virus Y* (PVY) epidemiology surveys in Kenya

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Potato virus Y (PVY) causes major yield and crop quality losses in the potato growing regions in Kenya. Use of resistant cultivars to the main PVY strains is considered the most sustainable and long term means of managing the disease. Starting cultivar resistance screening for PVY requires field surveys in the potato growing areas to understand occurrence, distribution and relative importance of the different PVY strains. Unfortunately, distance and remoteness of the potato growing regions often compromise quality of plant tissue samples for reliable serological and molecular detection especially if samples do not reach the laboratories in time. This work aims to identify sustainable and reliable alternatives for sample collection for PVY epidemiological surveys. Tobacco leaf samples infected with each of the four PVY strains (PVY^{N}, PVY^{O}, PVY^{NTN} and PVY^{Wi}) were applied to two types of filter paper cards (Whatman's FTA® and ordinary filter paper-OFP). Two mm discs punched from the cards were incubated overnight in SEB and dilutions of the eluate were tested for PVY in DAS-ELISA, dot blot and in RT-PCR in comparison with equal amount of leaf sap and also tested for infectivity by bioassay on *N. tabacum* cultivar White Burley. The four PVY strains were successfully detected on PVY infected samples collected using FTA® and OFP paper in DAS-ELISA and RT-PCR but not in dot blot. In contrast, dot blot and DAS-ELISA gave comparable results when using leaf sap. Infection of healthy tobacco plants using sap of the four PVY strains eluted from both FTA® and OFP was successful and PVY was detected using DAS-ELISA at two weeks after inoculation. From this work, it was clear that PVY samples obtained from remote potato growing areas can either be stored in FTA® or OFP cards awaiting further serological and molecular analysis and subsequent use in PVY resistance screening. Though OFP appeared to be a cheaper alternative to FTA®, its ability to retain a longer period of time still needs to be investigated and compared to FTA® cards.

Q-bank and VirusCollect: fulfilling the need for a common reference collection of plant viruses and viroids

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The availability and accessibility of suitably characterised plant virus and viroid reference isolates is vital for research and diagnostic laboratories. The maintenance of such collections has come under severe pressure due to a reduction in the number of scientists (in particular virologists) and associated budgets. As a result there is a need for international collaboration.
The Euphresco project VirusCollect aimed to establish a common reference collection of viruses and viroids by linking collections maintained by individual institutions via Q-bank.
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). Over a thousand virus species are included and relevant information for each species is provided. The inclusion of additional data and corresponding nucleotide sequences will allow provisional identification of 'unknown' virus isolates using the 'search' and 'BLAST' functions of Q-bank. Moreover, information on the availability of over 500 virus isolates from international laboratories can be obtained.
Within the VirusCollect project, standard operating procedures (SOP's) were developed and implemented by participating laboratories to guarantee the quality of isolates and data. In addition, more than 60 virus isolates of phytosanitary and/or economic importance were characterised and the corresponding data included in Q-bank. Characterised isolates were adequately stored to maintain their viability and guarantee the availability for future reference and use.
VirusCollect has enabled the first step in collaboration between curators and standardisation of maintenance of virus collections. Fundamental standards laid the basis for improving the quality of individual collections and the layout of Q-bank as a platform to share data and information. The achievements towards a common reference collection were appreciated and resulted in a follow-up project, Euphresco VirusCollect II, in which eight countries have expressed an interest to join.

Detection of *Potato spindle tuber viroid* (PSTVd) using newly generated primers and probe by real- time RT- PCR method

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Potato spindle tuber viroid (PSTVd) belonging to family of *Pospiviroidae* and genera of *Pospiviroid* is known causing potato spindle tuber disease. PSTVd is an agent listed quarantine organism for many regions, including the European Union. Furthermore PSTVd is listed in Regulation on Plant Quarantine of "Harmful organisms that have limited existence in Turkey, that are subject to quarantine and that hinder importation" (Annex 2. B). PSTVd isn't detected by using serological methods due to lacking of coat protein. Starting point for the control of PSTVd is the use of a rapid and reliable detection method. With this in mind a one-step real-time RT-PCR assay based on TaqMan™ has been widespread in recent years. In this study, it was aimed that to generate new and alternative primers and probe which detects most of isolate of PSTVd in the world. The primers were designed by using full genome of PSTVd from NCBI (National Center for Biotechnology Information) and using several softwares such as BLAST (Basic Local Alignment Search Tool). The BLAST showed that the primers strongly matched with wide range of isolates of PSTVd. The primers were performed by using one step real time RT-PCR (TaqMan hydrolyse probe) method that it was perfectly matched with PSTVd isolates which were isolated tomato and potato plants. After getting the results, the method was validated to be used in The Quarantine laboratories for detection of PSTVd. The BLAST was also showed that the primers can match by slight chance with *Tomato chlorotic dwarf viroid* (TCDVd), *Mexican papita viroid* (MPVd) and *Columnea latent viroid* (CLVd). Need to conclude that the primers are strongly specific just for detection of PSTVd. So that, real time RT-PCR analysis for TCDVd, MPVd and CLVd have still been performed.

Keywords: Detection, newly generated primers, *Potato spindle tuber viroid*, real time RT- PCR, TaqMan hydrolyse probe

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