Potato mop top virus, a soilborne virus transmitted by Spongospora subterranea: distribution, problems and possible solutions

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1. Etiology and symptoms
2. Geographical distribution
3. Diagnostics
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1. ETIOLOGY and SYMPTOMS
Foliar symptoms caused by PMTV are rare in Scandinavia, except in Norway.

Foliar symptoms occur in Scotland.
Potato mop-top virus (PMTV)

- Infects roots and tubers, but only rarely the green parts of the plant
- Causes necrotic rings and arcs inside tubers and on tuber surface
Other causal agents of spraing symptoms

*Tobacco rattle virus* (TRV) 
(nematode-transmitted)

*Potato virus Y* (PVY) 
(aphid-transmitted)
Saturna is a PMTV-sensitive cultivar, but resistant to TRV.

Therefore, the spraing symptoms observed in Saturna are likely caused by PMTV.

Figure 1 Characteristic spraing symptoms in the flesh of PMTV-infected potato tubers (upper image) and the consequent quality losses experienced in the production of potato chips (crisps) (lower image) from cv. Saturna.
Symptoms in tubers
Immunostaining with antibodies to PMTV coat protein

Infected (necrotic arcs pointed out by arrows)

Healthy

Detection of PMTV based on symptoms can be highly unreliable!

Latvala-Kilby et al. 2009, Phytopathology 99:519-531
Figure 3 The mean incidence of spraing symptoms in tubers of 20 potato cultivars grown in the same PMTV-infested test field in Finland for 3–10 years (number of years is indicated at the top of each bar) during the period 2000–2009. The disease incidence of each cultivar was compared with that of cv. Saturna. In group b, the incidence of symptoms was similar to Saturna, whereas in groups a and c, the incidence of symptoms was lower and higher than in Saturna, respectively. Note that symptomless PMTV infections were not considered. For example, 54–55% of the tubers of cv. Kardel were infected with PMTV in 2006 and 2007, but 90–100% of the infected tubers were symptomless (Latvala-Kilby et al., 2009).
Potato mop-top virus (PMTV)

Transmitted by zoospores, and retained infective in the resting spores, of the soilborne microbe Spongospora subterranea
NATURAL RESERVOIRS OF PMTV

Black nightshade
(Solanum nigrum)

(Photo: Steen Lykke Nielsen)

PMTV was detected in the roots of 19 % (n = 222) of the black nightshade plants growing at edges of potato fields in Denmark
EFFICIENT SAMPLING for SURVEYING FIELDS for PMTV

Sporangia of *S.s.* are concentrated in the soil on tubers →

Sampling of soil falling under the potato grading machine

Bait plant roots tested for PMTV
2. GEOGRAPHICAL DISTRIBUTION
Distribution of *Potato mop-top virus* as known in 2005

- 1960s
- 1980s
- 2002

**South America**

- Hinostrava & French 1972;
- Salazar & Jones 1975

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E.L. Calvert & B.D. Harrison 1966
R.A.C. Jones, J.I. Cooper…
Survey of PMTV in the Baltic Sea region

"Enhanced control of *Potato mop-top virus* in the Nordic and Baltic Sea region"

2005-2010

GOALS:

- Defined knowledge on geographic distribution of PMTV
  - Study on genetic variability (all countries where PMTV is found)
- Improved diagnostic methods for PMTV
- Improved resistance to PMTV in potatoes
Enhanced control of *Potato mop-top virus* in the Nordic and Baltic Sea region

**MEANS:**

i) Utilisation of the latest biotechnological methods and molecular tools

ii) Research training

iii) Platform for institutional collaboration within the Baltic Sea region (universities, research institutes and private enterprises)

Annual project meetings
Funding arrangements

ScanBalt

Roles of donors:

NKJ – research activities (2005-2008)
ScanBalt – workshops and training (2005-2010)
NOVA – education of doctoral students (2005-2010)

AB-RMS
Agrobiotechnology focused on root-microbe systems
Seed producers

- Pohjoisen Kantaperuna Ltd.
  Finland, Sweden, Russia

Breeders

- Graminor
  (BASF, Svalöf Weibull AB)
  Norway, Germany, Sweden
  (Kesko)
- DANESPO
  Denmark

Food industry

- Chips AB
  Finland, Sweden, Norway, Denmark, Lithuania, Latvia, Estonia, Russia
- Kraft Foods
  Estrella/Maarud Agro Group
  Norway, Sweden, Finland, Denmark, Estonia
- A/S Latfood
  Latvia

Agrochemical industry

- Kemira Grow-How
  Finland, Denmark, Estonia, Lithuania, Poland
Occurrence of PMTV, as known at the end of 2008.
Distribution of PMTV in Denmark

Red: PMTV detected.  Yellow: PMTV not detected

Data:
Steen Lykke Nielsen
Final project meeting in Helsinki, February 17-18, 2009

Occurrence of PMTV as known in 2016

- USA & Canada 2004
- Costa Rica 2008
- Colombia 2013*
- Chile 2016
- Japan since 1980s
- China 2013
- Pakistan 2013

* a new pomo-like virus infecting potatoes

Europe since 1960s
3. DIAGNOSTICS
Serological detection (ELISA) of PMTV in potato

Would it be possible to test PMTV in sprouts rather than the tuber?

Sprouts grown in dark
Sprout sap interferes less than tuber sap in ELISA

Detection with PMTV CP-specific monoclonal antibodies

Latvala-Kilby et al. 2009, Phytopathology 99:519-531
Latvala-Kilby et al. 2009, Phytopathology 99:519-531
Dilution of sprout sap / Pooling of samples

Latvala-Kilby et al. 2009, Phytopathology 99:519-531
Main functions of proteins encoded by the three viral (+)ssRNAs

**Multiplication**

- **RNA 1**
  - MetT
  - -148K
  - RNA 1
  - Hel
  - Pol
  - RT 206K
  - Val
  - 6.043 kb

**Vector transmission**

- **RNA 2**
  - -CP
  - RT 91K
  - RNA 2
  - 20K
  - Val
  - 3.134 kb

**Movement**

- **RNA 3**
  - TGB
  - 51K
  - RNA 3
  - 21K
  - 13K
  - 8K
  - Val
  - 2.964 kb

Savenkov et al. 1999
Sandgren et al. 2001
Phylogenetic analysis of CP and RT domain (RNA 2)

RNA2 CP

RNA2 RT

**Potato mop-top virus characterization and differentiation**

Table 3. Genomic RNA type of *Potato mop-top virus* based on the phylogenetic grouping and sequence similarity scanning.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Country of origin</th>
<th>RNA1</th>
<th>RNA2</th>
<th>RNA3</th>
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<td>A</td>
</tr>
</tbody>
</table>

*aSequence accession numbers are the same as shown in Table 1; -, sequence unavailable.*

Novel pomo-like viruses associated with potato in Colombia

SUMMARY/Genetic variability:

- Two types (genetic lineages) of PMTV RNA2 and RNA3: their all four different combinations have been detected in tubers but biological differences, if any, remain to be shown.

- Within each of the three PMTV RNAs genetic variability is limited.

- Two newly found PMTV-like viruses infect potatoes in Colombia
SUMMARY (1)

1. Foliar symptoms caused by PMTV are sporadic and irregularly observed.

2. Symptomless infections in tubers are very common, both in the field and in storage. Virus-specific testing of tubers for PMTV is necessary (like with other potato viruses): DAS-ELISA (MAbs), RT-PCR or IC-RT-PCR.

3. Soil testing for PMTV can be enhanced by collecting soil under potato grading machines and testing samples using bait plants – bait plant roots will be tested for PMTV.

4. Viruliferous sporangia in the soil on seed tubers may be the most important means by which PMTV spreads to new areas. Transmission of PMTV from the seed tuber to progeny tubers via stolons seems less efficient than S.s.-mediated transmission by zoospores.

5. It will be an important goal to prevent spread of PMTV to the PMTV-free areas.
6. All potato cultivars are susceptible to PMTV but their tolerance varies, which is observed as differences in the proportion of infected tubers developing symptoms. Symptom expression varies from year to year and is unpredictable.

7. True resistance to PMTV in potato cultivars will be the only sustainable solution to problems caused by the spraing disease. Potato germplasm should be screened for resistance and suitable accessions included in breeding programmes. Genetic engineering of cultivars for PMTV resistance could be used, too.

8. The limited genetic variability of PMTV implies that resistance breeding should provide an efficient means to control PMTV.
SUMMARY (2)

6. All potato cultivars are susceptible to PMTV but their tolerance varies, which is observed as differences in the proportion of infected tubers developing symptoms. Symptom expression varies from year to year and is unpredictable.

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→ COULD WE MANIPULATE PLANT FACTORS (GENES) REQUIRED BY PMTV FOR EFFICIENT INFECTION OF POTATO PLANTS,

CONVERT SUSCEPTIBILITY FACTORS TO RESISTANCE FACTORS?
Coordinated functions of TGB proteins

Model: TGBp3 and TGBp2 of PMTV mediate the transport of a TGBp1-containing ribonucleoprotein complex to and through the plasmodesmata, whereas TGBp2 and TGBp3 are not transported to the adjacent cell.

(Zamyatnin et al. 2004, MPMI 17: 921-930)
TGB proteins must be expressed at suitable relative molarities for viral movement to occur.

TGBp3 should be expressed at 10-fold lower levels than TGBp2, and 100-fold lower levels than TGBp1 (Tilsner et al. 2010). Careful regulation of expression levels seems pivotal.

→Could we interfere with the regulation of viral functions, e.g., by affecting plant factors possibly needed in the regulation?

Tool for the study: infectious clone of PMTV

Infectious PMTV was generated using transcripts of the full-length cDNA clones of RNA1, RNA2 and RNA3.
Protein phosphorylation is a typical mechanism to regulate functions

Is TGBp3 phosphorylated?

The putative phosphotyrosine sites in PMTV TGBp3 were predicted using NetPhos 2 and Scansite.

p3 detected with anti-pY antibodies in a PMTV-infected plant
Infectious clone of PMTV was engineered to express Myc-tagged TGBp3
Tyrosine-phosphorylated higher molecular weight host proteins were also detected.

Samuilova et al. 2013, J. Virol. 87:4313-4321
Multiplication and/or cell-to-cell movement of PMTV is inhibited, when tyrosine residues of TGBp3 are substituted for alanine

A. Detection of PMTV in inoculated (I) leaves (cell-to-cell movement) and upper leaves (S) following long-distance movement, by RT-PCR shows that both functions are affected in all mutant viruses.

(P.S. No infectivity at all was observed with virus mutants with Tyr120 substituted for alanine.)

B. Confocal microscopy of *N. benthamiana* leaves inoculated with PMTV that express GFP fused to TGBp1.

- **GFP-PMTV** expresses wild-type TGBp3 (large number of cells infected by cell-to-cell movement).
- **GFP-PMTV21K_{87-89A}** : only single cells infected, no cell-to-cell movement.
  (GFP-PMTV21K_{120A} : no infection detected)

Samuilova et al. 2013, J. Virol. 87:4313-4321
**Future prospects:**

Proteins of many mammalian viruses are phosphorylated by tyrosine kinases, and kinase inhibitors can increase survival of the host by reducing viral load and dissemination of the virus to distal tissues.

It is conceivable that inhibition of tyrosine kinases required for phosphorylation of viral proteins could have a role to play in combating viral infections also in plants.
Thank you!

The Viikki Life Science Campus of University of Helsinki, Finland


