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Utilizing plants defensive mechanism to detect viruses – from theory to practice

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2nd N'TOP Stakeholder meeting

March 17th, 2022

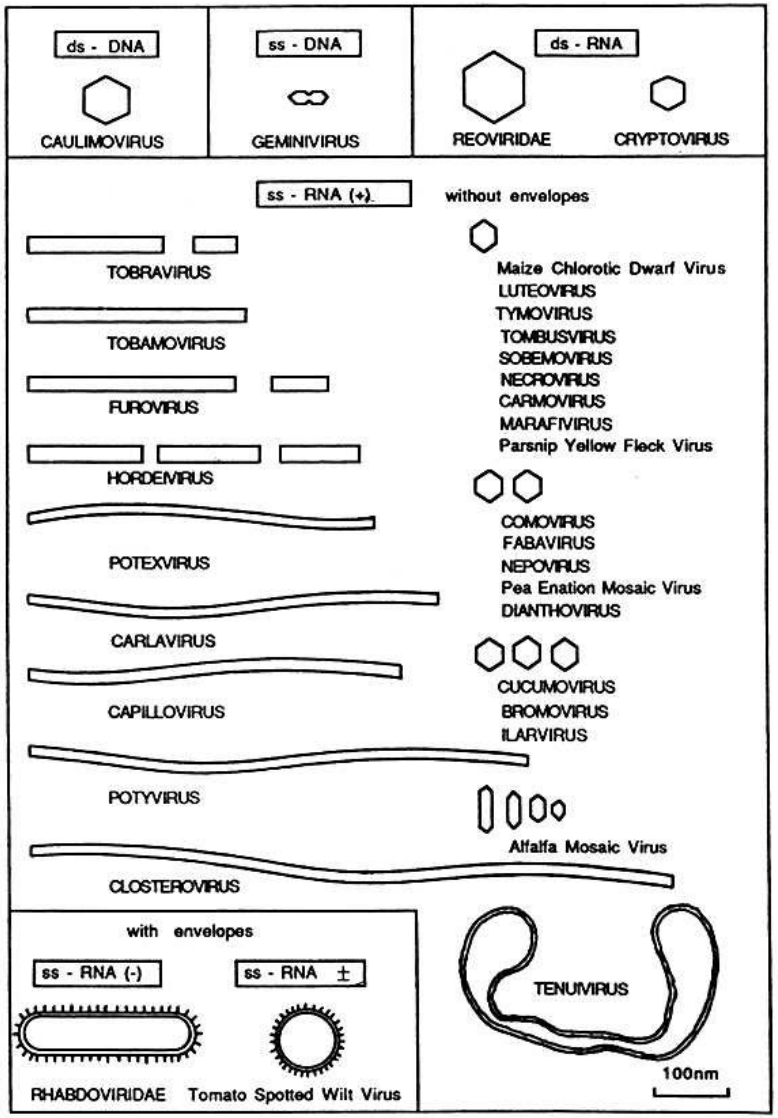


Figure 5.1 The families and groups of viruses infecting plants. Outline diagrams are drawn approximately to scale. (Courtesy R. I. B. Francki.) One additional group, the commelina yellow mottle virus group with dsDNA and bacilliform particles, was approved by the I.C.T.V. in August 1990.

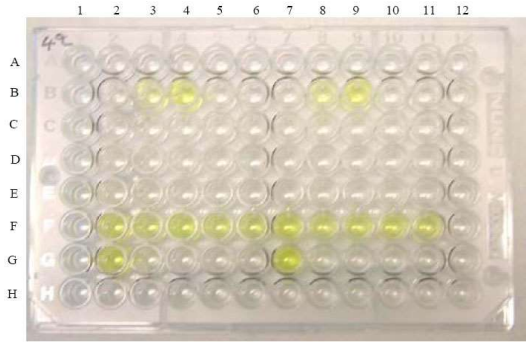
Potato viruses

Alfalfa mosaic virus (AMV), *Alfamovirus, Bromoviridae*
Andean potato latent virus (APLV), *Tymovirus, Tymoviridae*
Andean potato mottle virus (APMV), *Comovirus, Comoviridae*
Arracacha virus B (AVB), tentative *Cheravirus, Sequiviridae*
Beet curly top virus (BCTV), *Curtovirus, Geminiviridae*
Cucumber mosaic virus (CMV), *Cucumovirus, Bromoviridae*
Eggplant mottled dwarf virus (EMDV), *Nucleorhabdovirus, Rhabdoviridae*
Potato aucuba mosaic virus (PAMV), *Potexvirus, Flexiviridae*
Potato black ringspot virus (PBRSV), *Nepovirus, Comoviridae*
Potato deforming mosaic virus = *Tomato yellow vein streak virus* (ToYVSV), *Begomovirus, Geminiviridae*[d]
Potato latent virus (PotLV), *Carlavirus, Flexiviridae*
Potato leafroll virus (PLRV), *Polerovirus, Luteoviridae*
Potato mop-top virus (PMTV), *Pomovirus*, -
Potato rough dwarf virus = Potato virus P, tentative *Carlavirus*[e]
Potato virus A (PVA), *Potyvirus, Potyviridae*
Potato virus M (PVM), *Carlavirus, Flexiviridae*
Potato virus S (PVS), *Carlavirus, Flexiviridae*
Potato virus T (PVT), *Trichovirus, Flexiviridae*
Potato virus U (PVU), *Nepovirus, Comoviridae*
Potato virus V (PVV), *Potyvirus, Potyviridae*
Potato virus X (PVX), *Potexvirus, Flexiviridae*
Potato virus Y (PVY), *Potyvirus, Potyviridae*
Potato yellow dwarf virus (PYDV) *Nucleorhabdovirus, Rhabdoviridae*
Potato yellow mosaic virus (PYMV), *Begomovirus, Geminiviridae*
Potato yellow vein virus (PYVV), tentative *Crinivirus, Closteroviridae*
Potato yellowing virus (PYV), tentative *Alfamovirus*[f]
Solanum apical leaf curl virus (SALCV), tentative *Begomovirus*
Sowbane mosaic virus (SoMV), *Sobemovirus*, -
Tobacco mosaic virus (TMV), *Tobamovirus*, -
Tobacco necrosis virus (TNV), *Necrovirus, Tombusviridae*
Tobacco rattle virus (TRV), *Tobravirus*, -
Tobacco ringspot virus (TRSV), *Nepovirus, Comoviridae*
Tobacco streak virus (TSV), *Ilarvirus, Bromoviridae*
Tomato black ring virus (TBRV), *Nepovirus, Comoviridae*
Tomato mosaic virus (ToMV), *Tobamovirus*, -
Tomato mottle Taino virus (ToMoTV), *Begomovirus, Geminiviridae*
Tomato spotted wilt virus (TSWV), *Tospovirus, Bunyaviridae*

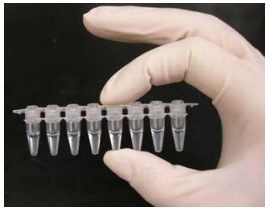
Currently used methods: test selected based on symptoms or legistlation

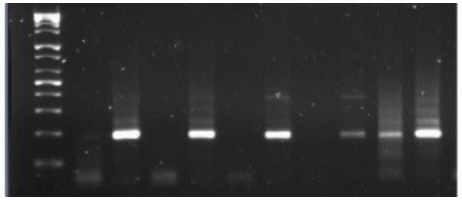
Symtoms + host = Virus present?

Selection of test plants



Selection of antibodies (ELISA)





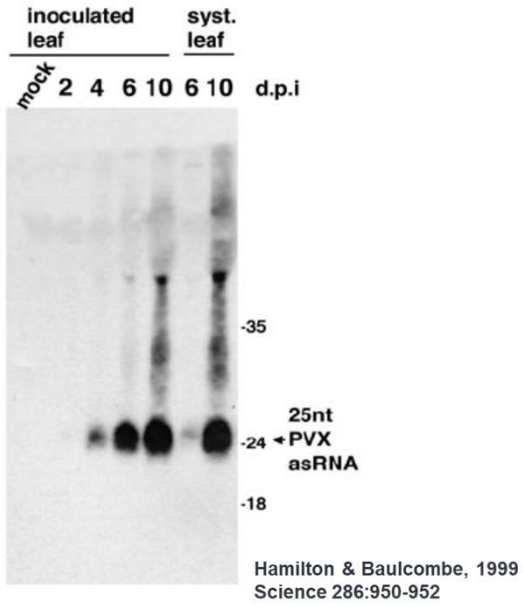
Selection of primers (PCR) and probe (real time PCR)

Tests are mainted for the most common viruses and for the viruses mentioned in legistlation.

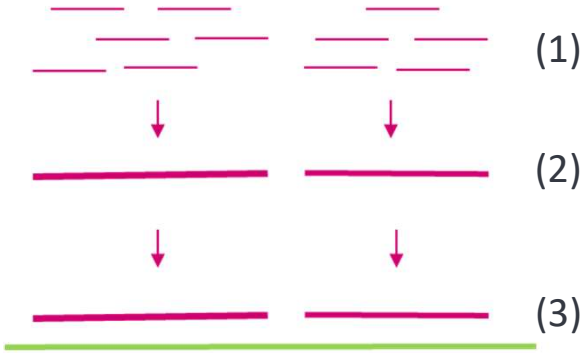
Small RNA sequencing

- All plants use RNA silencing to defend against viruses

- RNA silencing causes accumulation of (viral) small RNA in the plant



- These small RNAs can be sequenced (1) and contiquous sequences (contigs) can be build using bioinformatics (2). Contigs can be aligned to viral sequences in databases (3).



Small RNA sequencing (and other HTS methods)

- Detection of known, unexpected and ’new-to-science’ viruses
  - No need to decide which viruses are tested
- Already in wide use in research purposes

DISEASE NOTES

First Report of *Tobacco rattle virus* Infecting *Lysimachia nummularia* in Finland

J. Santala, J. Jukkala, J. Tuomola, and J. P. T. Valkonen

Affiliations

Published Online: 9 Dec 2019 | https://doi.org/10.1094/PDIS-03-19-0464-PDN

DISEASE NOTE

OPEN

First Report of Soybean Dwarf Virus Infecting White Clover (*Trifolium repens*) in Finland

A. Luoto, M. Lehtonen, J. P. T. Valkonen, and J. Santala

Affiliations

Published Online: 3 Dec 2021 | https://doi.org/10.1094/PDIS-04-21-0822-PDN

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Research project:
Viruses in Finnish potato production

Samples

Pre-basic and basic seed	Other seed classes	Ware potato	Wild plants
172 samples obtained from official seed samples arriving to Finnish Food Authority	64 samples from field inspections	94 samples obtained from official plant health samples arriving to Finnish Food Authority	241 samples belonging to 52 plant species collected from HG zone and other potato production areas

Samples analyzed using small RNA sequencing

Viruses threatening Finnish potato production. Assesment of the national seed potato legistlation.

Results

- From seed potato only PVY was found
  - PVY was found from the same samples as in official tests (ELISA)
- From ware potato also PMTV, PVS and PVM were found
  - PVS was found from 6 potato samples and PVM from 1 sample
  - PVS and PVM confirmed by real-time-PCR

Year of harvest	Number of samples	Proportion of individual samples containing viruses (estimated using Seedcalc 8 –software)			
		PVY	PMTV	PVS	PVM
2017	48	11,4 %	25,5 %	0 %	0 %
2018	46	45,1 %	4,9 %	10,7 %	2,4 %

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Routine use in plant health

COST-DIVAS: Deep Investigation of Virus Associated Sequences

- Application of deep sequencing methods in plant health
- Best practices and limits for the use of deep sequencing in rutine diagnostics
- E.g. proficiency test for the bioinformatics pipelines used in different laboratories (detection of viruses from ready-made data sets)
- Co-operation with EPPO to design quality assurance policy for small RNA sequencing

Bulletin OEPP/EPPO Bulletin (2018) **48** (2), 219–224 ISSN 0250-8052. DOI: 10.1111/epp.12472

High-throughput sequencing technologies for plant pest diagnosis: challenges and opportunities

A. Olmos[1], N. Boonham[2], T. Candresse[3], P. Gentit[4], B. Giovani[5], D. Kutnjak[6], L. Liefting[7], H.J. Maree[8], A. Minafra[9], A. Moreira[10], M.K. Nakhla[11], F. Petter[5], M. Ravnikar[6], B. Rodoni[12], J.W. Roenhorst[13], M. Rott[14], A.B. Ruiz-García[14], J. Santala[15], G. Stancanelli[16], R. van der Vlugt[17], C. Varveri[18], M. Westenberg[13], T. Wetzel[19], H. Ziebell[20] and S. Massart[21]

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High-throughput sequencing (HTS) technologies have revolutionized plant pest research and are now raising interest for plant pest diagnostics, with plant virus diagnostics at the forefront of development. However, the application of HTS in plant pest diagnostics raises important challenges that plant health regulators will have to address. Adapted infrastructures, technical guidelines and training are pivotal for further use and adoption of the HTS technologies in the phytosanitary framework.

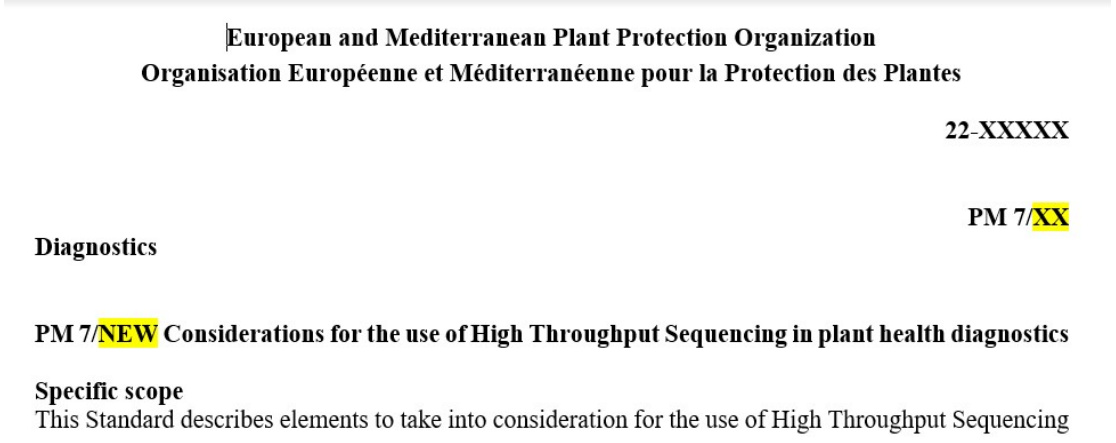
1. Introduction

Early diagnosis and a rapid response are crucial to reduce the risk of entry and spread of plant pests into an area. In 2016, the Commission on Phytosanitary Measures adopted a recommendation recognizing that 'pest diagnosis is a cross cutting issue that underpins most International Plant Protection Convention (IPPC) activities. In order to take action against a pest, it must be accurately identified. To enable safe trade, pest diagnosis must further be completed quickly and to a high level of confidence' (FAO, 2016). National Plant Protection Organizations (NPPOs) routinely perform pest diagnosis to support export certification, import inspections, pest surveillance and eradication programmes. Plant pests can be managed most effectively when control measures are implemented at an early stage of infestation. Plant pest diagnostics is based on the use of a range of methods underpinned by different biological principles (e.g. bioassay, biochemical, isolation/extraction methods, molecular methods, morphological and morphometric and serological methods), some of them being highly specific and others more generic. The ability to detect plant pests varies with the sensitivity and specificity of the detection tools used. The recent development of high-throughput sequencing (HTS) technologies, also called next-generation sequencing (NGS) or deep sequencing, has revolutionized the research on plant-associated organisms. These techniques are beginning to be

EPPO Bulletin 48, 219-224.

Rutine use requires harmonization

- To be used in routine testing, characteristics of a test have to be evaluated
  - Sensitivity
  - Specificity (inclusivity and exclusivity)
  - Repeatability
  - Reproducibility
- EPPO standard almoust ready
  - How to set up a HTS test
  - How to evaluate test characteristics
  - How to ensure traceability
  - When is additional testing required
  - How to report findings



European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

22-XXXXX

PM 7/XX

Diagnostics

PM 7/NEW Considerations for the use of High Throughput Sequencing in plant health diagnostics

Specific scope
This Standard describes elements to take into consideration for the use of High Throughput Sequencing

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Thank you!

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