

The plant virome: the quantum leap of HTS technologies and its consequences on plant health regulation

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1. Plant-virus equilibrium : wild ecosystems




Nearly always one or more asymptomatic host (no fitness penalty)

2

1. Plant-virus equilibrium : its disruption



- New host encountering
- Host jump leading to disease
- Mutation of host/virus

3

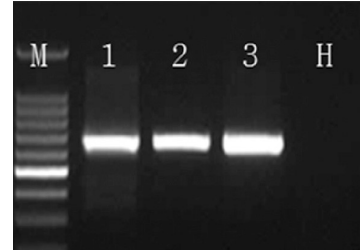
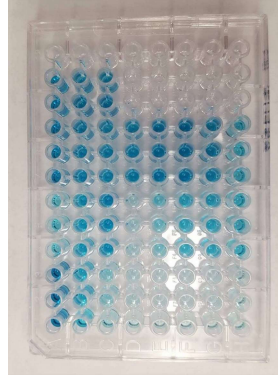
2. Virus detection



- Historically long process
- Disease-based
- Symptom etiology

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2. Virus detection: technical evolution



5

2. Virus detection: here comes the HTS technologies



- The virus discovery party !



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2. Virus detection: here comes the HTS technologies



Disease etiology



Healthy crops

Wild plants



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2. Virus detection: HTS technologies quantum leap

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PM 7/151 (1) Considerations for the use of high throughput sequencing in plant health diagnostics¹

First published: 28 October 2022 | <https://doi.org/10.1111/epp.12884>

- Used in several NRL in EU as well as in US, UK, Australia...
- ISO17025 accredited HTS-based tests



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3. What's the problem with HTS ?



Known virus
Known host

Known virus
Unknown host

Unknown virus

Contamination

9

3. What's the problem with HTS ?



Known virus
Known host

Known virus
Unknown host

Unknown virus

Contamination

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3. What's the problem with HTS ?



Known virus
Known host

- « Business as usual » (action if regulated)

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3. What's the problem with HTS ?



Known virus
Unknown host

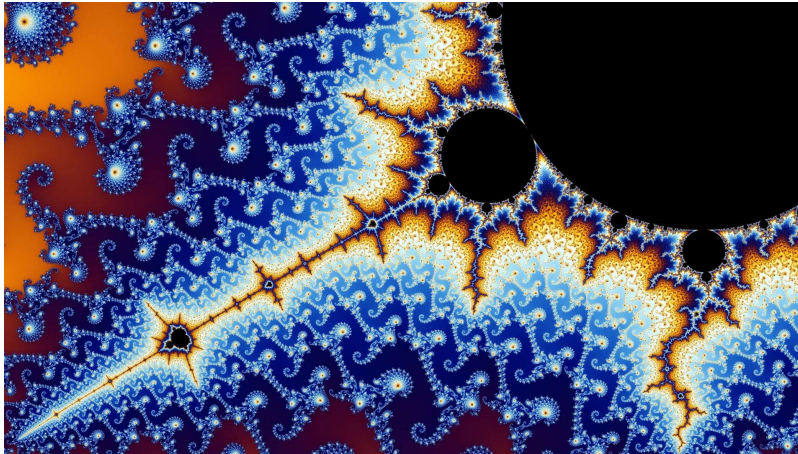
- Unexpected detection of regulated virus on a new host
- Trickier but remains within usual scope :
 - is it infectious from this material ? New pathway ?
 - Adapting trade regulation ? Quickly ?

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3. What's the problem with HTS ?



Unknown virus



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3. What's the problem with HTS ?



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in Microbiology

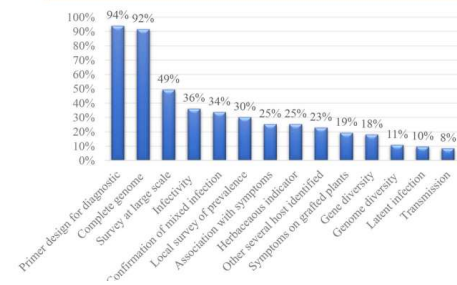
REVIEW
published: 19 November 2020
doi: 10.3389/fmicb.2020.592816



Is There a “Biological Desert” With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses

Wanying Hou^{1,2,3}, Shifang Li^{3,4*} and Sebastien Massart^{2*}

Unknown virus

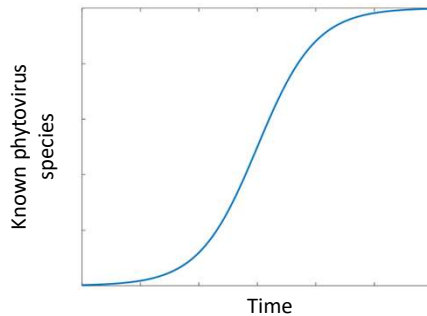


How to evaluate risks without or with minimal biological information ?

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3. What's the problem with HTS ?

- The rythm accelerates but will slow down



- But when ?

Unknown virus

- Wikipedia of plant viruses on earth



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4. How to deal with the new viruses : a framework

frontiers
in Microbiology

PERSPECTIVE
published: 24 January 2017
doi: 10.3389/fmicb.2017.00045



A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies

Sebastien Massart^{1*}, Thierry Candresse¹, José Gil², Christophe Lacomme¹, Lukas Predajna³, Maja Ravnikar⁴, Jean-Sébastien Reynard¹, Artemis Rumbou⁵, Pasquale Saldarelli⁶, Dijana Škorić⁷, Eeva J. Vainio⁸, Jari P. T. Valkonen⁹, Hervé Vanderschuren¹⁰, Christina Varveri¹¹ and Thierry Wetzal¹²

- Efficiency
- Multistakeholder approach
- Communication, communication & communication

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4. How to deal with the new viruses : limits



frontiers
in Microbiology

PERSPECTIVE
published: 03 January 2017
doi: 10.3389/fmicb.2017.00404

A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies

Sebastien Massart^{1*}, Thierry Candresse², José Gil³, Christophe Lacomme⁴, Lukas Predajna⁵, Maja Ravnikar⁶, Jean-Sébastien Reynard⁷, Artemis Rumbou⁸, Pasquale Saittarello⁹, Dijana Skoric¹⁰, Eeva J. Vainio¹¹, Jari P. T. Valkonen¹², Hervé Vanderschuren¹³, Christina Varren¹⁴ and Thierry Wetzlar¹⁵



- 3.5 years of researcher + international collaboration for one virus

Plant Disease • 2022 • 106:2797-2807 • <https://doi.org/10.1094/PDIS-12-21-2808-RE>

Research

e-Xtra*

Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time

Coline Temple¹, Arnaud G. Blouin^{1,2}, Kris De Jonghe³, Yuka Foucart⁴, Marleen Botermans⁴, Marel Westenberg⁴, Ruben Schone⁵, Pascal Gentil⁶, Michèle Vignac⁷, Eric Verdin⁸, Catherine Wajsbichel⁹, Heiko Zabel¹⁰, Yajaya Z. A. Gualter⁷, Anjad Zia¹¹, Xiao-Hua Yan¹², Katja R. Richter-Püggeter¹³, Roswitha Ulrich¹⁴, Mark Paul S. Rivasere¹⁵, Denis Kutnjak¹⁶, Ana Vucurcovic¹⁷ and Sebastien Massart^{1*}

Biological characterization of an emergent virus infecting vegetables in diversified production systems: physostegia chlorotic mottle virus

Coline Temple¹, Arnaud G. Blouin², Dieke Boezen³, Marleen Botermans⁴, Laurena Durant¹, Kris De Jonghe⁵, Pier de Koning⁶, Thomas Goedefroit⁷, Laurent Minee⁸, Stephan Steyer⁹, Eric Verdin⁸, Mark Zwart⁸, Sebastien Massart^{1*}

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SO WHAT ???



Raising WW use of HTS technologies

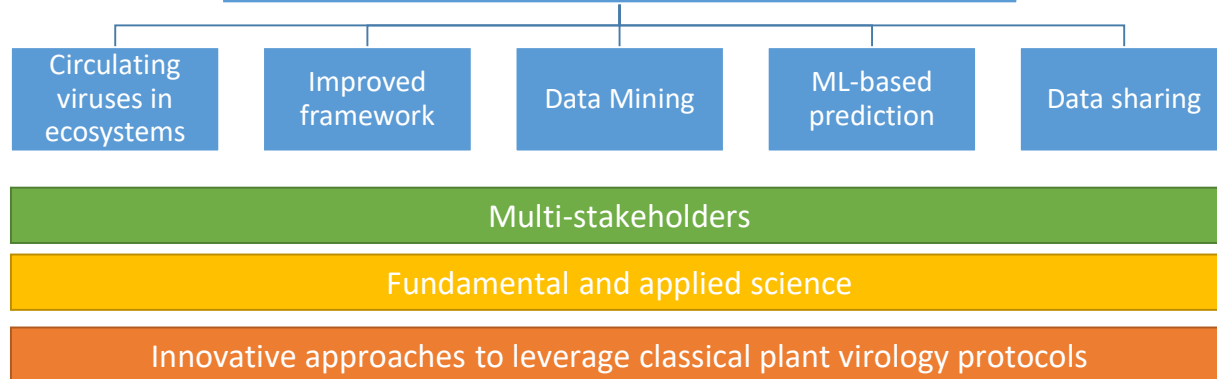
Absence of information for PRA and regulation

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5. What do we need ?



Information-based regulation and management of plant viruses

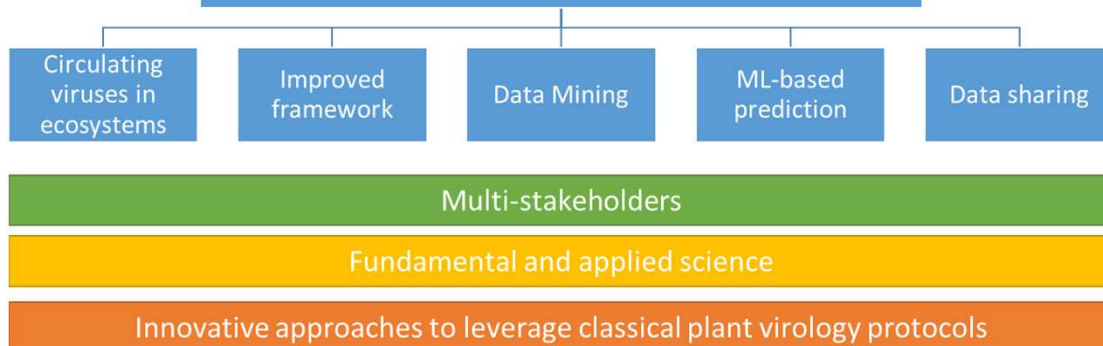


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5. What do we need ?



Information-based regulation and management of plant viruses



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5. What do we need ?

Circulating
viruses in
ecosystems

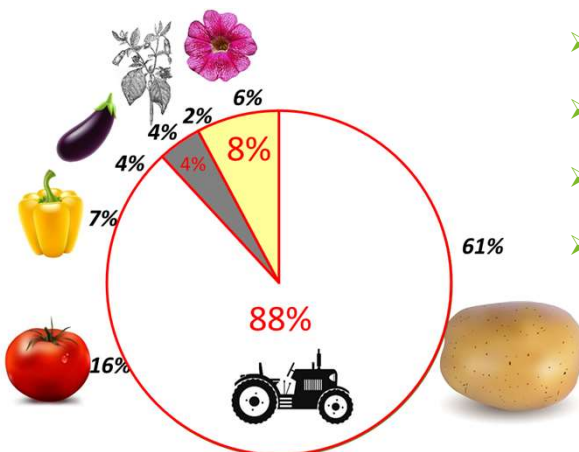


- Seviplant project: sequencing 17,600 plants



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Nation-wide blueprint of Solanaceae viruses



- Total 17,600 plants
- 24 different genera
- In *Solanum* alone, 14 species
- Intra-species diversity
 - *Solanum lycopersicum* > 60 cv.
 - *Solanum tuberosum* > 45 cv.
 - *Capsicum annuum* > 15 cv.



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Nation-wide blueprint of Solanaceae viruses



Potato	Tomato	Capsicum	Eggplant	Nightshade	Ornamental	Mix pool
PVY	PVY	CMV	CMV	PVY	PVY	PVY
AMV	CMV	PhCMoV	MeCSV	AMV	BrLV	CMV
PhCMoV	LRNV	PMMV	MPV	DMV-like	CDV	PLRV
PnLV	OLV-1	ToMV	OLV-1	MeCSV	CMV	ArMV
PVA	PhCMoV	SpLV	OMMV	SnIV-1	OLV-1	CMoV
PVV	SLRSV		PhCMoV	SVS	PLV	New marafivirus
TRV	SpLV		SnIV-1	CMV	PIVX	
PVS	TBRV		TBSV		PVM	
PLRV	ToCV		SLRSV		PVX	
ToMV	ToMV				PYV	
ToCV	TSWV				ToCV	
SnIV-1	PepMV				ToMV	
	OMMV				TRV	
	TNV				New carlavirus	
					New dianthovirus	

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Nation-wide blueprint of Solanaceae viruses: new species



Potato	Tomato	Capsicum	Eggplant	Nightshade	Ornamental	Mix pool
PVY	PVY	CMV	CMV	PVY	PVY	PVY
AMV	CMV	PhCMoV	MeCSV	AMV	BrLV	CMV
PhCMoV	LRNV	PMMV	MPV	DMV-like	CDV	PLRV
PnLV	OLV-1	ToMV	OLV-1	MeCSV	CMV	ArMV
PVA	PhCMoV	SpLV	OMMV	SnIV-1	OLV-1	CMoV
PVV	SLRSV		PhCMoV	SVS	PLV	New marafivirus
TRV	SpLV		SnIV-1	CMV	PIVX	
PVS	TBRV		TBSV		PVM	
PLRV	ToCV		SLRSV		PVX	
ToMV	ToMV				PYV	
ToCV	TSWV				ToCV	
SnIV-1	PepMV				ToMV	
	OMMV				TRV	
	TNV				New carlavirus	
					New dianthovirus	

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Nation-wide blueprint of Solanaceae viruses: new hosts

Potato	Tomato	Capsicum	Eggplant	Nightshade	Ornamental	Mix pool
PVY	PVY	CMV	CMV	PVY	PVY	PVY
AMV	CMV	PhCMoV	MeCSV	AMV	BrLV	CMV
PhCMoV	LRNV	PMMV	MPV	DMV-like	CDV	PLRV
PnLV	OLV-1	ToMV	OLV-1	MeCSV	CMV	ArMV
PVA	PhCMoV	SpLV	OMMV	SnIV-1	OLV-1	CMoV
PVV	SLRSV		PhCMoV	SVS	PLV	New marafivirus
TRV	SpLV		SnIV-1	CMV	PIVX	
PVS	TBRV		TBSV		PVM	
PLRV	ToCV		SLRSV		PVX	
ToMV	ToMV				PVY	
ToCV	TSWV				ToCV	
SnIV-1	PepMV				ToMV	
	OMMV				TRV	
	TNV				New carlavirus	
					New dianthovirus	

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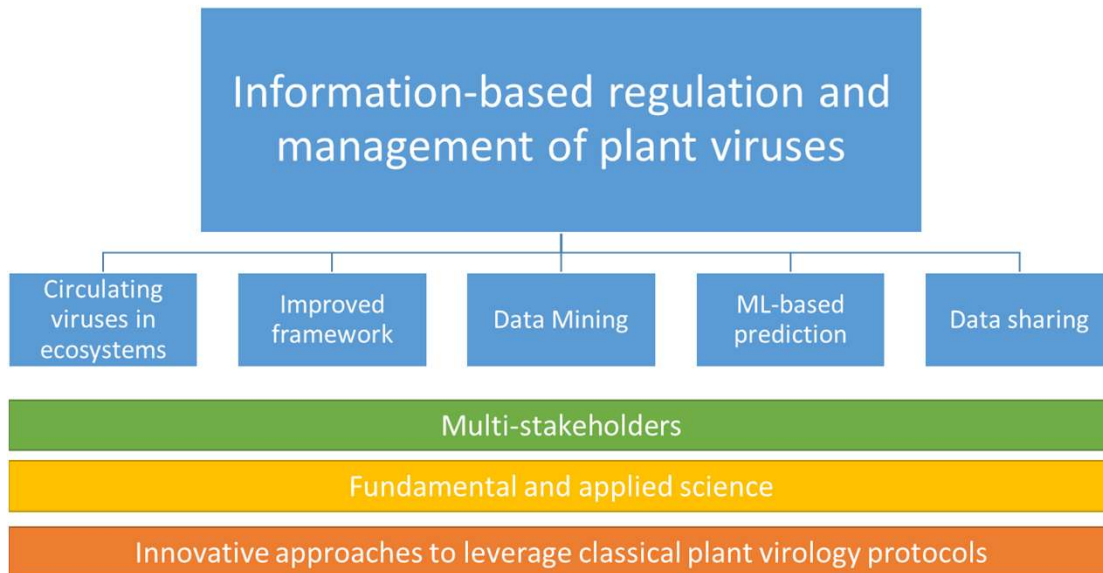


Nation-wide blueprint of Solanaceae viruses: new for Belgium

Potato	Tomato	Capsicum	Eggplant	Nightshade	Ornamental	Mix pool
PVY	PVY	CMV	CMV	PVY	PVY	PVY
AMV	CMV	PhCMoV	MeCSV	AMV	BrLV	CMV
PhCMoV	LRNV	PMMV	MPV	DMV-like		PLRV
PnLV	OLV-1	ToMV	OLV-1	MeCSV	CMV	ArMV
PVA	PhCMoV	SpLV	OMMV	SnIV-1	OLV-1	CMoV
PVV	SLRSV		PhCMoV	SVS	PLV	New marafivirus
TRV	SpLV		SnIV-1	CMV	PIVX	
PVS	TBRV		TBSV		PVM	
PLRV	ToCV		SLRSV		PVX	
ToMV	ToMV				PVY	
ToCV	TSWV				ToCV	
SnIV-1	PepMV				ToMV	
	OMMV				TRV	
	TNV				New carlavirus	
					New dianthovirus	

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5. What do we need ?



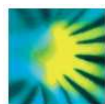
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5. What do we need ?

Data Mining



- SRA repository : Petabases
- Millions of sequencing dataset
- Transcriptome sequencing: hidden (asymptomatic) viruses



New Phytologist

Rapid report | Open Access |

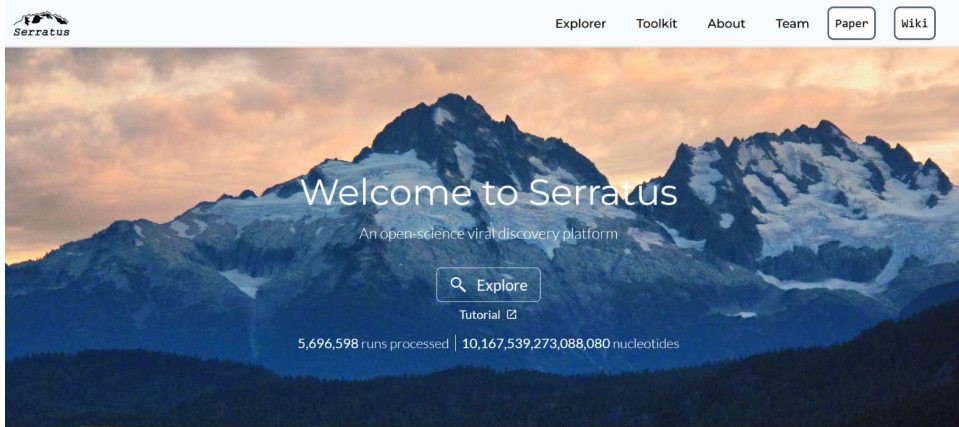
Arabidopsis latent virus 1, a comovirus widely spread in *Arabidopsis thaliana* collections

Ava Verhoeven, Karen J. Kloth, Anne Kupczok, Geert H. Oymans, Janna Damen, Karin Rijnsburger, Zhang JIang, Cas Deelen, Rashmi Sasidharan, Martijn van Zanten, René A. A. van der Vlugt ✉

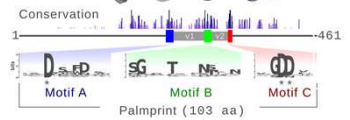
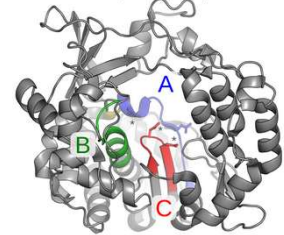
First published: 08 September 2022 | <https://doi.org/10.1111/nph.18466> | Citations: 1

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What do we need? SERRATUS: www.serratus.io



Viral RdRP (Poliovirus)



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SERRATUS: www.serratus.io : PhCoMV case



- ◆ Published data
- ◆ Historic sample < 2017
- ◆ Recent discovery > 2017

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Biological and genetic characterization of Physostegia chlorotic mottle virus in Europe based on host range, location, and time.

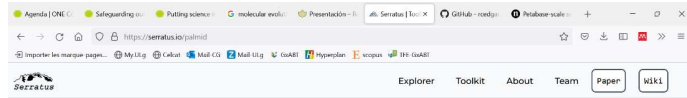
Coline Temple, Arnaud G. Blouin, Kris De Jonghe, Yoika Foucart, Marleen Botermans, Marcel Westenberg, Ruben Schoen, Pascal Gentit, Michele Visage, Eric Verdin, Catherine Wipf-Scheibel, Heiko Ziebell, Yahya Z. A. Gaafar, Denis Kutnjak, Ana Vučurović, ... See all authors

Published Online: 8 Apr 2022 | <https://doi.org/10.1094/PDIS-12-21-2800-RE>



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SERRATUS: www.serratus.io: PhCoMV case



palMID: Viral-RdRP Analysis
Sequence Submission

Info

Sequence, in FASTA format

>Enter your sequence (DNA / Protein)

Parsed FASTA:

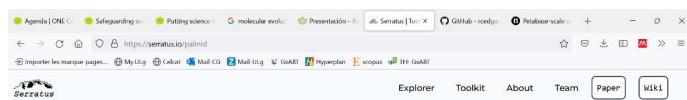
hash:

Analyze Sequence Load Example Clear



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SERRATUS: www.serratus.io: PhCoMV case



palMID: Viral-RdRP Analysis
Sequence Submission

Info

Sequence, in FASTA format

>Enter your sequence (DNA / Protein)

Parsed FASTA:

hash:

Analyze Sequence Load Example Clear



Polemonium pulcherrimum

Plant sample from *Polemonium pulcherrimum*

Identifiers BioSample: SAMN15153850, Sample name: Polemonium pulcherrimum, SRA: SRS6630477

Organism *Polemonium pulcherrimum*
cellular organism; Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Euphyllophyta; Spermatophyta; Magnoliopsida; Mesangiospermae; eudicotyledons; Gunnerales; Parangeliaceae; astrovites; Ericaceae; Polemoniaceae; Polemonium

Package [Plant_vision_1.0](#)

Attributes isolate not applicable
age not applicable
geographic location Not applicable
tissue leaves
biomaterial provider Bonn University Botanical Garden
specimen voucher Hong Ma L37

BioProject [PRJNA636634](#)
Retrieve [all samples](#) from this project

Submission [Eudon,Liberalitat, Calle Zhang](#) 2020-06-06

Accession SAMN15153850 ID: 15153850
BioProject SRA



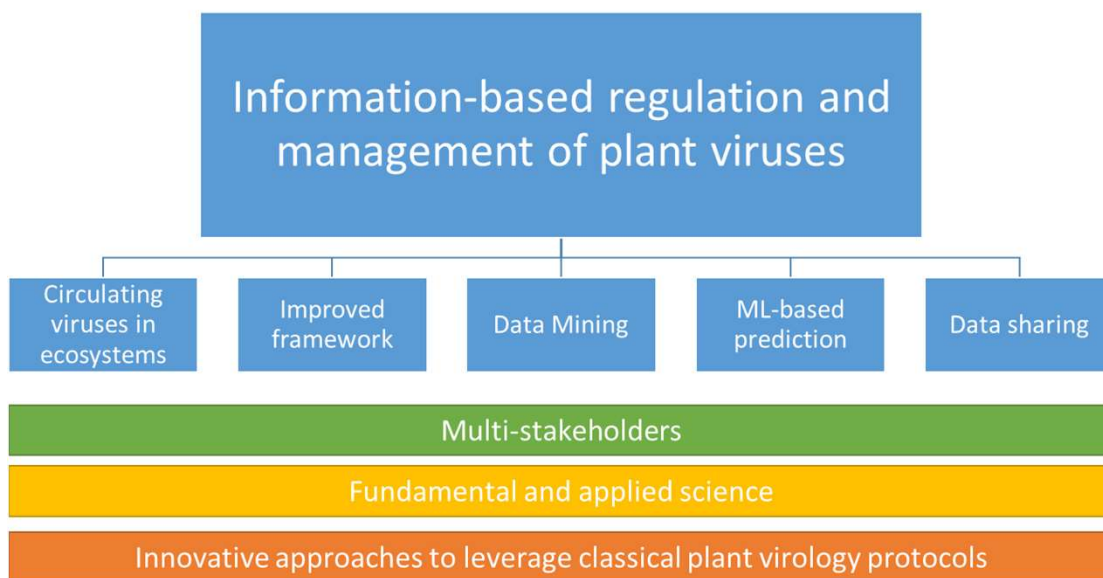
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SERRATUS: www.serratus.io: PhCoMV case

- Virus present in a new host family (*Polemoniaceae*) ?
- Virus present in a new continent (Asia) ?
- Contact researchers having deposited the data
- No way to confirm by RT-PCR
- Ethical and phytosanitary issues ?

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5. What do we need ?



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5. What do we need ?

ML-based
prediction



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HOME > SCIENCE > VOL. 362, NO. 6414 > PREDICTING RESERVOIR HOSTS AND ARTHROPOD VECTORS FROM EVOLUTIONARY SIGNATURES IN RNA VIRUS...

REPORT



Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes

SIMON A. BABAYAN¹, RICHARD J. ORTON¹, AND DANIEL G. STREICKER¹ [Authors Info & Affiliations](#)

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5. What do we need ?

ML-based
prediction



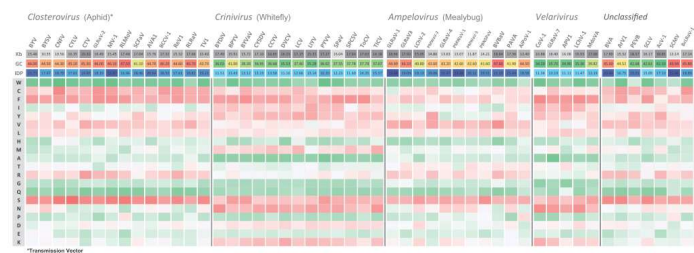
- For plant viruses ?
- A few preliminary studies :



CHAPTER TEN

Flexible spandrels of the global plant virome: Proteomic-wide evolutionary patterns of structural intrinsic protein disorder elucidate modulation at the functional virus–host interplay

Rachid Tahzima^{a,*}, Annelies Haegeman^b, Sébastien Massart^a, and Eugénie Hébrard^c



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5. What do we need ?

Data sharing



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5. What do we need ?

Data sharing



- Informal exchanges during conferences
- « On-the-go »
- DIY : excel file between scientists
- Expand geographical & host range + historical samples

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5. What do we need ?

Improved
framework

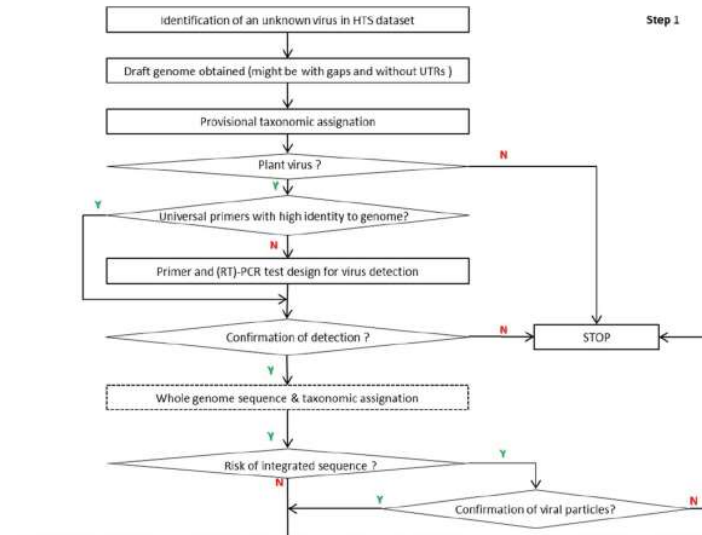


- An improved version of the framework
- In 4 steps
- Will be published in 2023



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5. What do we need ?



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5. What do we need ?

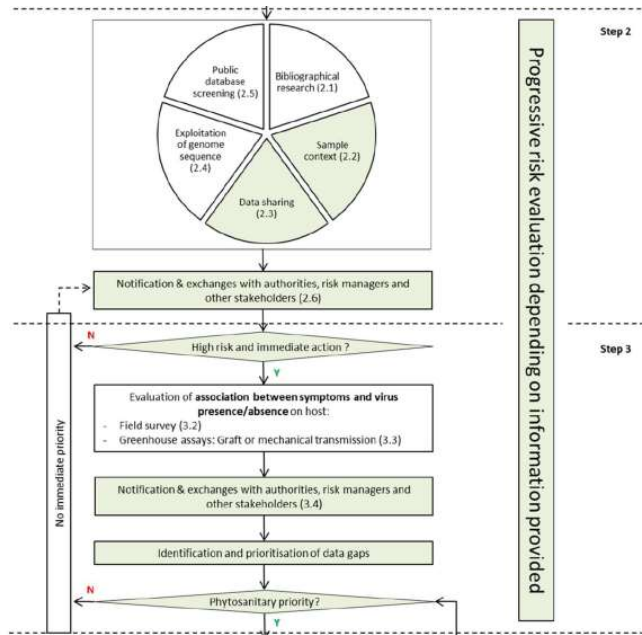
Circulating viruses in ecosystems

Improved framework

Data Mining

ML-based prediction

Risk management



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5. What do we need ?

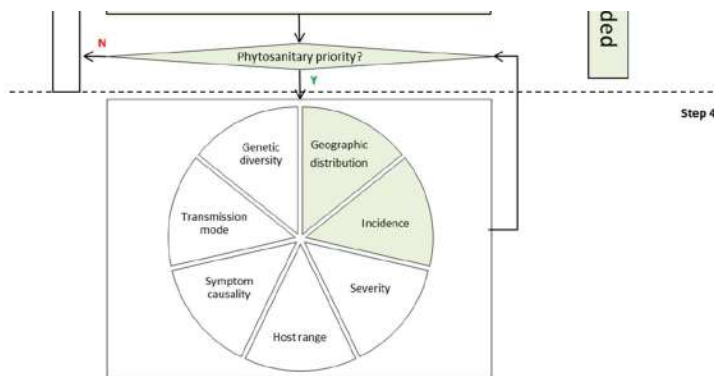
Circulating viruses in ecosystems

Improved framework

Data Mining

ML-based prediction

Risk management



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5. What do we need ?

Circulating
viruses in
ecosystems

Improved
framework

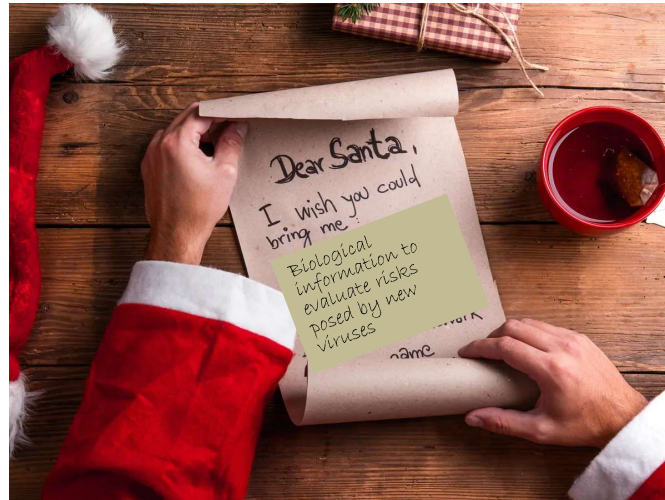
Data Mining

ML-based
prediction

Risk
management



- Will be published in 2023
- But...



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3. What's the problem with HTS ?



Known virus
Known host

Known virus
Unknown host

Unknown virus

Contamination

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3. What's the problem with HTS ?

- HTS is very sensitive -> risk of cross-sample contamination during analyses by researchers



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- When and re

Validation of high throughput sequencing as virus indexing test for *Musa* germplasm: performance criteria evaluation and contamination monitoring using an alien control



act plant health

Wei Rong, Johan Rollin, Marwa Hanafi, Nicolas Roux, and Sebastien Massart

3. What's the problem with HTS ?



ARTICLE

<https://doi.org/10.1038/s41467-022-28143-9> OPEN



The pollen virome of wild plants and its association with variation in floral traits and land use

Andrea M. Fetters^{1,2,3*}, Paul G. Cantalupo^{1,3}, Na Wei^{1,4}, Maria Teresa Sáenz Robles¹, Amber Stanley¹, Jessica D. Stephens^{1,5}, James M. Pipas¹ & Tia-Lynn Ashman^{1,5*}

Supplementary material



Virus family*	Virus genus†	Known virus	Region‡	Plant species§	No. segments recovered¶	Percent sequence coverage¶	No. alignments*	NCBI accession nos.
Bromovirus	Brome mosaic virus		CA	<i>Aquilegia canadensis</i>	2/3	23.75 – 31.41	22 – 29	NC_002026.1
			EDAFI	<i>Tiarellia cordifolia</i>	3/3	17.81 – 32.35*	18 – 30	NC_002027.1 NC_002028.2
Cucumovirus	Peanut stunt virus		EDAFI	<i>Vernonia gigantea</i>	1/3	31.31	18	NC_002040.1
			EDAFI	<i>Impatiens capensis</i>	2/3	9.88 – 22.11*	22809 – 23257	NC_022127.1 NC_022128.1
Bromoviridae	Ageratum latent virus		EDAFI	<i>Lotus corniculatus</i>	3/3	8.86 – 20.02*	66 – 1122	NC_022129.1
			CA	<i>Packera aurea</i>	2/3	20.61 – 61.58	35 – 174	NC_003465.1 NC_003480.1
Bromoviridae	Blackberry chlorotic ringspot virus		EDAFI	<i>Convolvulus arvensis</i>	2/3	29.24 – 63.23	48 – 113	
			EDAFI	<i>Impatiens capensis</i>	3/3	42.48 – 96.77	470785 – 20653066	NC_011553.1
			EDAFI	<i>Lotus corniculatus</i>	3/3	25.49 – 73.52	189 – 344694	NC_011554.1
			EDAFI	<i>Oenothera biennis</i>	2/3	36.86 – 63.01	73 – 347	NC_011554.1
			EDAFI	<i>Solidago</i> sp.	3/3	13.16 – 71.05*	47 – 210	NC_011555.2
Illarvirus	Parietaria mottle virus		EDAFI	<i>Vernonia gigantea</i>	2/3	26.57 – 57.38	47 – 104	
			EDAFI	<i>Impatiens capensis</i>	2/3	8.16 – 22.66*	42 – 69875	NC_005848.1 NC_005849.1

3. What's the problem with HTS ?

SCIENTIFIC OPINION



ADOPTED: 21 November 2019

doi: 10.2903/j.efsa.2020.5928



Contamination

Pest categorisation of non-EU viruses of *Rubus L.*

EFSA Panel on Plant Health (PLH),
 Claude Bragard, Katharina Dehnen-Schmutz, Paolo Gonthier, Marie-Agnès Jacques,
 Josep Anton Jaques Miret, Annemarie Fejer Justesen, Alan MacLeod, Christer Sven
 Magnusson, Panagiotis Milonas, Juan A Navas-Cortes, Stephen Parnell, Roel Potting,
 Philippe Lucien Reignault, Hans-Hermann Thulke, Wopke Van der Werf, Antonio Vicent Civera,
 Jonathan Yuen, Lucia Zappalà, Thierry Candresse, Elisavet Chatzivassiliou, Franco Finelli,
 Stephan Winter, Domenico Bosco, Michela Chiumenti, Francesco Di Serio, Franco Ferilli,
 Tomasz Kaluski, Angelantonio Minafra and Luisa Rubino

Table 18.3: Blackberry chlorotic ringspot virus (BCRV)

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest	Key uncertainties
Identity of the pest (section 3.1)	The identity of BCRV is established and diagnostic techniques are available	The identity of BCRV is established and diagnostic techniques are available	Absence of a proven diagnostic protocol
Absence/presence of the pest in the EU territory (section 3.2)	BCRV has been reported in 1 MS (UK) but its presence is considered restricted	BCRV has been reported in 1 MS (UK) but its presence is considered restricted	More widespread and unreported presence in the EU
Regulatory status (section 3.3)	BCRV can be considered as regulated in Annex IAI as 'Non-European viruses and virus-like organisms of <i>Cydonia</i> Mill., <i>Fragaria</i> L., <i>Malus</i> Mill., <i>Prunus</i> L., <i>Pyrus</i> L., <i>Ribes</i> L., <i>Rubus</i> L. and <i>Vitis</i> L.'	BCRV can be considered as regulated in Annex IAI as 'Non-European viruses and virus-like organisms of <i>Cydonia</i> Mill., <i>Fragaria</i> L., <i>Malus</i> Mill., <i>Prunus</i> L., <i>Pyrus</i> L., <i>Ribes</i> L., <i>Rubus</i> L. and <i>Vitis</i> L.'	BCRV not explicitly mentioned in Directive 2000/29/EC

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Conclusion

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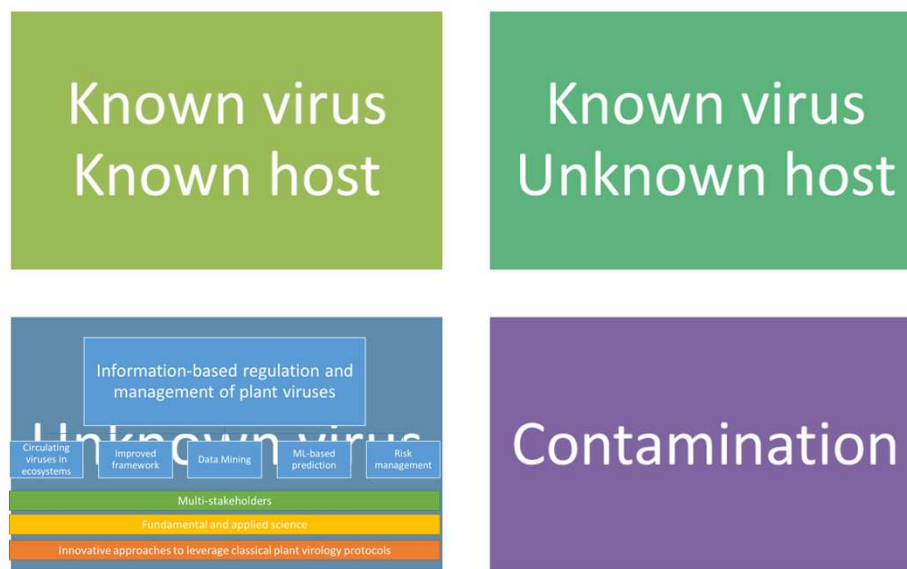
Conclusion

1. After a decade of use in plant health research, HTS technologies are progressively transferred to plant health diagnostics;
2. Guidelines have been written by a large panel of experts to facilitate the adoption of HTS technologies and they are now publicly available
3. The expected wider application of HTS technologies by diagnosticians and researchers can impact the current plant health policies and regulation as well as the trade of goods.
4. This impact represents a key challenge but also an opportunity for a future knowledge-driven plant health protection

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Conclusion: impact on plant health policies



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Thanks for the support

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A team work in the lab

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Thank you for your attention

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