Next generation diagnostics: from continental viromes to field level molecular diagnostics

Jan Kreuze

What is the International Potato Center (CIP)

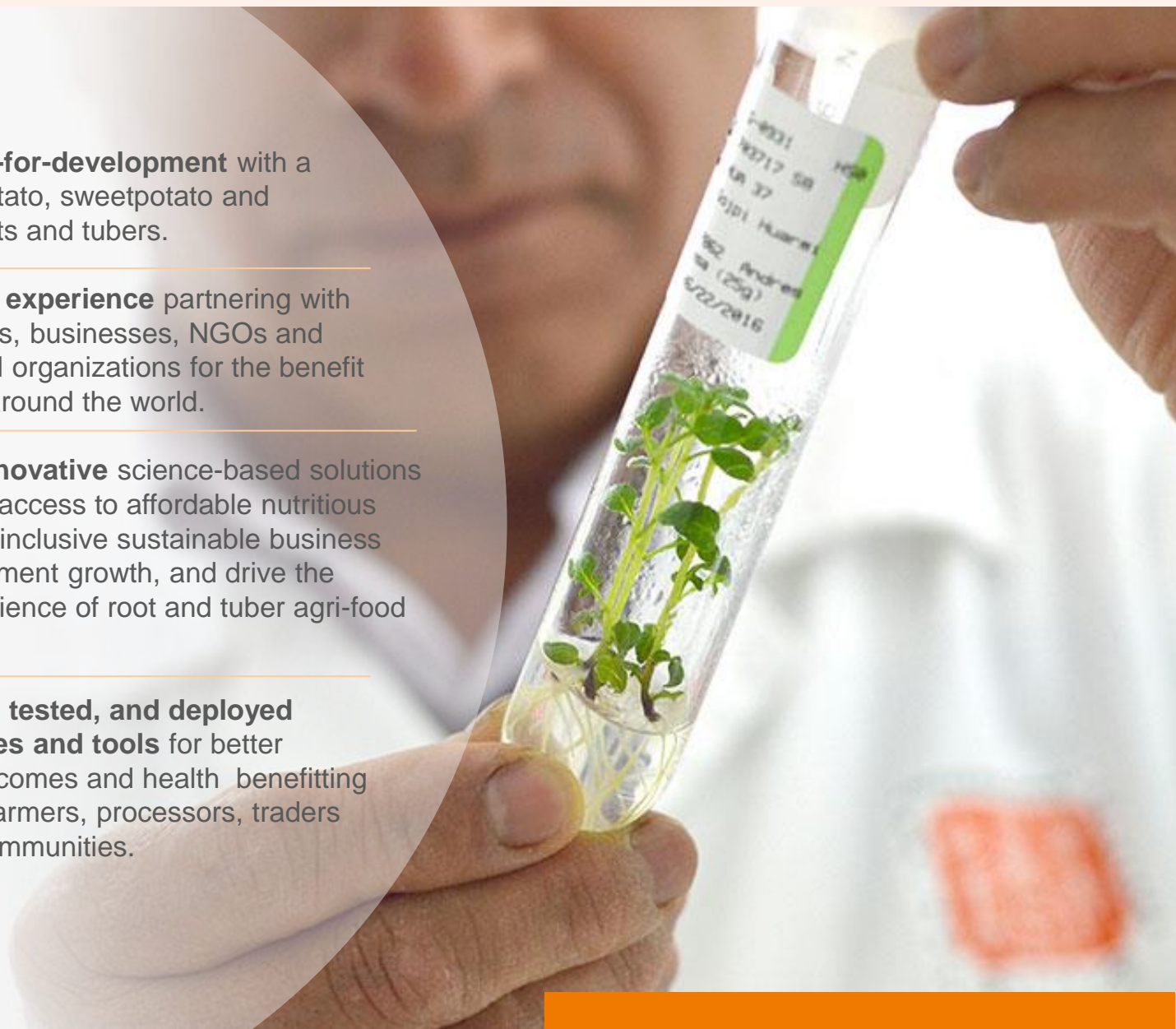**A research-for-development** with a focus on potato, sweetpotato and Andean roots and tubers.

50 years of experience partnering with governments, businesses, NGOs and international organizations for the benefit of farmers around the world.

50 YEARS

Delivers innovative science-based solutions to enhance access to affordable nutritious food, foster inclusive sustainable business and employment growth, and drive the climate resilience of root and tuber agri-food systems.
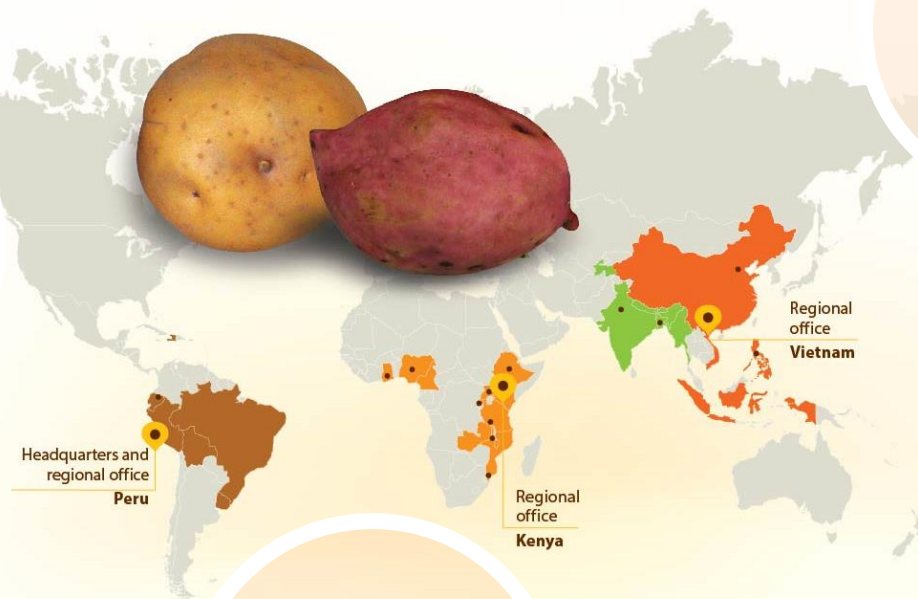
Developed, tested, and deployed technologies and tools for better harvests, incomes and health benefitting millions of farmers, processors, traders and their communities.

What is the International Potato Center (CIP)

Proven experience scaling science innovations and approaches.

Headquartered in Lima, Peru, CIP has a presence in 20+ countries in Africa, Asia and Latin America.

Regional office
Vietnam

Headquarters and regional office
Peru

Regional office
Kenya

A CGIAR research centers: global research partnership for a food-secure future.

CIP: Tapping the potential of root and tuber crops

CIP institutional goals

SDGS

Improved food and nutritional security through the introduction of healthier diets to vulnerable populations (women, young and displaced people).

Increased livelihood and employment opportunities and wide-scale development of inclusive value chains in rural and urban areas.

Sustainable intensification and diversification of agri-food systems through climate-resilient and biodiverse agriculture.
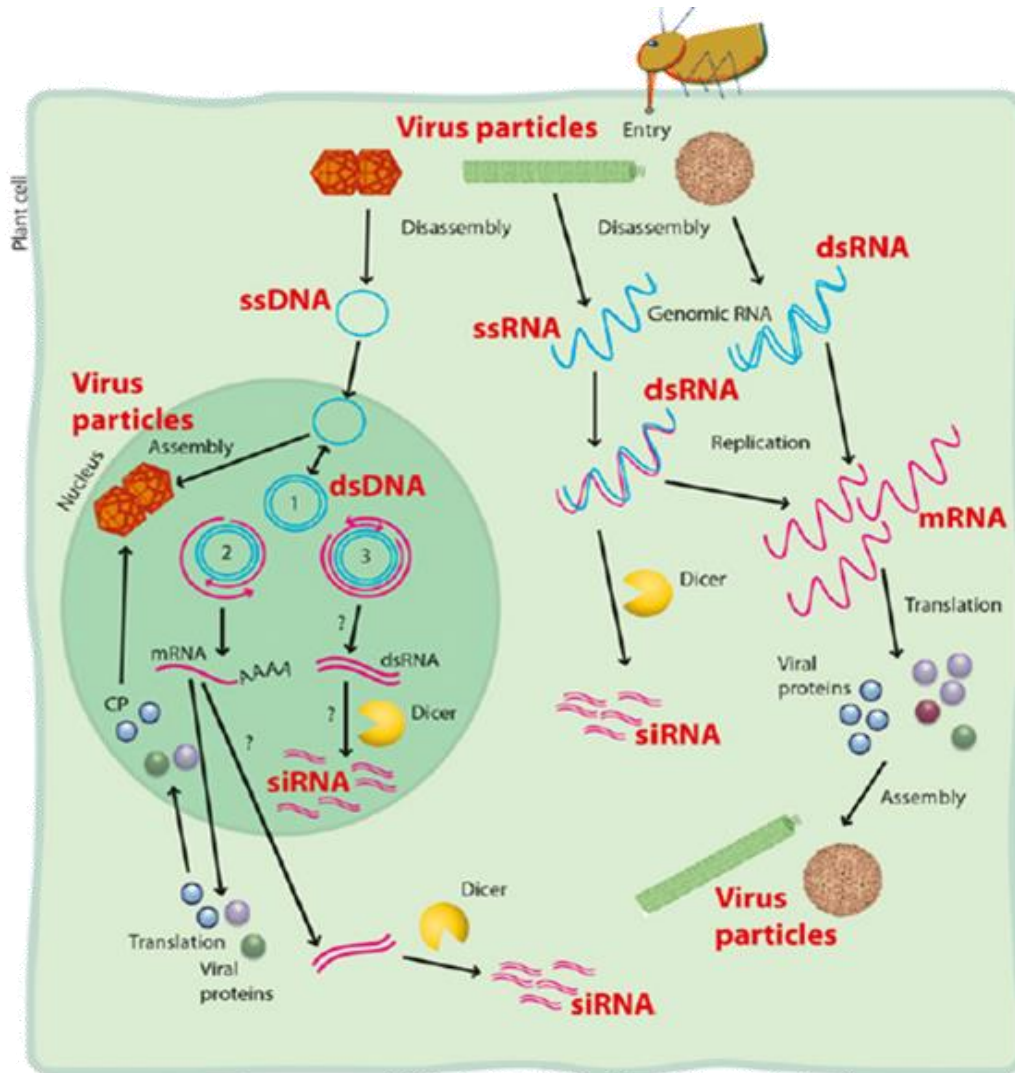
Aligned to the Sustainable Development Goals

Next generation diagnostics

Better understanding of the major diseases contributing to seed degeneration, their epidemiology and cheap rapid accurate diagnostics

Appropriate diagnostics technologies

Characterization & surveillance of (new & emerging) pathogens

Predicting risk
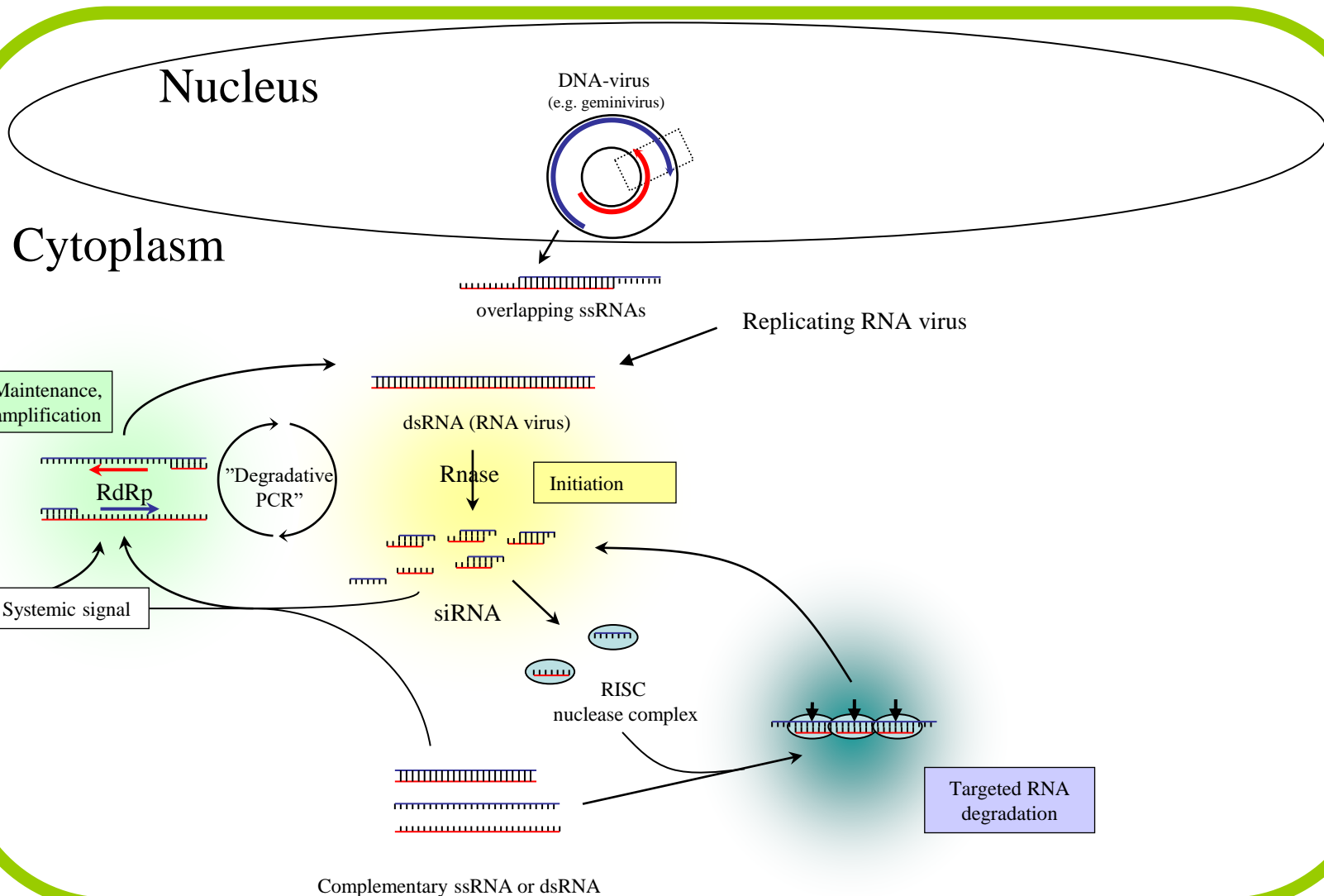

disease management
germplasm exchange
crop improvement

How to use high throughput sequencing?
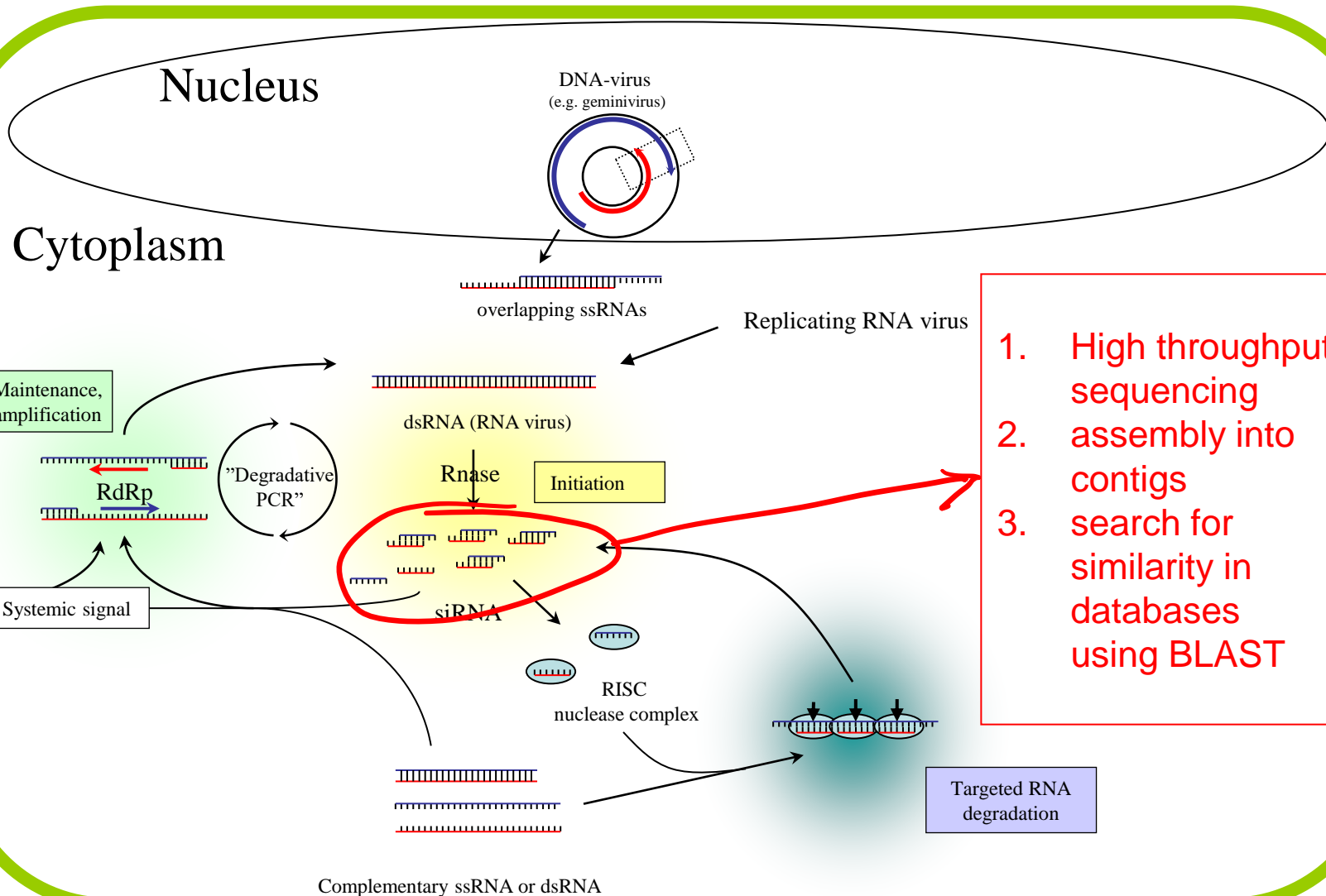

✓Total DNA or RNA or ribosome depleted RNA

✓Virion-associated nucleic acid (VANA) from virus particle

✓Double stranded RNA (dsRNA)

✓Virus-derived small interfering RNAs (siRNA)

Anti-viral RNA silencing in eukaryotic organisms

Nucleus

DNA-virus
(e.g. geminivirus)

Cytoplasm

overlapping ssRNAs

Replicating RNA virus

Maintenance,
amplification

dsRNA (RNA virus)

Rnase

Initiation

RdRp

"Degradative
PCR"

siRNA

Systemic signal

RISC
nuclease complex

Targeted RNA
degradation

Complementary ssRNA or dsRNA

Anti-viral RNA silencing in eukaryotic organisms

Nucleus

DNA-virus
(e.g. geminivirus)

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Replicating RNA virus

Maintenance,
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dsRNA (RNA virus)

RdRp

"Degradative
PCR"

Rnase

Initiation

Systemic signal

siRNA

RISC
nuclease complex

Targeted RNA
degradation

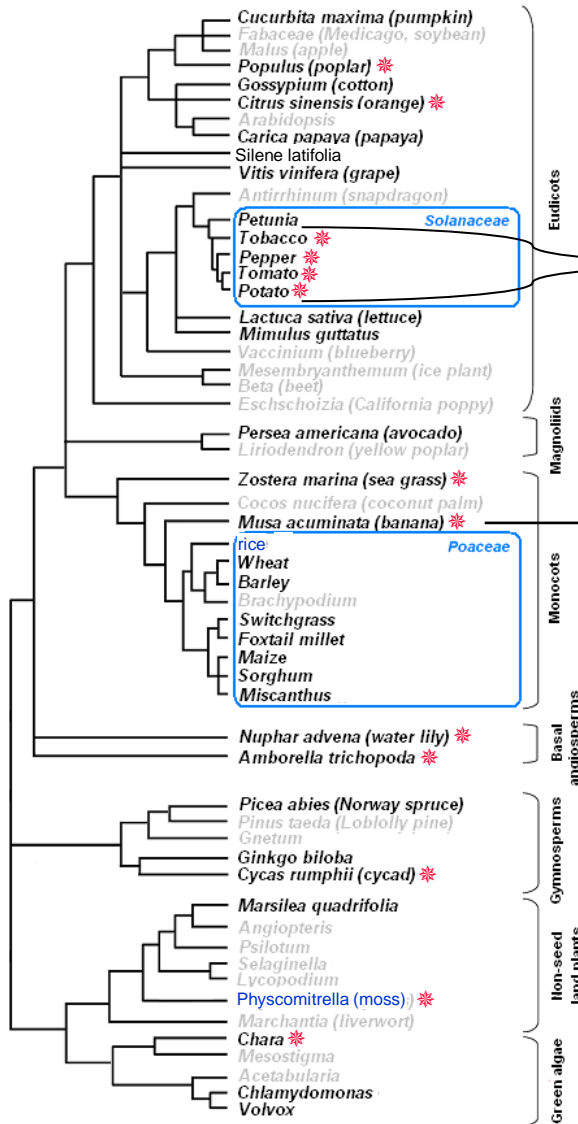Complementary ssRNA or dsRNA

1. High throughput sequencing
2. assembly into contigs
3. search for similarity in databases using BLAST

Number of contigs assembled by Velvet using 21–24 nt sRNA, or only 22 nt sRNA sequences, with virus specific hits as identified using Translated Nucleotide Blast (Blastx).

Plant infected with	siRNAs sequenced	Contigs identified	Contigs with Blastx hits 21–24 nt sRNA $k=15$, cov $=30$[a]	Contig sizes 21–24 nt sRNA $k=15$, cov $=30$[a]	Contigs with Blastx hits 21–24 nt sRNA $k=15$, cov $=3$[b]	Contigs with Blastx hits only 22 nt sRNA $k=15$, cov $=3$[b]
SPFMV	1,275,673	Total contigs	239		1633	431
		SPFMV	25	($\leq$949)	71	78
		SPCSV	0	–	0	0
		Badnavirus	38	($\leq$256)	62	55
		Mastrevirus	5	($\leq$210)	6	5
SPCSV	1,271,382	Total contigs	283		1675	285
		SPFMV	0	–	0	0
		SPCSV	10	($<$70)	64	12
		Badnavirus	44	($\leq$181)	63	44
		Mastrevirus	8	($\leq$260)	10	6
SPFMV +SPCSV	1,067,577	Total contigs	221		1363	581
		SPFMV	20	($\leq$1600)	43	51
		SPCSV	2	($<$81)	41	12
		Badnavirus	38	($\leq$266)	63	51
		Mastrevirus	5	($\leq$210)	8	5

SPFMV-Piu3
10996 bp

Sweetpotato pakakuy virus A & B

SPSMV-1
2,602bp
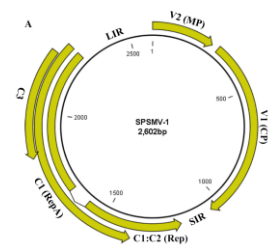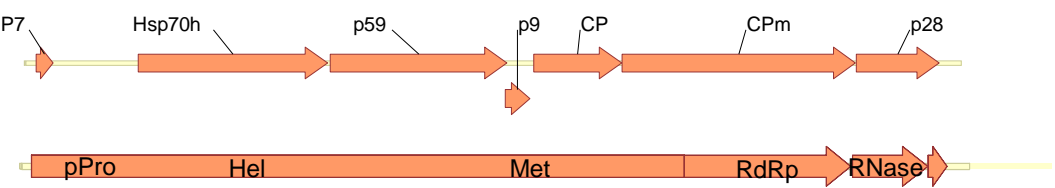
Other plant species

Comparative sequencing of plant small RNAs
http://smallrna.udel.edu/

TVCV (integrated)

Integrated badnaviruses

✷ similarity to caulimoviridae
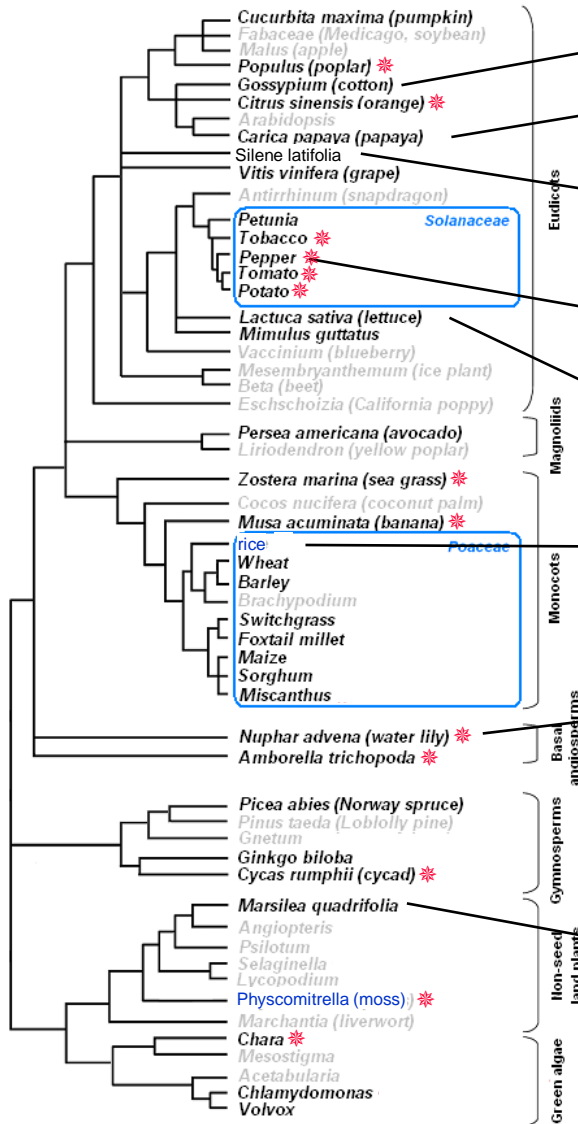
✓ new totivirus

✓ Papaya ring spot virus: 99.6% coverage

White Campion

✓ new partitivus
✓ new totivirus

✓ Bell pepper endornavirus (Endornavirus)

✓ Lettuce big-vein associated virus (Varicosavirus)
✓ Mirafiore lettuce virus (Ophiovirus)

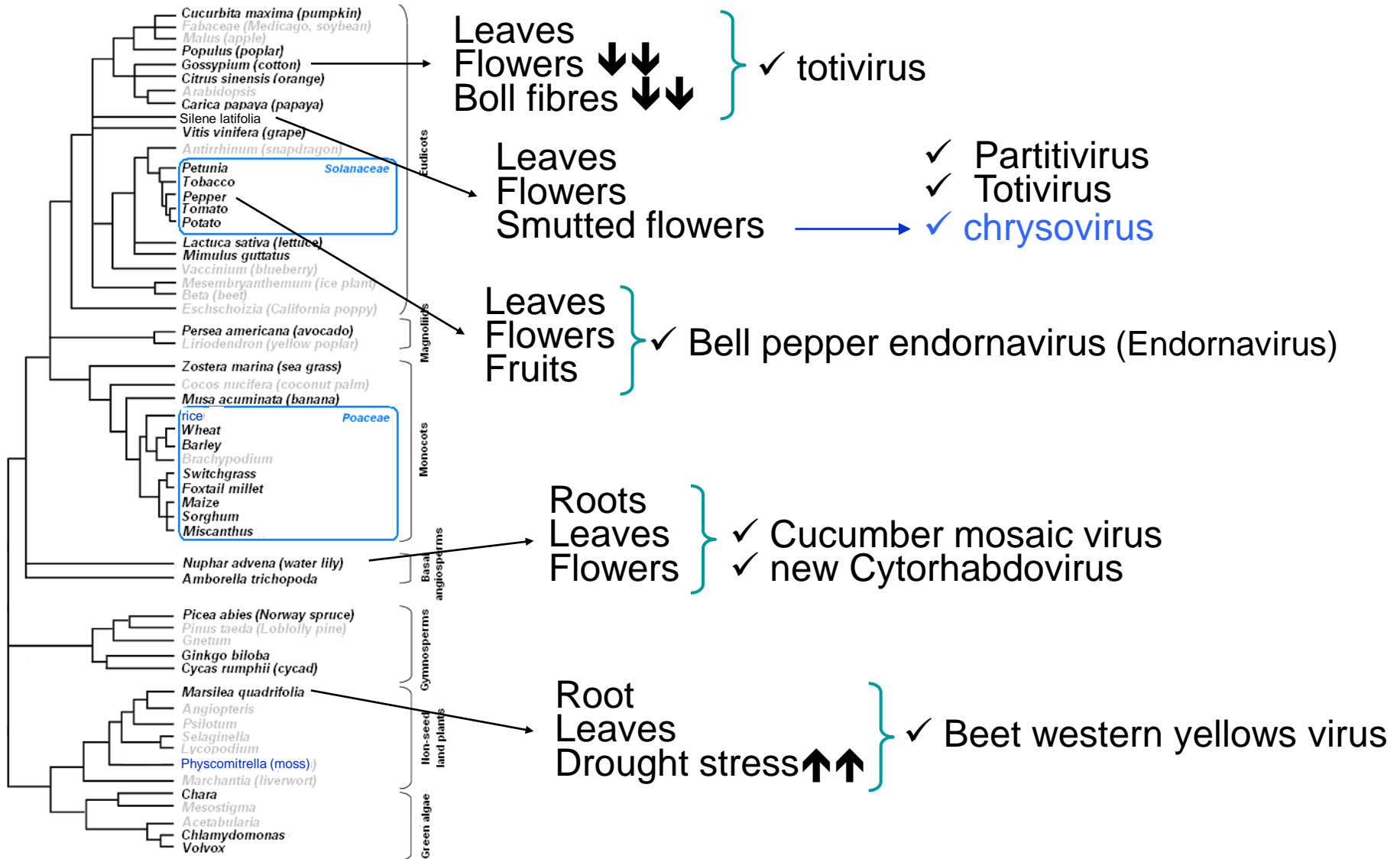✓ Rice ragged stunt virus (Oryzavirus; Reoviridae)
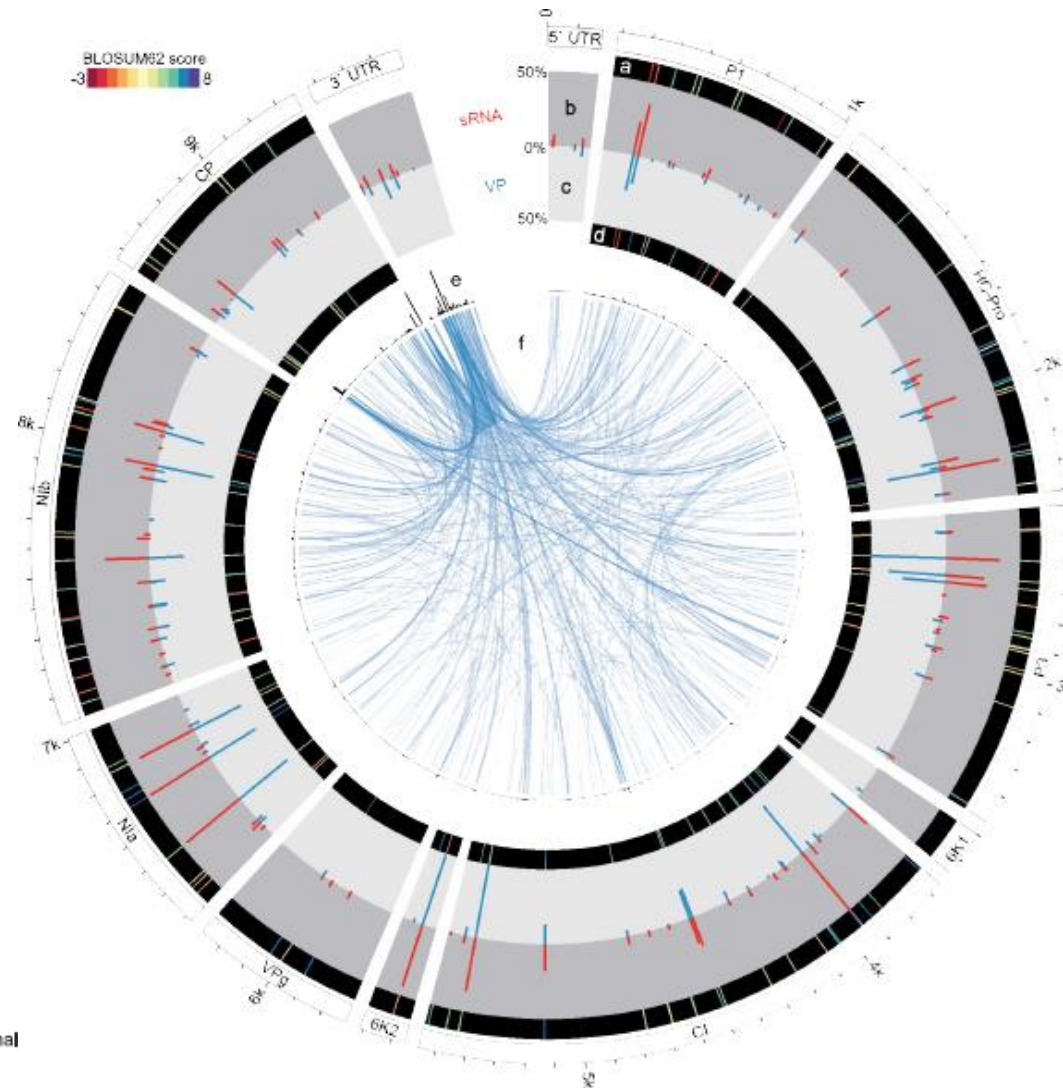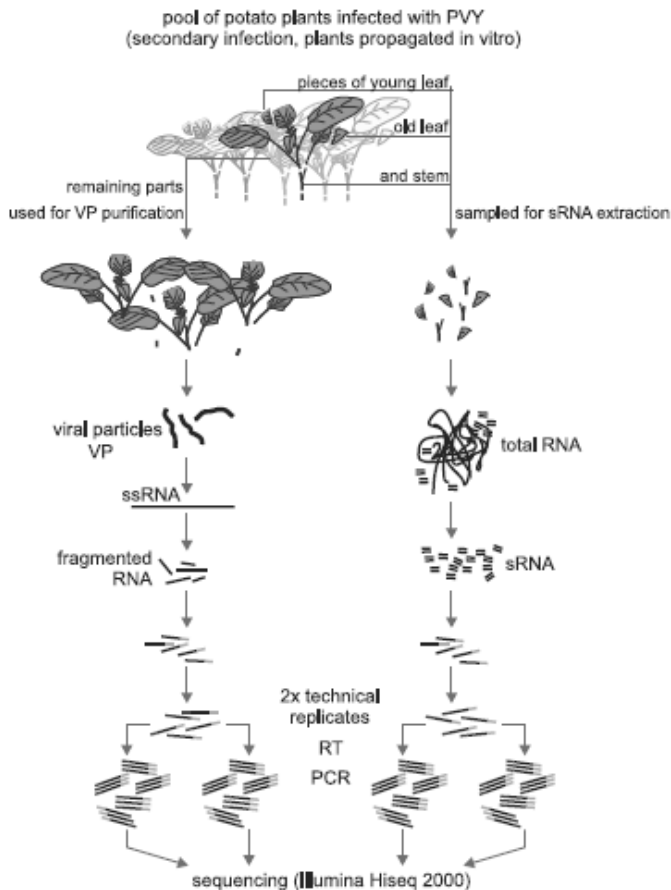
Spatterdock

✓ Cucumber mosaic virus
✓ new Cytorhabdovirus

European waterclover

✓ Beet western yellows virus

❋ similarity to Caulimoviridae

Tree labels:
Cucurbita maxima (pumpkin)
Fabaceae (Medicago, soybean)
Malus (apple)
Populus (poplar) ❋
Gossypium (cotton)
Citrus sinensis (orange) ❋
Arabidopsis
Carica papaya (papaya)
Silene latifolia
Vitis vinifera (grape)
Antirrhinum (snapdragon)
Petunia
Tobacco ❋   Solanaceae
Pepper ❋
Tomato ❋
Potato ❋
Lactuca sativa (lettuce)
Mimulus guttatus
Vaccinium (blueberry)
Mesembryanthemum (ice plant)
Beta (beet)
Eschschoizia (California poppy)
Persea americana (avocado)
Liriodendron (yellow poplar)
Zostera marina (sea grass) ❋
Cocos nucifera (coconut palm)
Musa acuminata (banana) ❋
rice   Poaceae
Wheat
Barley
Brachypodium
Switchgrass
Foxtail millet
Maize
Sorghum
Miscanthus
Nuphar advena (water lily) ❋
Amborella trichopoda ❋
Picea abies (Norway spruce)
Pinus taeda (Loblolly pine)
Gnetum
Ginkgo biloba
Cycas rumphii (cycad) ❋
Marsilea quadrifolia
Angiopteris
Psilotum
Selaginella
Lycopodium
Physcomitrella (moss) ❋
Marchantia (liverwort)
Chara ❋
Mesostigma
Acetabularia
Chlamydomonas
Volvox

Eudicots · Magnoliids · Monocots · Basal angiosperms · Gymnosperms · Non-seed land plants · Green algae

Leaves
Flowers ⬇⬇
Boll fibres ⬇⬇ ⟩ ✓ totivirus

Leaves
Flowers   ✓ Partitivirus
Smutted flowers ✓ Totivirus
→ ✓ chrysovirus

Leaves
Flowers ⟩ ✓ Bell pepper endornavirus (Endornavirus)
Fruits

Roots
Leaves ⟩ ✓ Cucumber mosaic virus
Flowers ✓ new Cytorhabdovirus
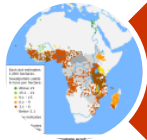
Root
Leaves ⟩ ✓ Beet western yellows virus
Drought stress⬆⬆

The African sweetpotato virome

Conduct unbiased geo-referenced sample collection of field-grown sweetpotatoes throughout Africa

Simplified procedure for RNA processing, purification and storage, and small RNA library construction for sweetpotato.

Computational methods to efficiently process and assemble siRNA into complete viral genomes (virome) by siRNA deep sequencing on samples.
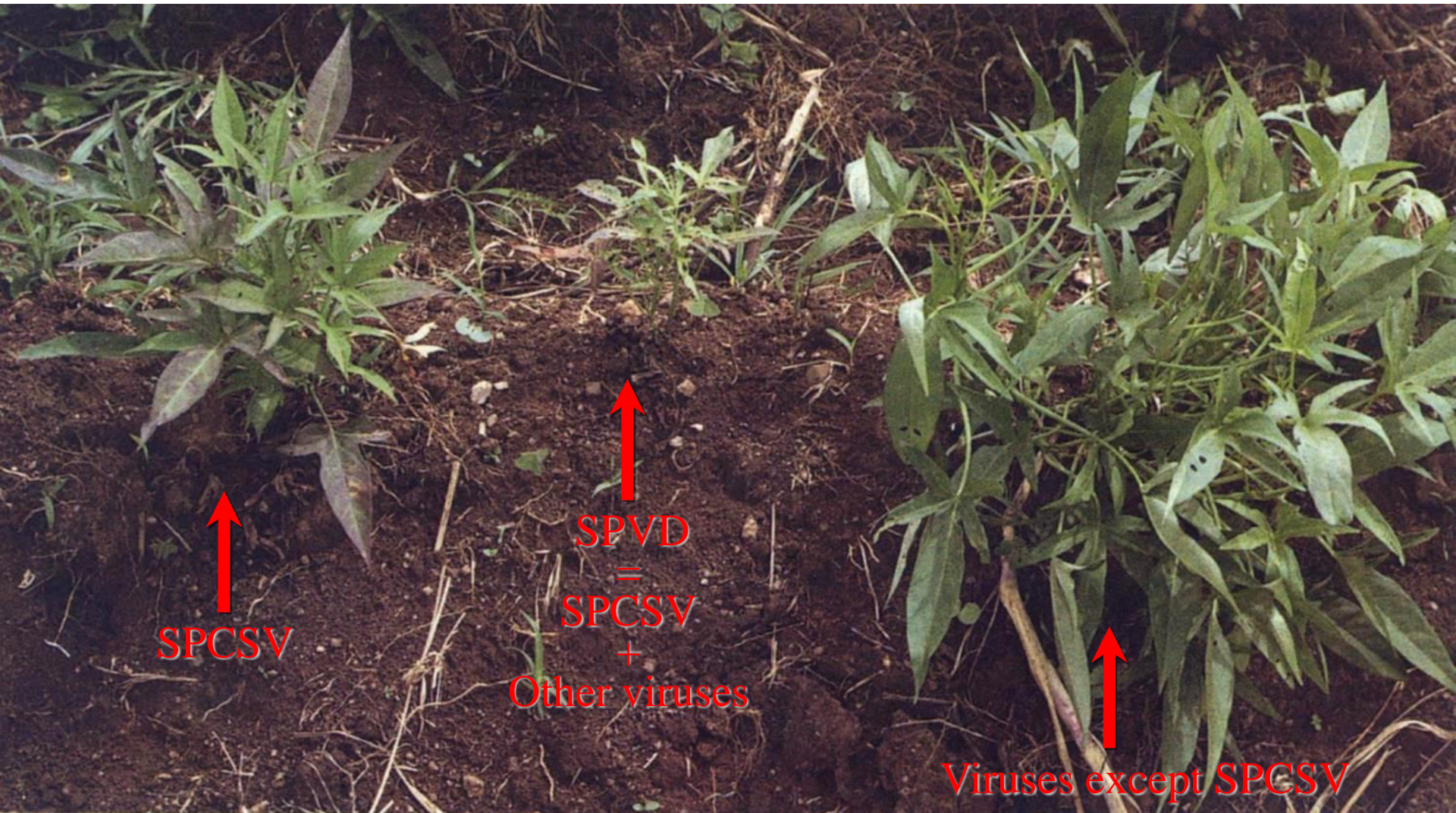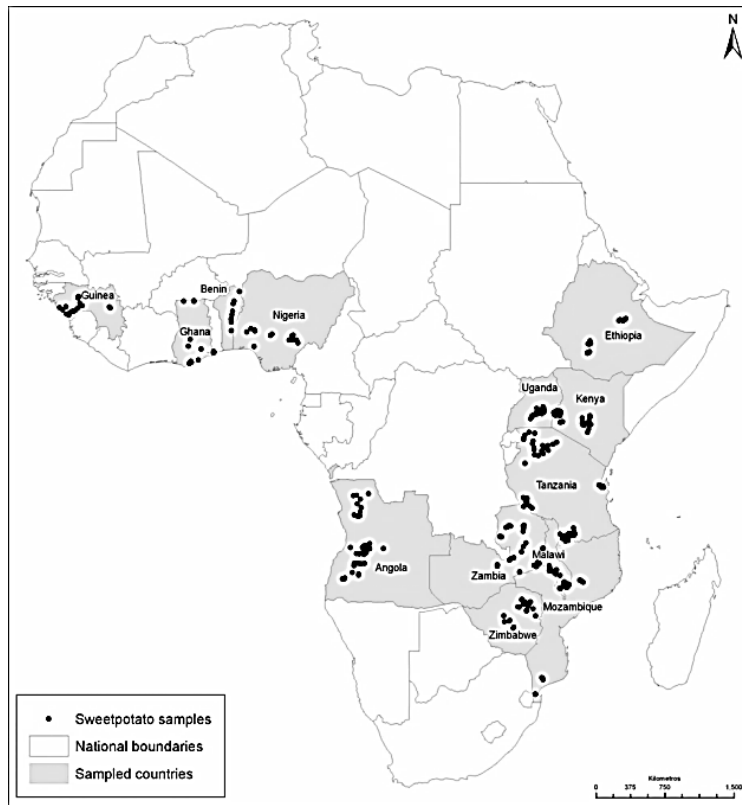

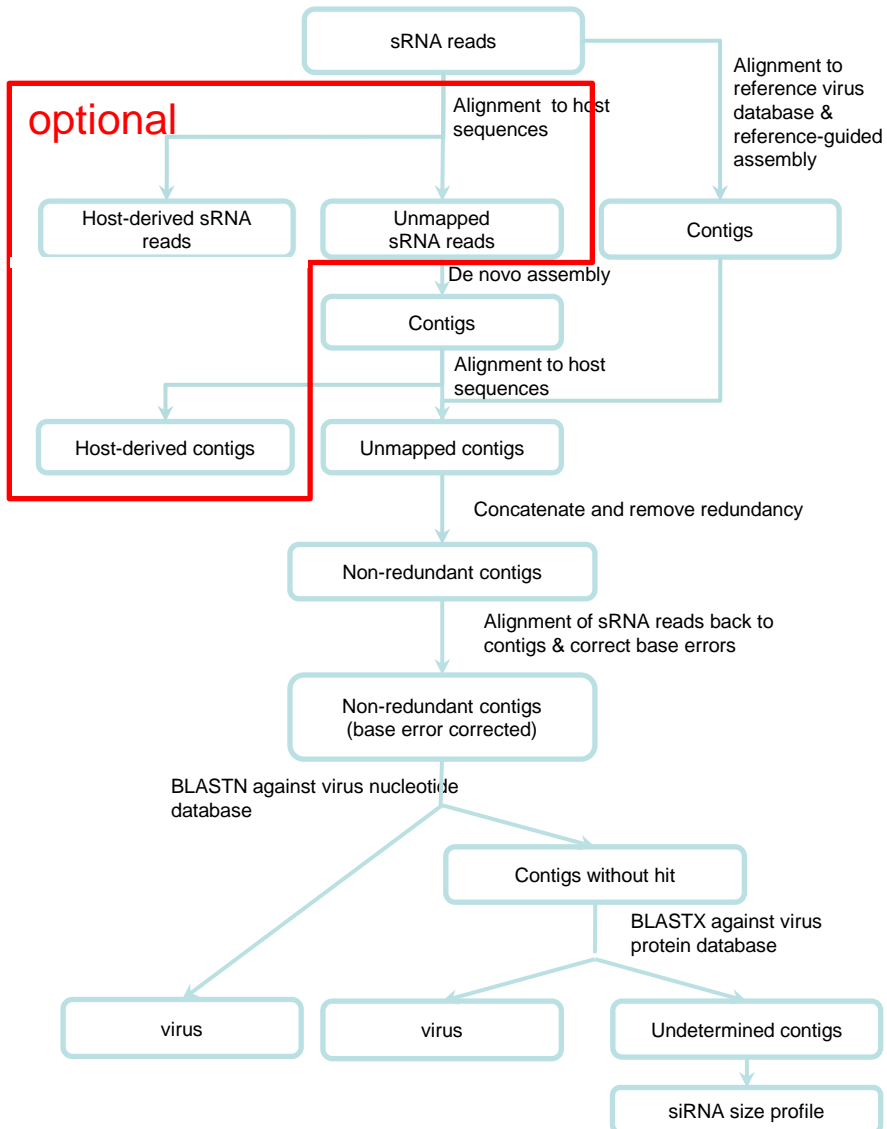Assembly of information into database
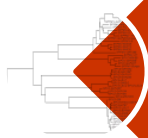
Analyze data

Viruses of sweetpotato

SPCSV

SPVD
=
SPCSV
+
Other viruses

Viruses except SPCSV

~2000 samples and bioinformatics pipeline for virus identification using siRNA assembly and genome subtraction

Small RNA sequence analysis pipeline: VirusDetect_v1.6

optional

sRNA reads

Alignment to host sequences

Alignment to reference virus database & reference-guided assembly

Host-derived sRNA reads

Unmapped sRNA reads

Contigs

De novo assembly

Contigs

Alignment to host sequences

Host-derived contigs

Unmapped contigs

Concatenate and remove redundancy

Non-redundant contigs

Alignment of sRNA reads back to contigs & correct base errors

Non-redundant contigs (base error corrected)

BLASTN against virus nucleotide database

Contigs without hit

BLASTX against virus protein database

virus

virus

Undetermined contigs

siRNA size profile

Reference	Length	Coverage (%)	#contig	Depth	Depth (Norm)	%Identity	%Iden Max	%Iden Min	Genus	Description
FJ150422	4806	4806 (100)	11	675.9	18.5	94.64	96.00	84.82	NA	Drosophila A virus isolate HD, complete genome.
GQ342962	3260	3257 (99.9)	3	524.3	14.4	98.11	98.75	97.03	NA	Drosophila melanogaster birnavirus SW-2009a strain DBV segment A, complete sequence.
GQ342963	3014	3014 (100)	1	854.8	23.4	98.47	98.47	98.47	NA	Drosophila melanogaster birnavirus SW-2009a strain DBV segment B, complete sequence.
KF947078	13534	1773 (13.1)	27	35.2	1.0	99.10	100	97.14	NA	Spodoptera frugiperda rhabdovirus isolate Sf, complete genome.
GQ257737	12333	12333 (100)	6	661.2	18.1	96.87	99.29	91.67	NA	Nora virus isolate Umea 2007, complete genome.
M32779	2225	2112 (94.9)	7	20.4	0.6	99.57	100	97.69	alphabaculovirus	Autographa californica nucleopolyhedrovirus insertion element IFP2.2 genomic sequence.
EF690537	3107	3089 (99.4)	5	654.2	17.9	94.82	96.59	94.12	alphanodavirus	Flock house virus isolate TNCL segment RNA1 protein A mRNA, complete cds.
EF690538	1383	1383 (100)	1	378.8	10.4	94.44	94.44	94.44	alphanodavirus	Flock house virus isolate TNCL segment RNA2 protein alpha mRNA, complete cds.
AF014388	9264	9244 (99.8)	3	1096.2	30.1	98.03	98.21	96.88	cripavirus	Drosophila C virus strain EB, complete genome.

AF014388
CONTIG220
CONTIG156
CONTIG50
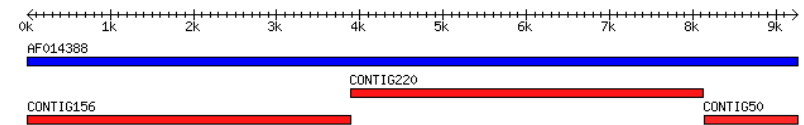
Order	Query ID	Query Start	Query End	Subjct Start	Subjct End	Identity	E value	Strand
1	CONTIG50	1	1121	8144	9264	1086/1121(96%)	0.0	1

Alignment:

```
Query: 1    tgaggttgaatatgctcgtattgttgcgcaagtaatgggtgaagatgtagctattcaaag 60
            |||||||||| ||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct: 8144 tgaggttgaagatgctcgtattgttgcgcaagtaatgggtgaagatttagctattcaaag 8203

Query: 61   aaacgatgctcaacatggtgttcatccaatgaccatagacactcataagattgactcaaa 120
            ||||||||||||||||||||||||||||||||||||||||| ||||||||||| |||||||
Sbjct: 8204 aaacgatgctcaacatggtgttcatccaatgactatagacactcataagatcgactcaaa 8263
```

sRSA: a didactic explanation

Extraction & library prep

Host genome subtraction

de novo Assembly

Reference guided Assembly

depth

contigs

Coverage & identity

Reference database

http://bioinfo.bti.cornell.edu/virome/index
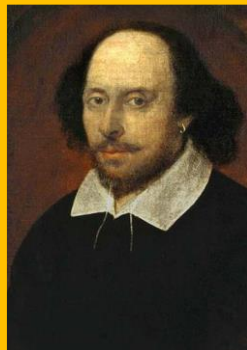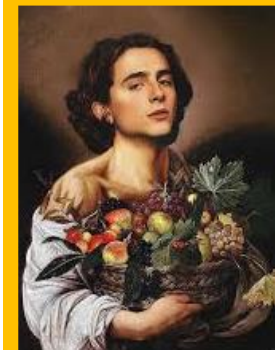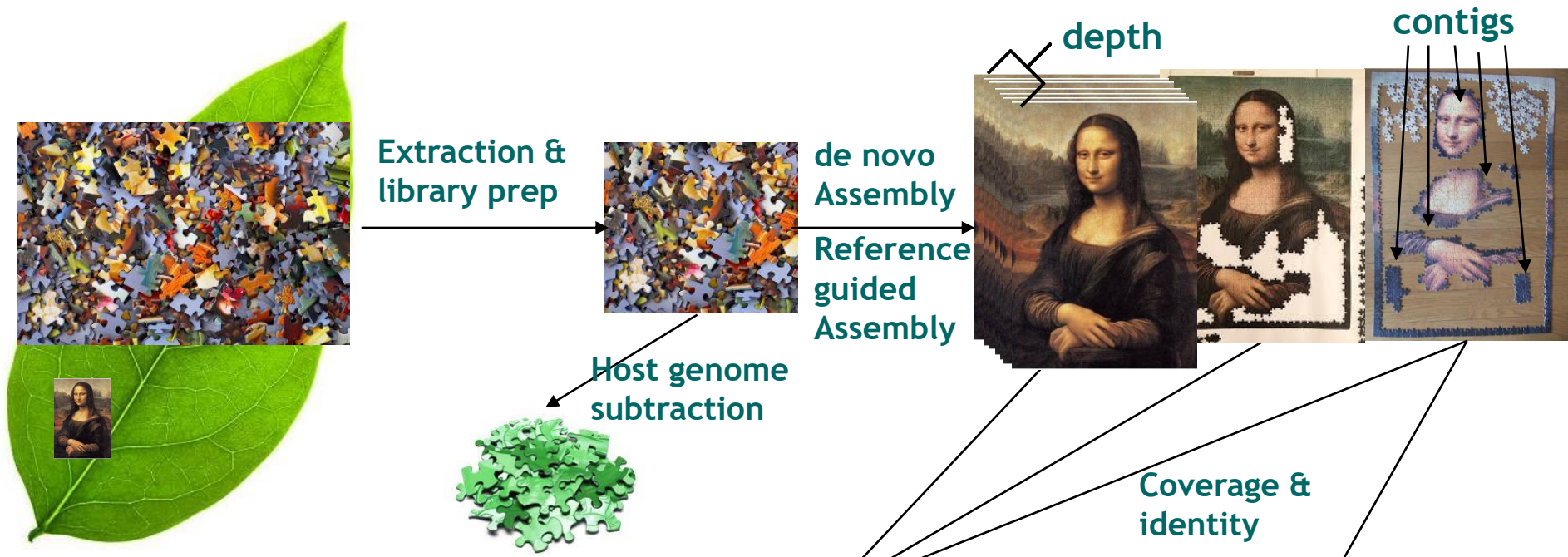
Results: the viruses
3193 viruses from 1168 samples
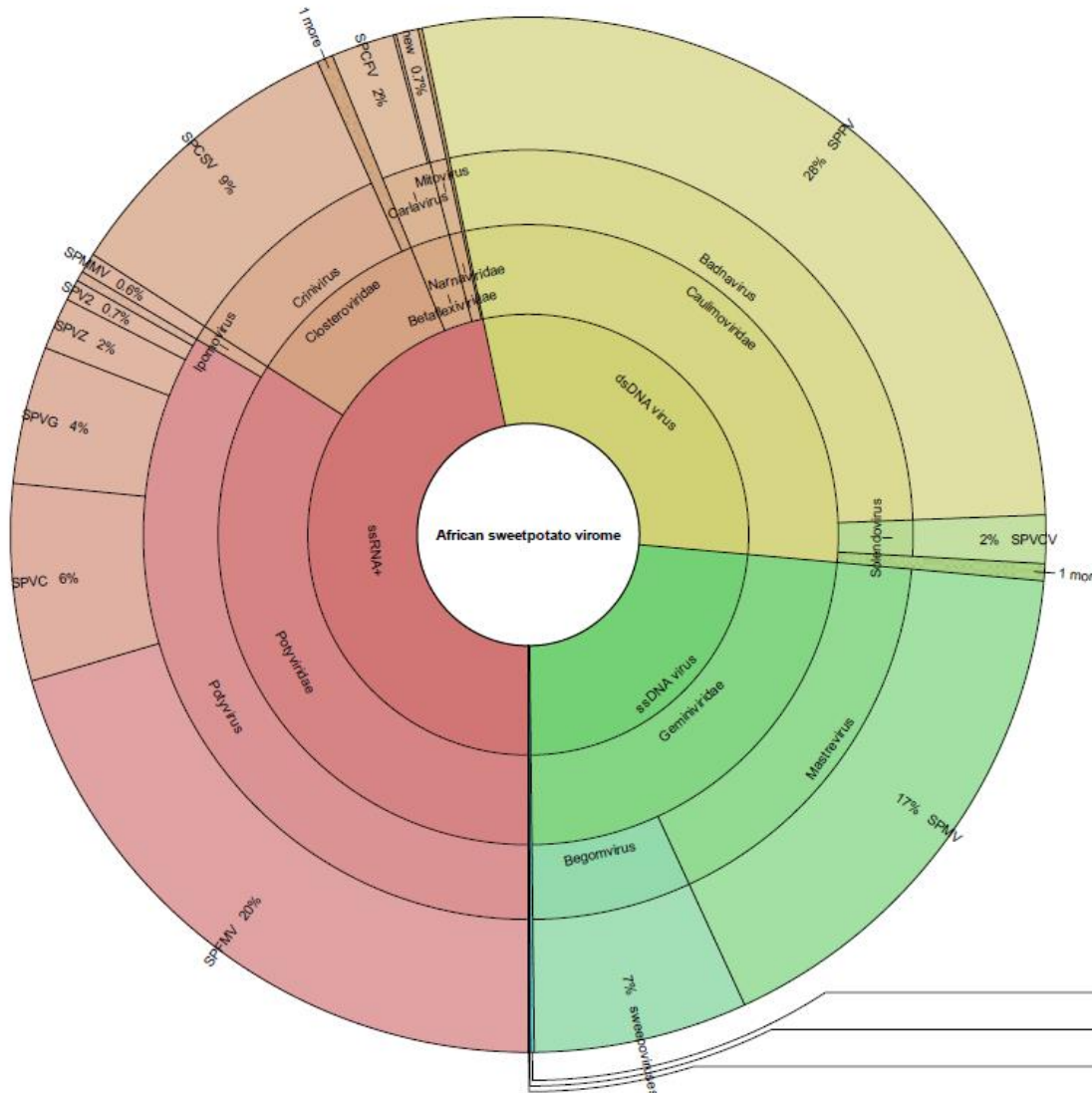
New viruses:
Potyviruses: 3
Ampeloviruses: 3
Mitoviruses: many
Nepovirus
Soymovirus
Emaravirus
Begomoviruses
Alphasatellites

e-**X**tra*

Badnaviruses of Sweet Potato: Symptomless Coinhabitants on a Global Scale

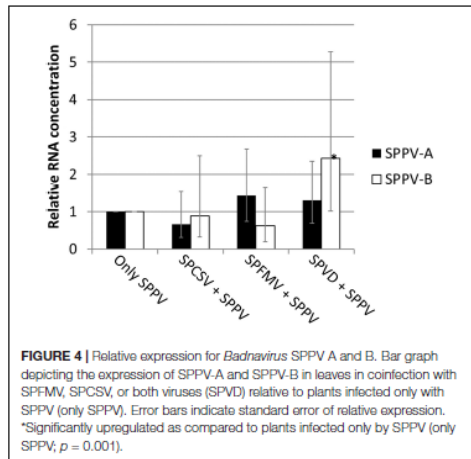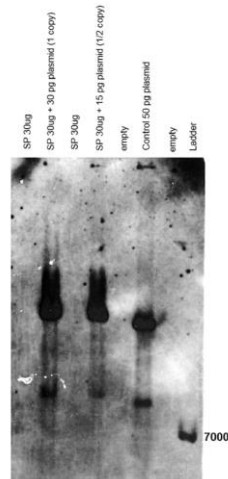Jan F. Kreuze*‡, Ana Perez‡, Marco Galvez Gargurevich† and Wilmer J. Cuellar†

Storage Root Yield of Sweetpotato as Influenced by *Sweetpotato leaf curl virus* and Its Interaction With *Sweetpotato feathery mottle virus* and *Sweetpotato chlorotic stunt virus* in Kenya

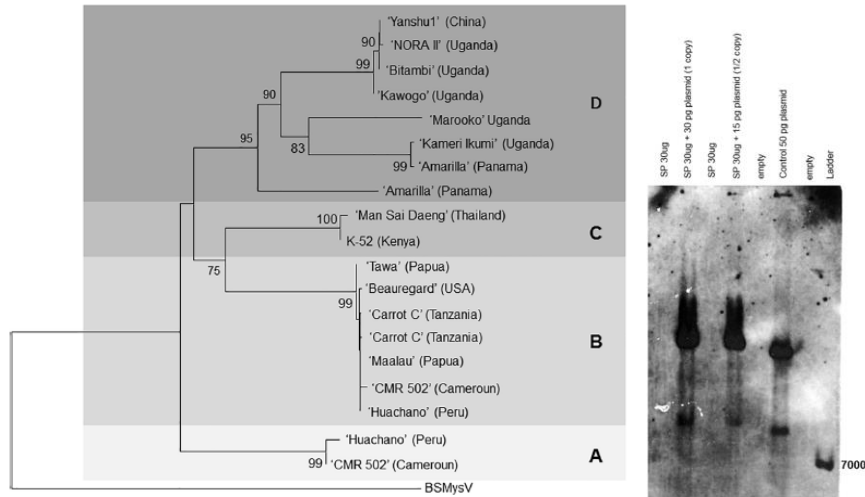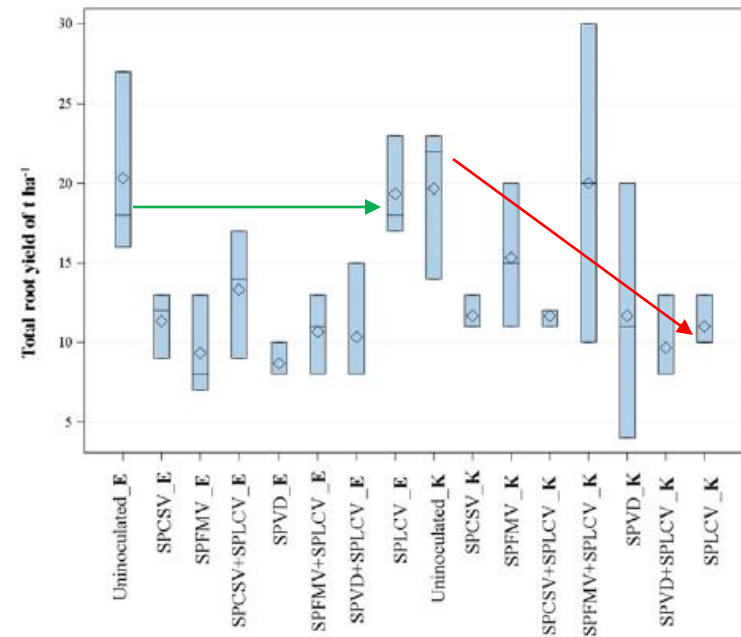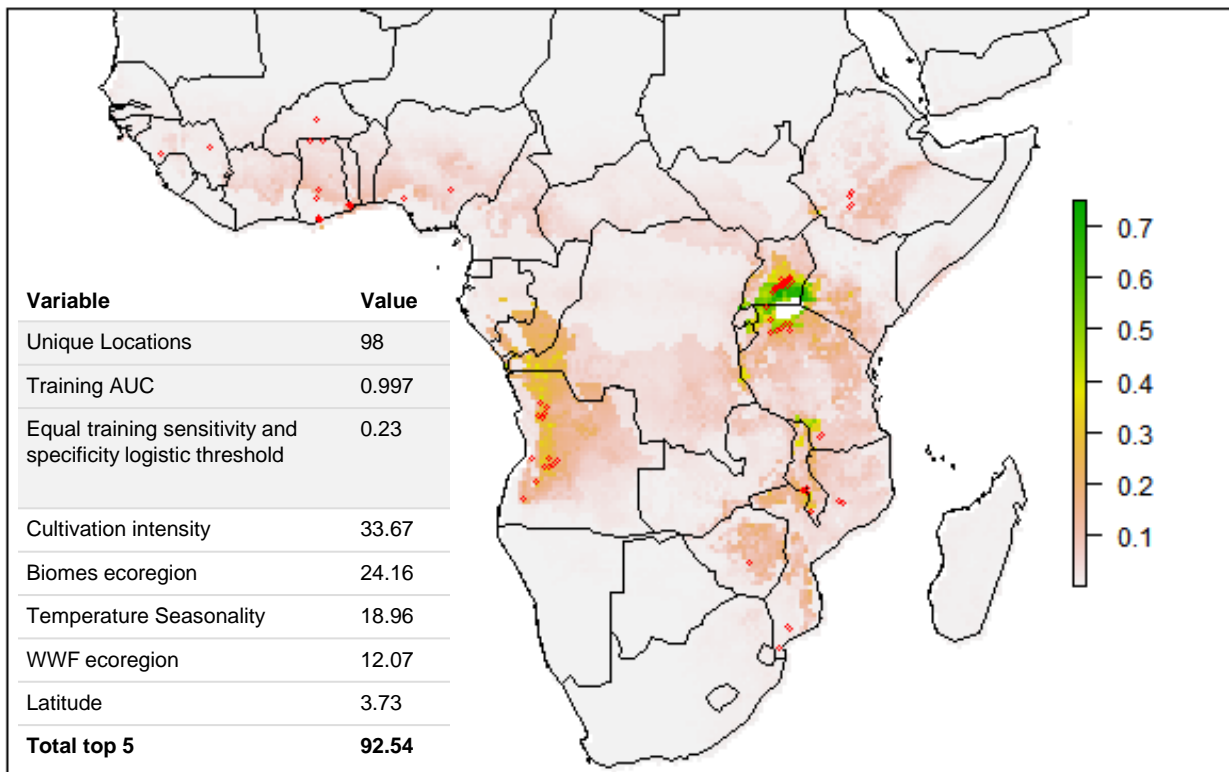Bramwel W. Wanjala,[1,2] Elijah M. Ateka,[2] Douglas W. Miano,[3] Jan W. Low,[1] and Jan F. Kreuze[4,†]

FIGURE 4 | Relative expression for *Badnavirus* SPPV A and B. Bar graph depicting the expression of SPPV-A and SPPV-B in leaves in coinfection with SPFMV, SPCSV, or both viruses (SPVD) relative to plants infected only with SPPV (only SPPV). Error bars indicate standard error of relative expression. *Significantly upregulated as compared to plants infected only by SPPV (only SPPV; $p = 0.001$).
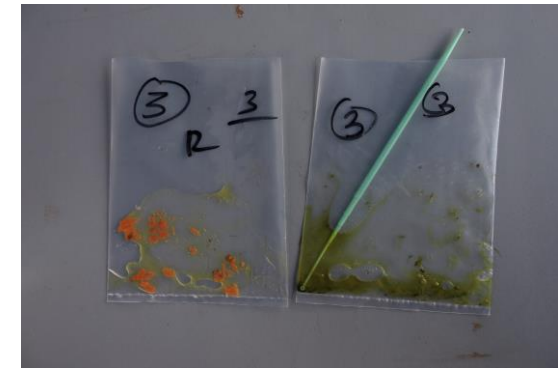
Can we make predictions about likely occurance beyond evaluated areas?

Example for begomoviruses: Niche analysis using MaxEnt modeling and bioclimatic-ecosystem variables

Variable	Value
Unique Locations	98
Training AUC	0.997
Equal training sensitivity and specificity logistic threshold	0.23
Cultivation intensity	33.67
Biomes ecoregion	24.16
Temperature Seasonality	18.96
WWF ecoregion	12.07
Latitude	3.73
Total top 5	**92.54**

Field LAMP kit

Prototype LAMP field kit

For simple sap extraction

Alkaline PEG extraction buffer

Sample maceration bags

Discardable inoculation loop for transferring sample & mixing

Tube with water for sample dilution

Macerate sample in bag, dilute 1/10 with water and transfer 1ul to reaction tube using inoculation loop

Discardable pipette for adding extraction buffer & reaction buffer

Realtime Genie

Portable and battery operated

Mix and add to tubes using pipette

Run in realtime Genie and read results on screen

Lyphilized LAMP reagents: primers & enzyme

LAMP buffer

Reaction tubes

Lyophilized reagents

BioRanger

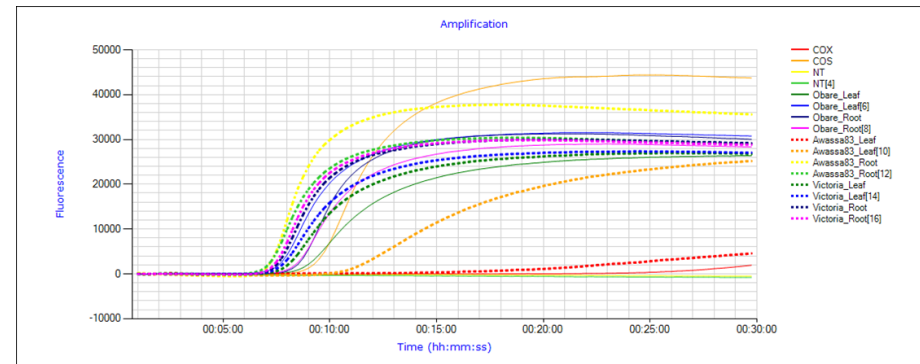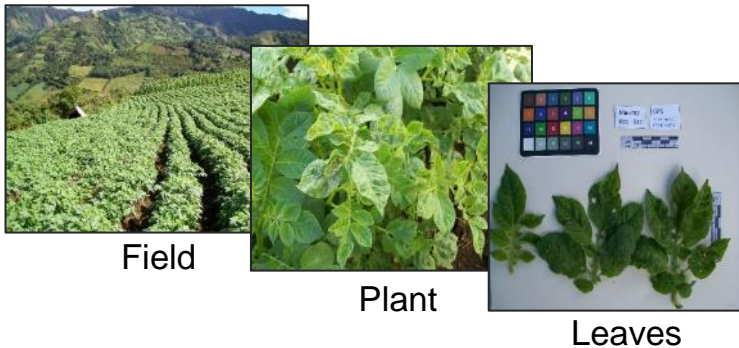Easy to interpret

Amplification

The Peruvian potato virome

- ## Sample collection:
  994 geo-referenced potato leaf samples (Cajamarca, Huanuco, Junin, Huancavelica, Cusco)

Field

Plant

Leaves

Drying

Weighting

Peruvian potato virome - Web platform

Baseline to monitor changes in the future

General results
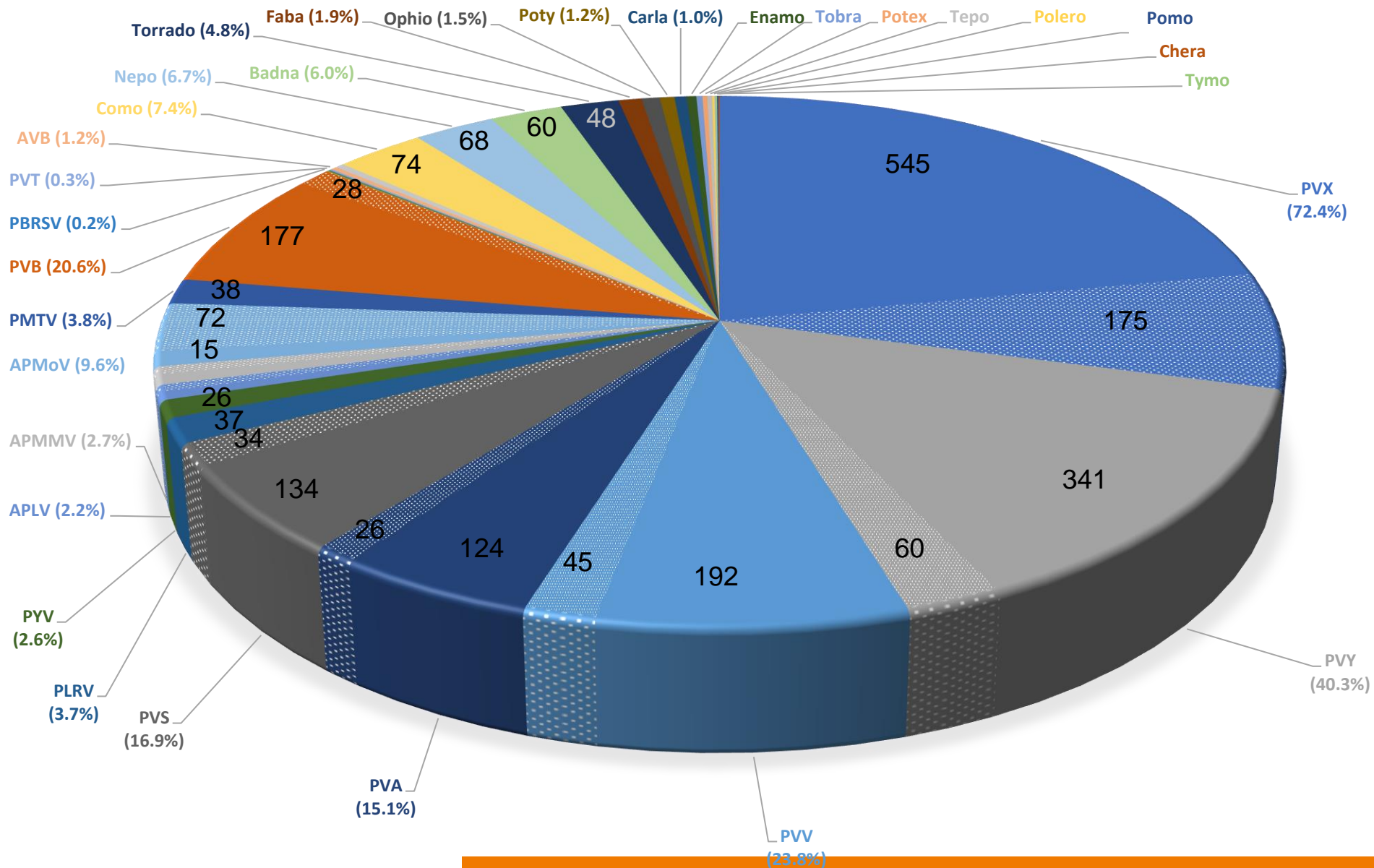
virus
Apurímac
Cajamarca
Cusco

Samples: 552
PVY: 235
Genomes: 32

nOphiovirus 2
nOphiovirus 3
nSecovirus 2
APMMV 1 nOphiovirus 1
PMTV 3 PMTV 2
PMTV 1
PVA
PVV
PLRV
APLV
PVB 1
PVB 2
nNepovirus 2 nBadnavirus
nNepovirus 1
PVX
PVY
nVaricosavirus
AVB 1
nComovirus 2
nComovirus 1
nCheravirus 2
PVT nReovirus 8
GBLV
PVS
nFabavirus 1
nTorradovirus
AVB 2 FClLV 1
FClLV 2
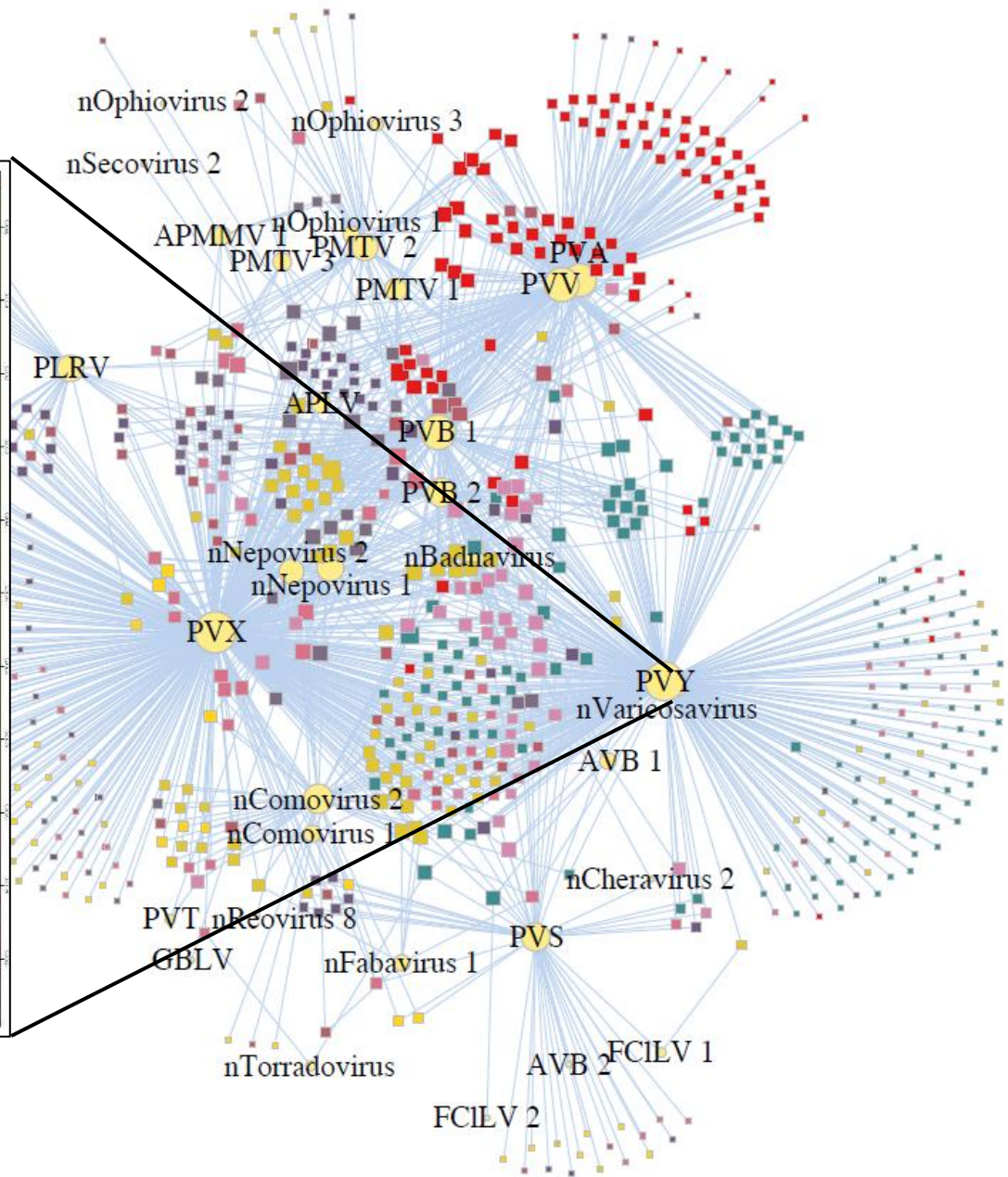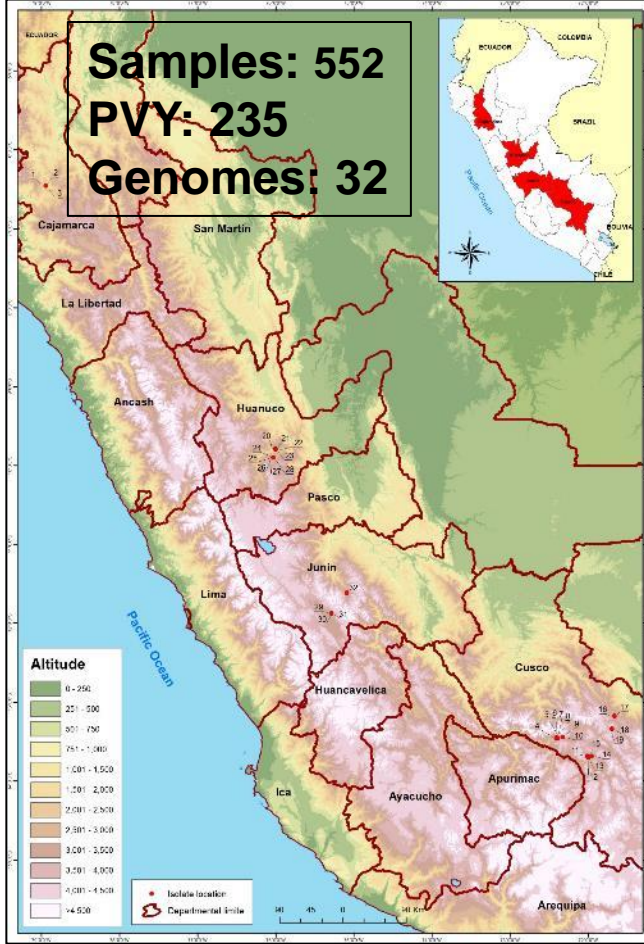
Phylogeny
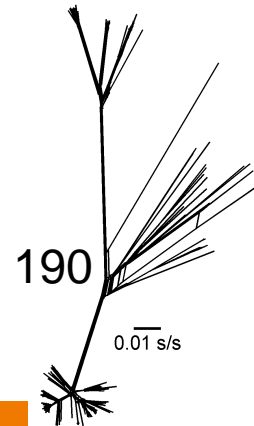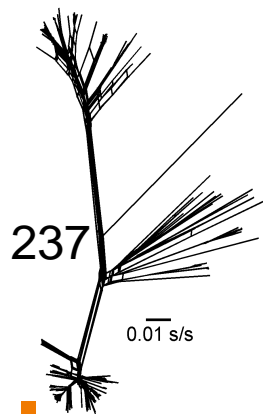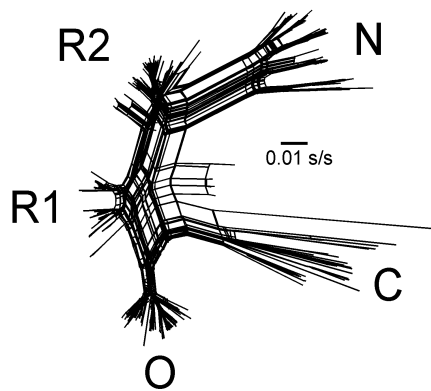
Identifying temporal signals using TempEst

(125) 10 Peru, 1 Brazil
(11)

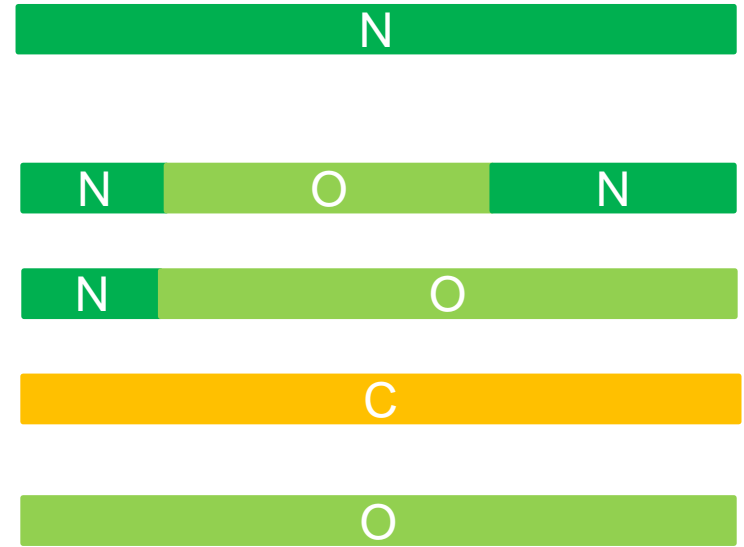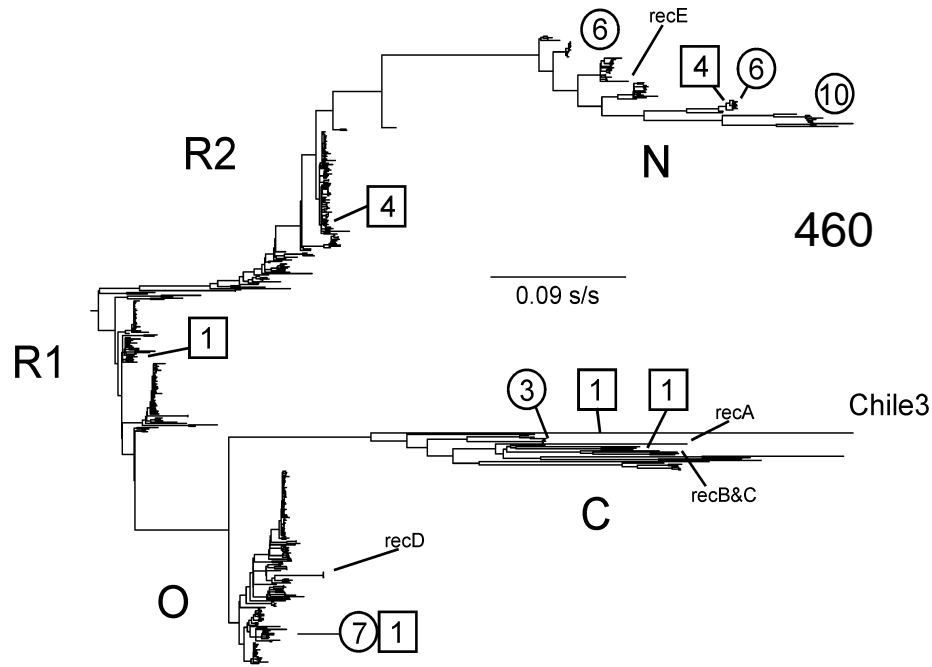| Accession Code	host	phylogroup
see legend	mostly S.t.t.	O1
see legend	mostly S.t.t.	O2
KP691329	S.t.t.	
KP691324	S.t.t.	
KP691321	S.t.t.	O3
AJ890348	S.t.t.	
MH795843	S.t.t x S.t.a.	
MH795841	S.t.t x S.t.a.	
MH795842	S.t.t x S.t.a.	
MF134864	P. peru	
MF134862	P. peru	
KF770835	C. ann	
KY847935	N. tab	
KX580384	S. lyco	
DQ309028	N. tab.	
AF463399	N. tab	C
KP691320	S. lyco	
KP063211	N. tab	
EU482153	S. lyco	
AJ439545	S. lyco	
EU563512	S.t.t.	
AJ439544	C. ann	
FJ214726	C. bacc	Chile 3
MH795866	S.t.t. x S.t.a.	
MH795867	S.t.t. x S.t.a.	
MH795864	S.t.t. x S.t.a.	
MH795868	S.t.t. x S.t.a.	N1
MH795870	S.t.a.	
MH795869	S.t.t. x S.t.a.	
MH795865	S.t.t x S.t.a..	
MH795872	S.t.a.	
MH795861	S.t.t.	
MH795857	S.t.t. x S.t.a.	
MH795860	S.t.t. x S.t.a.	
MH795859	S.t.t. x S.t.t.	
MH795858	S.t.t. x S.t.a.	N2
MH795850	S.t.t. x S.t.a.	
KT290511	S. lyco	
KR270797	S. lyco	
KX531041	S.t.t.	
KU043114	S.sisym	
MH795846	S.t.t. x S.t.a.	
MH795862	S.t.t. x S.t.a.	
X97895	S.t.t.	
CS434575	S.t.t.	
AM268435	S.t.t.	
KU724101	N.tab	N3
MH795863	S.t.t. x S.t.a.	
MH795844	S.t.t. x S.t.a.	
MH795848	S.t.t. x S.t.a.	
MH795847	S.t.t. x S.t.a.	
JQ969036	S.t.t.	
AY884983	S.t.t.	
KY847985	S.t.t.	
KY847988	S.t.t.	
KY847986	S.t.t.	
KY847984	S.t.t.	

Evaluated twelve combinations of substitution models, three clock and four population growth models

Parameter				
Demographic model	Constant population size	Expansion growth	Exponential growth	Bayesian skyline plot
Path sampling (BF)	141.31	99.47	107.51	<u>179.87</u>
Stepping-stone sampling (BF)	152.94	<u>260.15</u>	110.3	185.96
TMRCA (95% CI)	1873 (1090-2608)	1841 (1157-2622)	1663 (1071-2384)	1879 (1192-2659)
TMRCA effective sample size	239	238	247	261
Substitution rate (nt/site/year)	9.66×10^{-5} (7.10×10^{-5} – 1.23×10^{-4})	9.30×10^{-5} (6.79×10^{-5} – 1.18×10^{-4})	9.89×10^{-5} (7.40×10^{-5} – 1.25×10^{-4})	9.16×10^{-5} (6.90×10^{-5} – 1.15×10^{-4})

Estimated TMRCA of significan nodes

The recombinants

NxO

recE

⑥

4 ⑥

⑩

R2

4

N

0.09 s/s

1

R1

CxO –'pepper veinal necrosis' - 1981

③ 1 1

Chile3

recA

recB&C CxO – Tannat, Uruguay

C   CxO – Tobacco, USA

recD OxN -1969

O

⑦ 1

N O

N O N
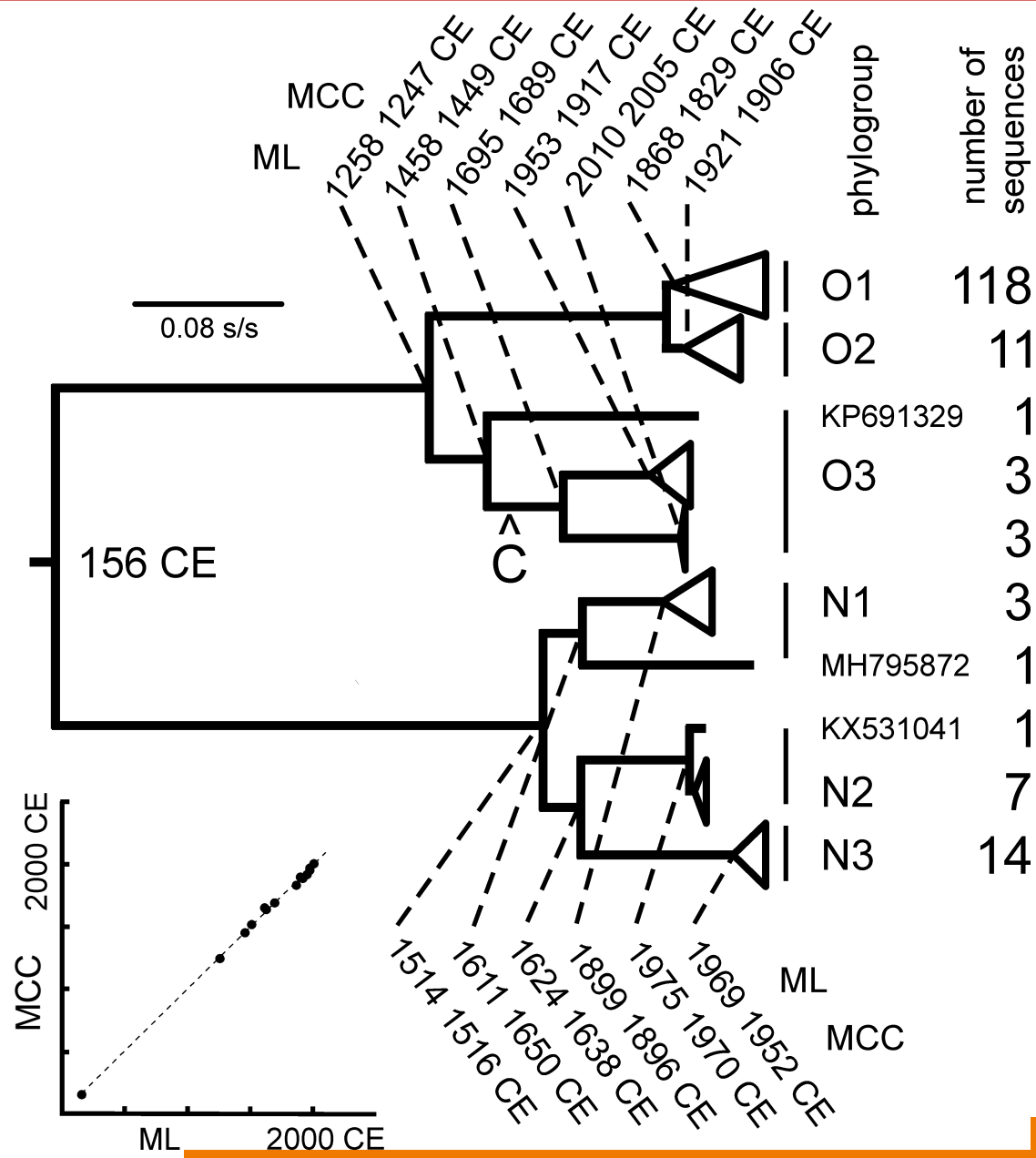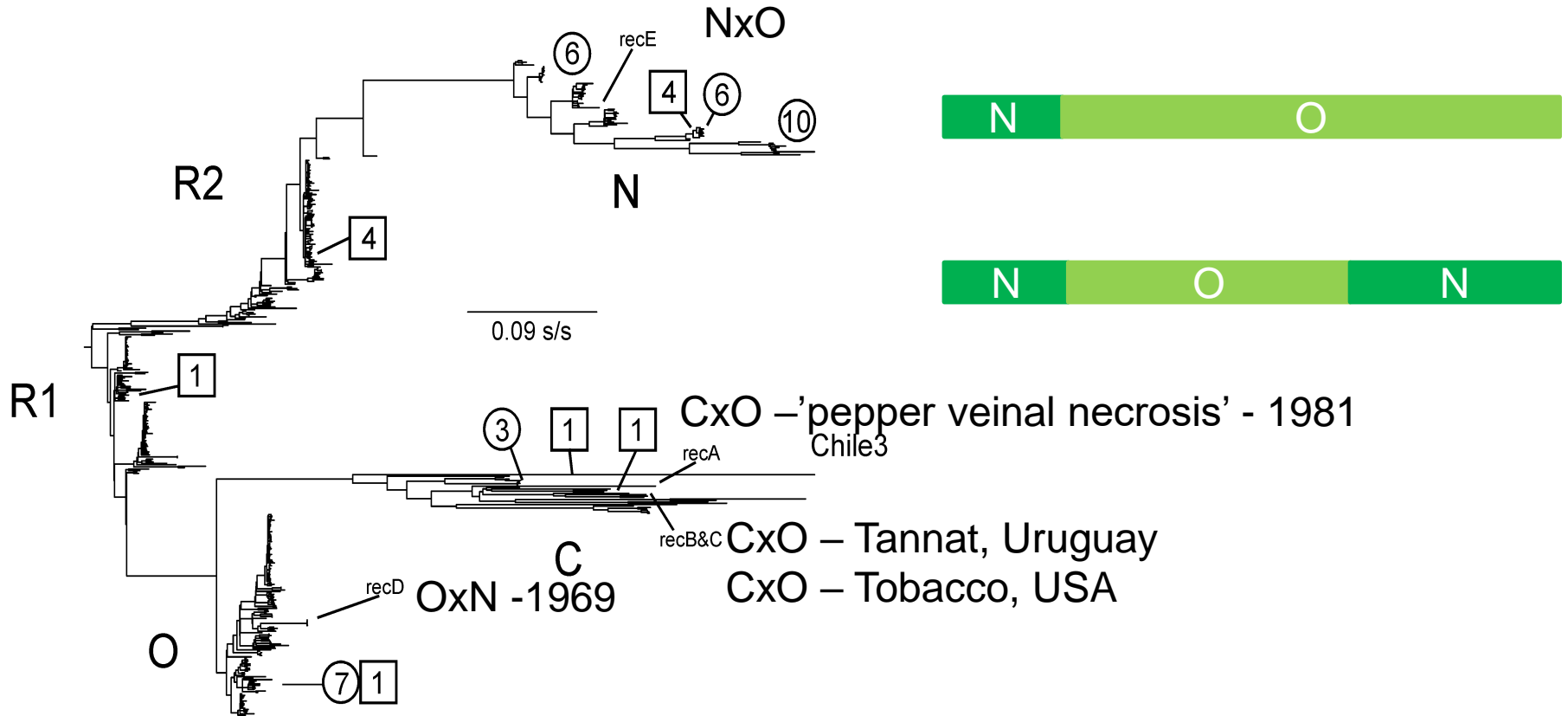
Are any of the new viruses of relevance?
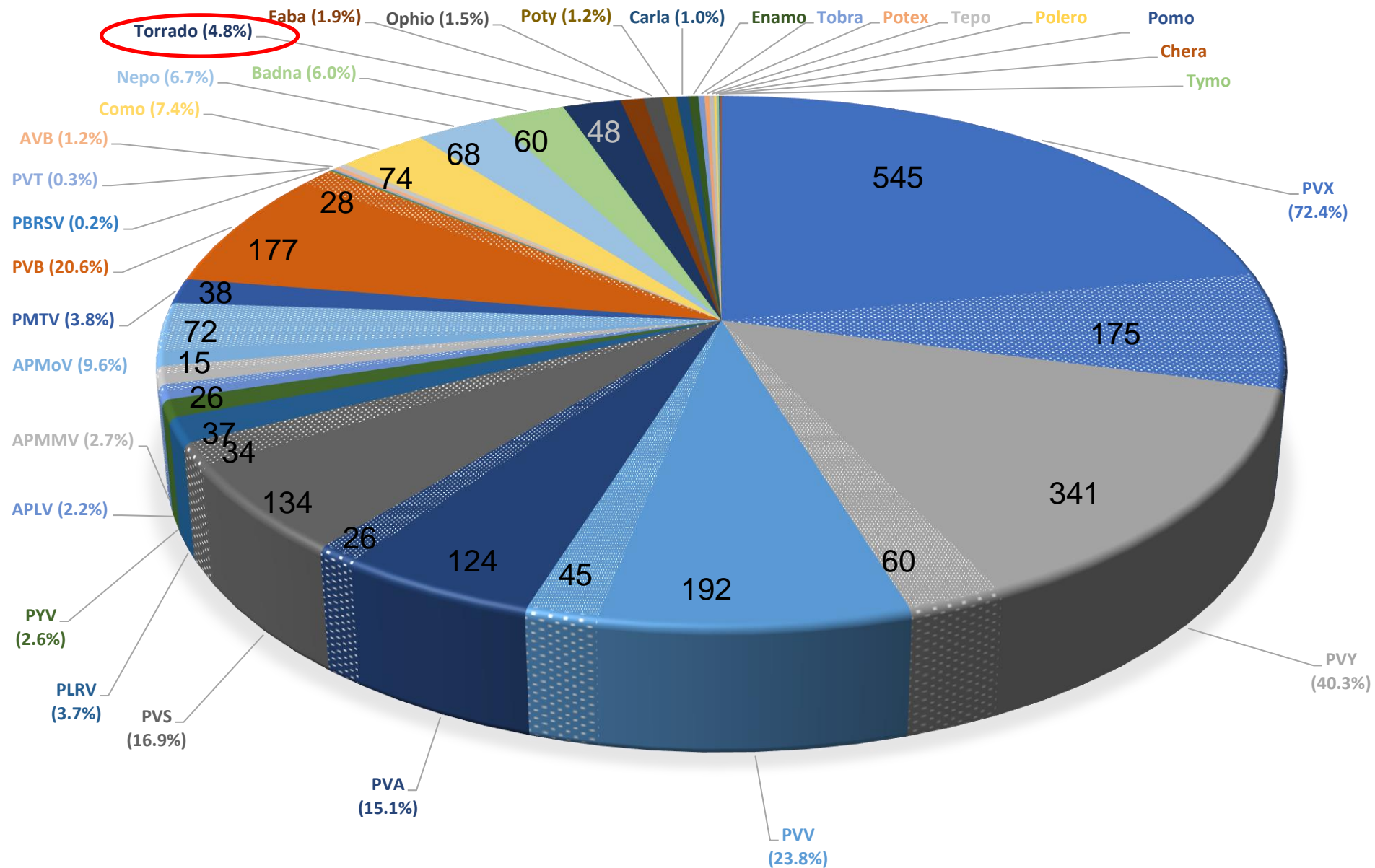
Rugose stunting disease of potato: the history of the emergence and decline of a viral disease

- Observed since 1990 in Arequipa, Moquegua and Tacna and studied as SB26 and SB29

- Isometric virus, transmitted by transmitted by brown leafhoppers (*Russelliana solanicola*) and generated losses of between 35-85% depending on the variety

- Farmers changed their planting dates to manage the disease or quit potato al together and it is no longer considered a problem

- It was never determined what type of virus it was

Potato rugose stunting virus is a torradovirus and present in low frequencies in potatoes throughout Peru
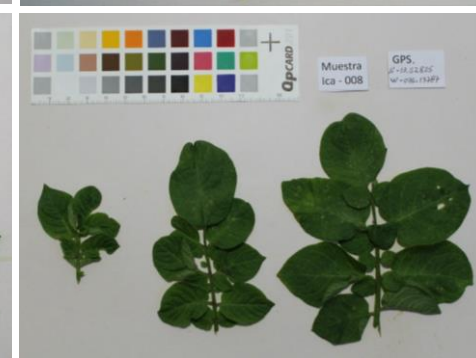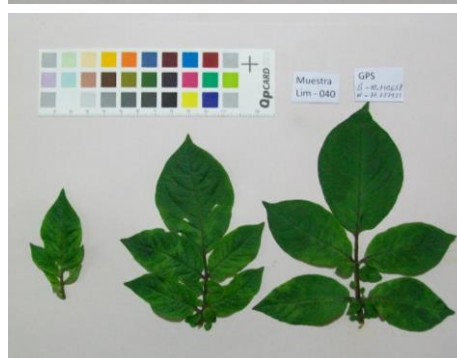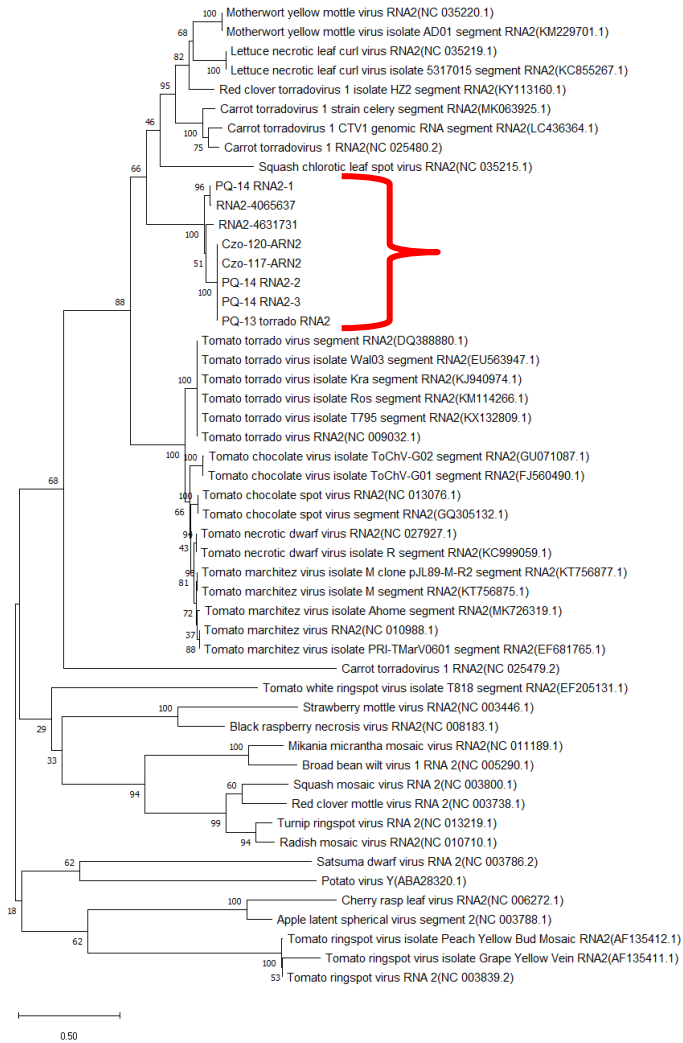
Can it be used for Diagnostics?

❖ **Technical challenges**
- Laboratory protocol?
- Bioinformatic algorithms?

❖ **Performance evaluation**
- Sensitivity variability?
- Specificity?
- Reproducibility?
- Repeatability?

❖ **Routine analysis**
- Contamination ?

Current indexing process

sweetpotato

In vitro plantlets → PROPAGATION → **"A"**

Growing in jiffy strips (30 days)

Growing in pots (30-45 days)

Symptoms, NCM-ELISA, PCR (Begomovirus)

10—15 NODES

Symptoms

High probability of detection

GRAFTING
Held grafted plants for a minimum of 30 days

Ipomoea setosa (3 weeks old or 15-20 cm height)

IF NEGATIVE

Prune and allow regrowing (30-45 days)

Symptoms, NCM-ELISA PCR (Begomovirus)

10—15 NODES AGAIN

Symptoms

High probability of detection

GRAFTING
30 days

Ipomoea setosa (3 weeks old or 15-20 cm height)

IF NEGATIVE MULTIPLY AND DISTRIBUTE FROM IN VITRO "A"

5- 6 months (US$ 110- 120)

potato

Health Testing for in vitro potato plantlets

Stock in IV Bank

Mother tube
1 month

Mother plant

TEST A

ELISA: PVS, PVY, PVX, AP LV, PLRV, APMV, PYV, PVV, and AVB.
NASH: PSTVd, PVT

Planting in pots in greenhouse

TEST B

HR: indexation using 10 species
Visual inspection
Phytoplasm?

C. Yrouye, G.Muller, C. Chuquillanqui, July 2006

1 month   2 months   1 month

NCM-ELISA is performed for 10 viruses (SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCSV, SPCFV, SPC6V, SPCV, and CMV).

sRSA for virus indexing: How is it done, what does it cost, how long does it take

2 weeks, 96 samples

~ 30 US$/sample

Cut and purify 20-30 nt band, prepare library

Library Preparation

RNA extraction

Send to sequencing provider

3-5 days

<1 US$/sample

Sequencing by Synthesis

Bio-informatics:
VirusDetect v1.0

<1 day

cue + 3 days

~20 US$/sample
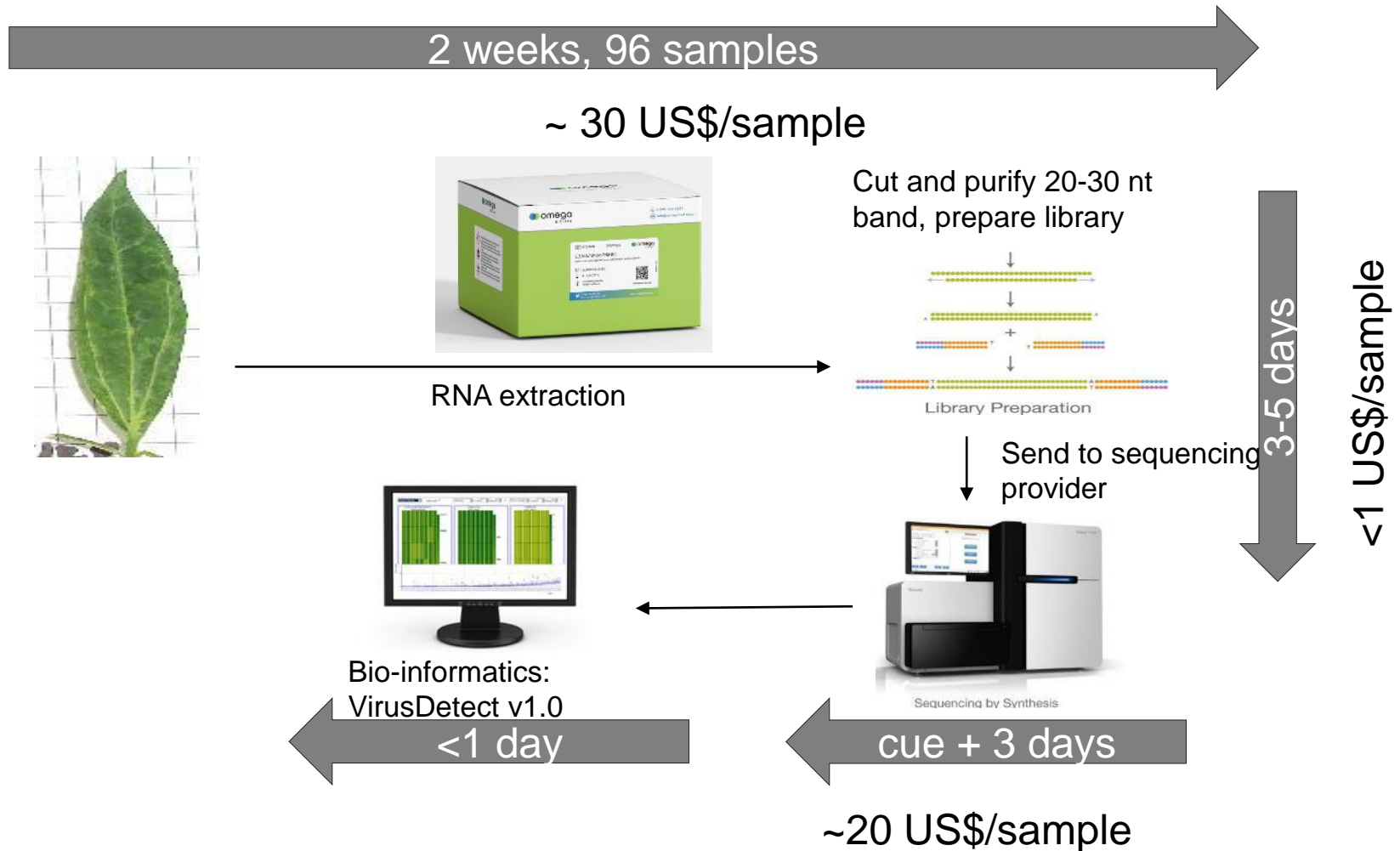
Lab ID	Building the contigs						BLAST comparison		
	Software	Extension step	Minimal contig length (nt)	k-mer range	Removal redundant reads	Host filtering	Method	Database	Cut off used
A	Velvet/Oases then Seqman	N	100	13-21	Y	Y	MegaBLAST + BLASTN	GenBank (nr)	None
B	CLC Genomics Workbench	Y	60	Variable	N	N	BLASTX + BLASTN	GenBank (viruses and viroids)	e-value of 10^{-3}
C	CLC Genomics Workbench	N	30	16-19	N	N	BLASTN	Local databases of complete virus and viroid genomes mined from Genbank	None
D	Velvet/Assembly Assembler	Y	38	(9-)11-25	N	Y	BLASTX + BLASTN	GenBank (viruses and viroids)	None
E	CLC Genomics Workbench	N	50	15-21	N	N	BLASTX + BLASTN	GenBank (nr + nt)	None
F	Velvet	Y	26	13-17	N	N	BLASTX + BLASTN	GenBank (nr)	e-value of 10^{-2} Homology cut off >80%, 100% coverage for known viruses identification
G	Velvet	N	N	17	N	N	BLASTN +TBLASTX of non-ID contigs	Local databases (viruses and viroids; ribosomal RNA; host) and GenBank (nr) for TBLASTX	Sequencing depth >5
H	Velvet/Assembly Assembler	N	21	7-21	N	N	BLASTX + BLASTN	Genbank (nr)	None
J	Velvet	Y (Only the 2,5M depth)	40	13-15-17	N	N	BLASTN/X/P	GenBank (nr)	Default parameters, e-value 10^{-1}
K	Mapping and then de novo assembly with Velvet	N	31	13-15-17	Y	N	MegaBLAST / BLASTN	GenBank (viruses and viroids)	e-value 10^{-10} Homology >95%
M	Velvet	N	29	15-17	N	N	BLASTX + BLASTN	GenBank (viruses and viroids)	e-value 10^{-4} (BLASTX) e-value 10^{-6} (BLASTN)
N	Velvet and BWA backtrack	N	50	11-19	Y	N	BLASTX + BLASTN	GenBank (viruses and viroids)	Bit score >= 30
O	CLC Genomics Workbench	N	50	16-19	N	N	BLASTN	GenBank (nt)	e-value 10^{-3} Homology >85%
P	Velvet/Assembly Assembler	N	21	9-31	N	N	BLASTX + BLASTN	GenBank (nr + nt)	e-value 10^{-3}
R	Mapping against refseqdB, and de novo assembly, both in CLC Genomics Workbench	Y	21	12	N	Y	BLASTX + BLASTN	GenBank (nr)	None
S	VirusDetect	N	40	9-19	N	Y	BLASTX + BLASTN	GenBank (nt + nr)	Sequencing depth >5; reference genome coverage >10%; e-value 10^{-5}
T	Blasting raw reads against Genbank (nt) and de novo assembly with CLC Genomics Workbench	N	50	17	N	N	BLASTN	Genbank (nt) + Refseq virus and viroids	e-value 10^{-4}
V	AByss	N	16	16	N	N	BLASTN	GenBank (nt)	e-value 10^{-5}
W1	CLC Genomics Workbench	N	60	14	N	N	BLASTX	GenBank (viruses and viroids)	e-value 10^{-3}
W2	CLC Genomics Workbench	N	60	Variable	N	N	BLASTX	GenBank (viruses and viroids)	e-value 10^{-3}
X	CLC Genomics Workbench	Y	21	17	N	N	BLASTN + BLASTX	GenBank virus + viroid	e-value 10^{-3}

Bio-informatics pipelines: How consistent are they?

LAB ID	SENSITIVITY				FALSE DISCOVERY RATE		
	2,500,000	250,000	50,000	AVERAGE	2,500,000	250,000	50,000
A	90%	53%	10%	51%	0%	0%	0%
B	80%	35%	30%	48%	0%	0%	0%
C	80%	71%	60%	70%	0%	0%	0%
D	100%	82%	50%	77%	17%	7%	9%
E	80%	82%	30%	64%	0%	0%	0%
F	100%	88%	80%	89%	0%	0%	0%
G	100%	53%	20%	58%	0%	0%	0%
H	70%	65%	30%	55%	0%	0%	0%
J	100%	94%	70%	88%	0%	0%	9%
K	90%	71%	40%	67%	0%	0%	0%
M	90%	94%	50%	78%	0%	6%	18%
N	90%	82%	30%	67%	0%	0%	0%
O	40%	41%	20%	34%	0%	0%	0%
P	70%	59%	20%	50%	0%	0%	0%
R	100%	100%	100%	100%	9%	6%	9%
S	100%	100%	50%	83%	0%	0%	0%
T	100%	100%	90%	97%	0%	0%	0%
V	80%	88%	60%	76%	0%	0%	0%
W1	90%	82%	40%	71%	0%	0%	0%
W2	90%	82%	60%	77%	0%	0%	0%
X	80%	71%	30%	60%	0%	8%	27%
AVERAGE	87%	76%	46%	70%	GLOBAL FDR RATE: 1.9%		
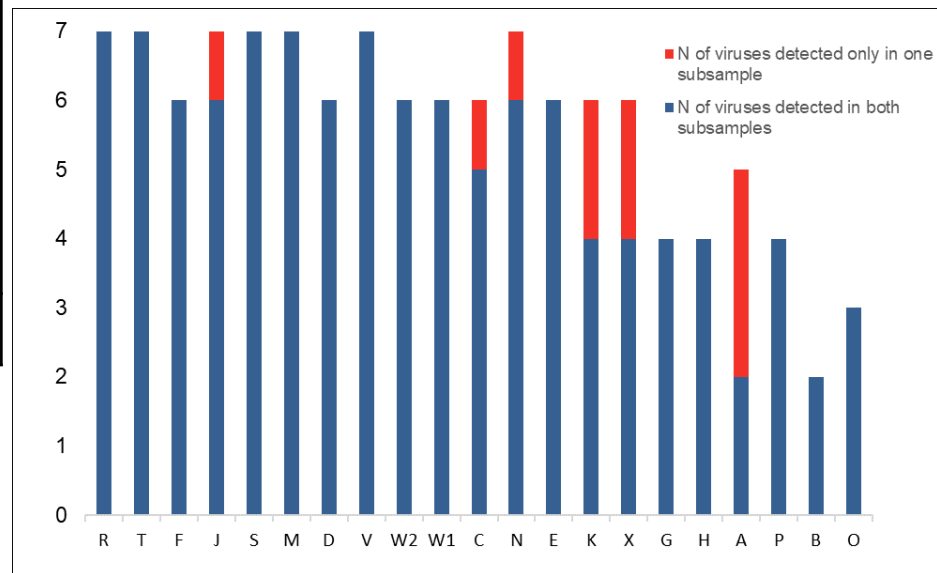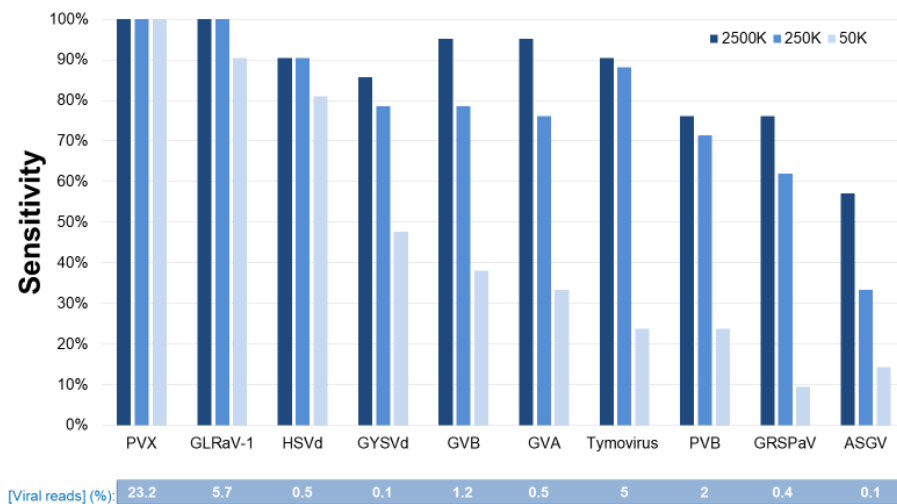
VirusDetect-Windows
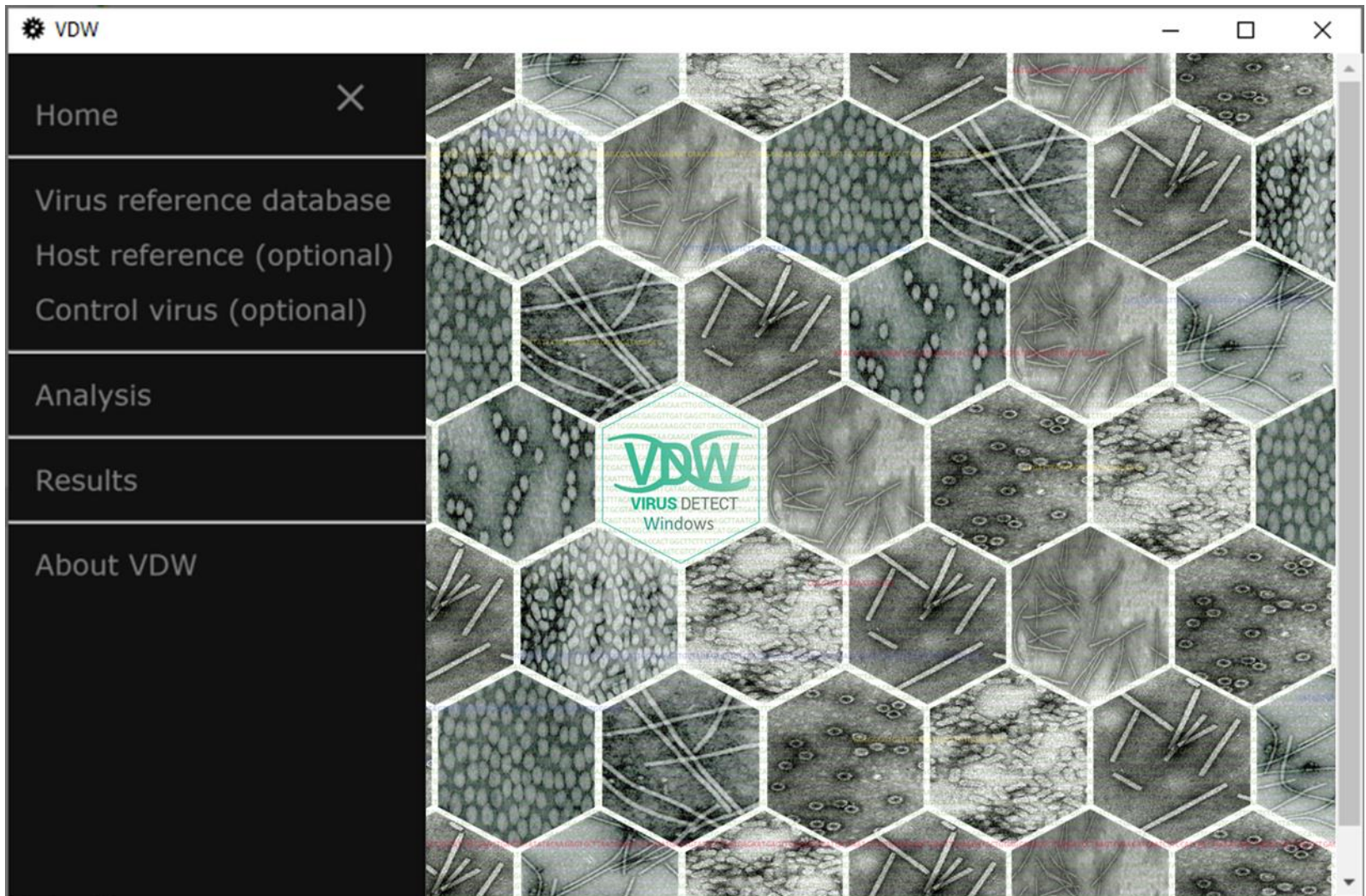
The International Potato Center (known by its Spanish acronym CIP) is a research-for-development organization with a focus on potato, sweetpotato, and Andean roots and tubers. CIP is dedicated to delivering sustainable science-based solutions to the pressing world issues of hunger, poverty, gender equity, climate change and the preservation of our Earth's fragile biodiversity and natural resources.

www.cipotato.org

CIP is a member of CGIAR

CGIAR is a global agriculture research partnership for a food secure future. Its science is carried out by the 15 research centers who are members of the CGIAR Consortium in collaboration with hundreds of partner organizations.

www.cgiar.org