



Agricultural viromics: from continental viromes to field level molecular diagnostics

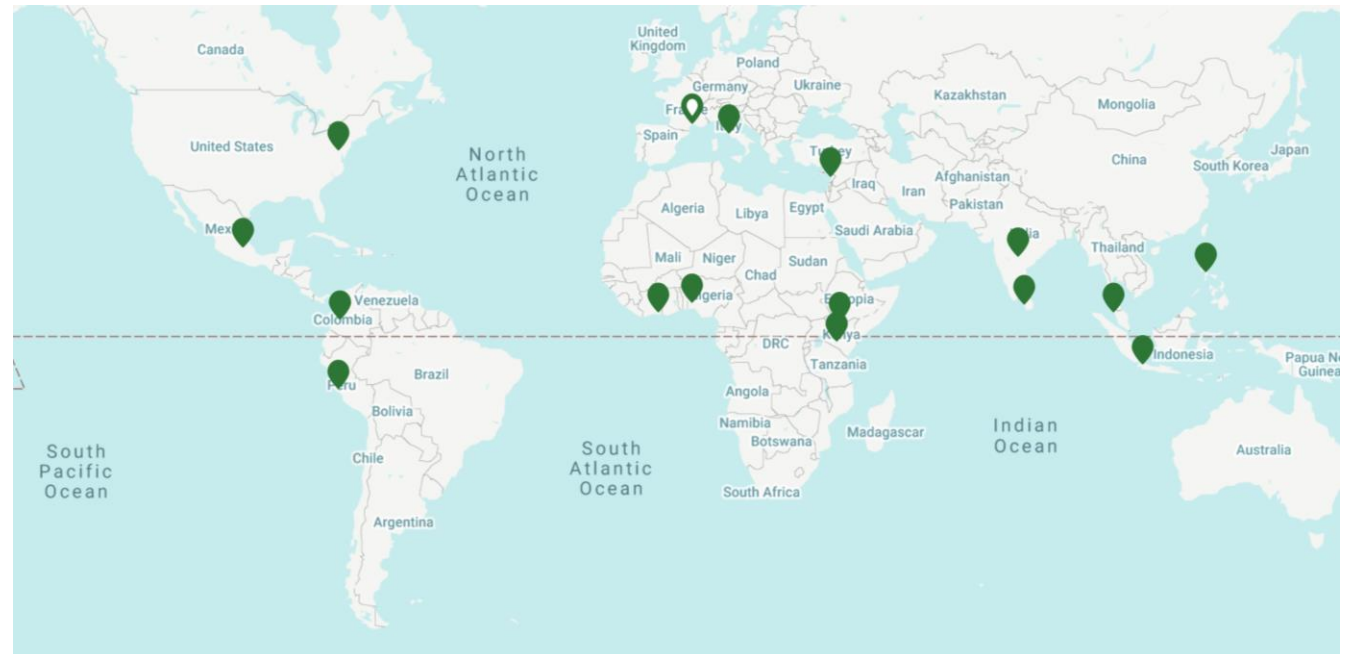
Jan Kreuzer



Fall KSPF conference, 2022



One CGIAR

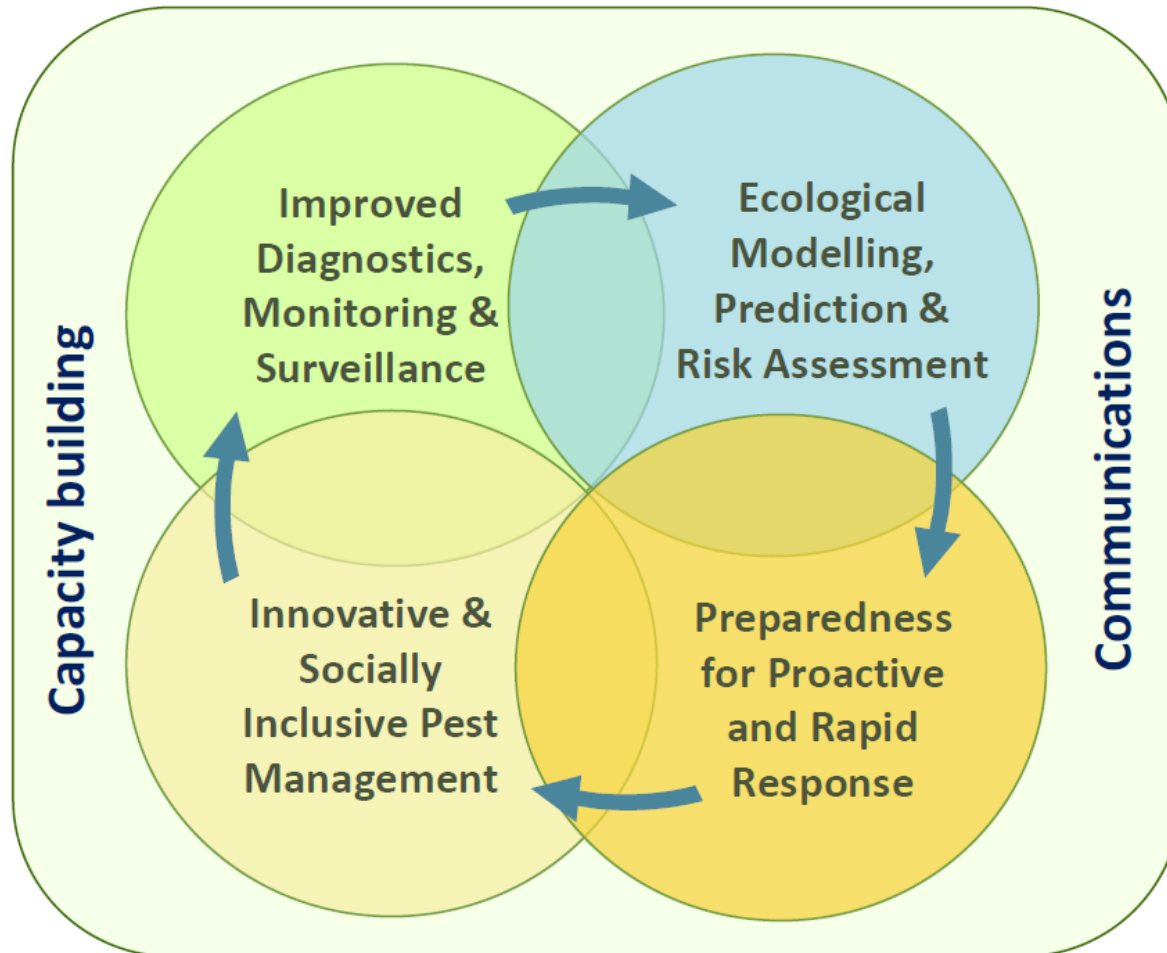


Plant Health Management under Changing Climates

Need for a Revitalized Strategy and Innovative Approaches



Plant Health and Rapid
Response to Protect
Food Security and
Livelihoods

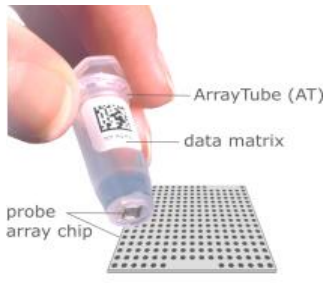


Next generation diagnostics

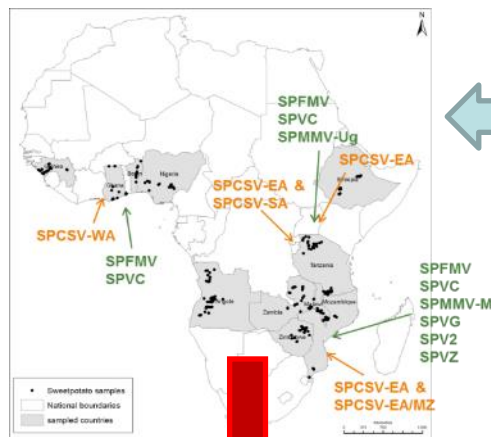
Better understanding of the major diseases contributing to seed degeneration, their epidemiology and cheap rapid accurate diagnostics

Appropriate diagnostics technologies

Figure 2 Components of prototype field LAMP kit for detection of sweetpotato viruses.

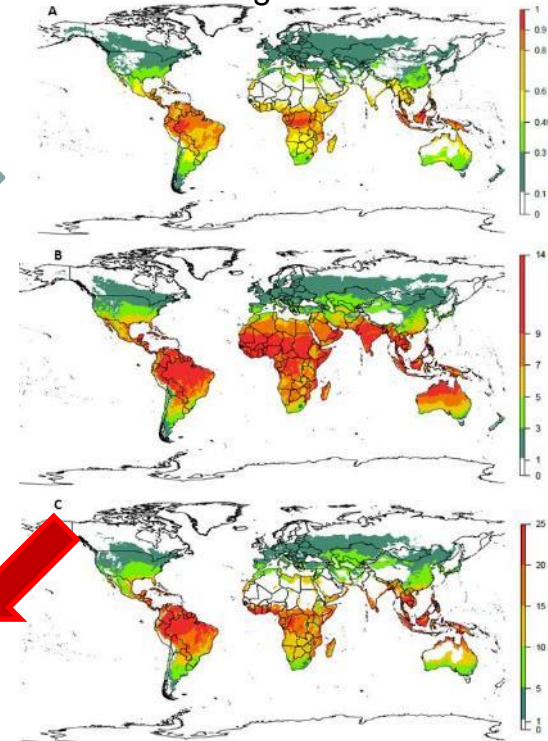


Characterization & surveillance of (new & emerging) pathogens

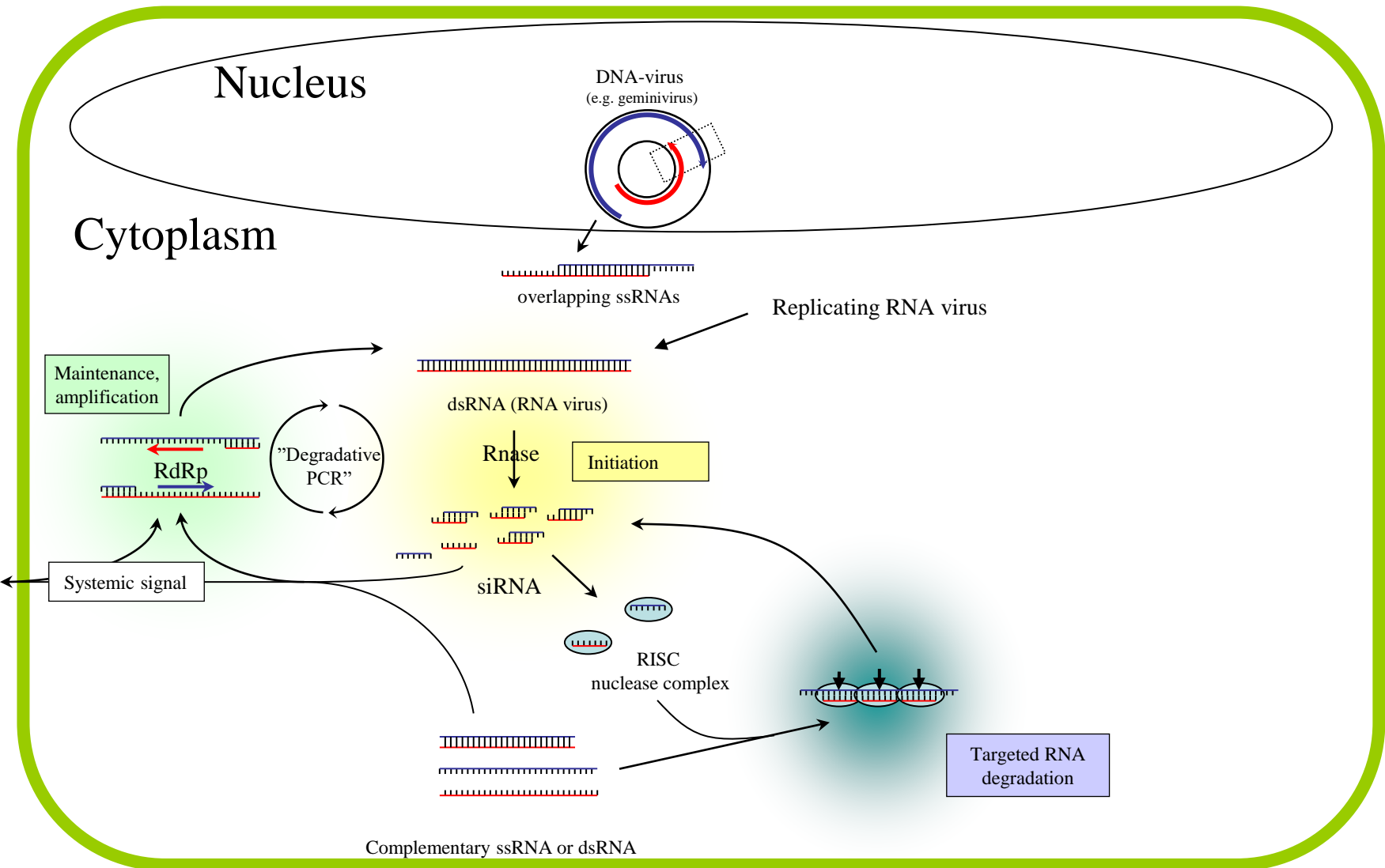


disease management
germplasm exchange
crop improvement

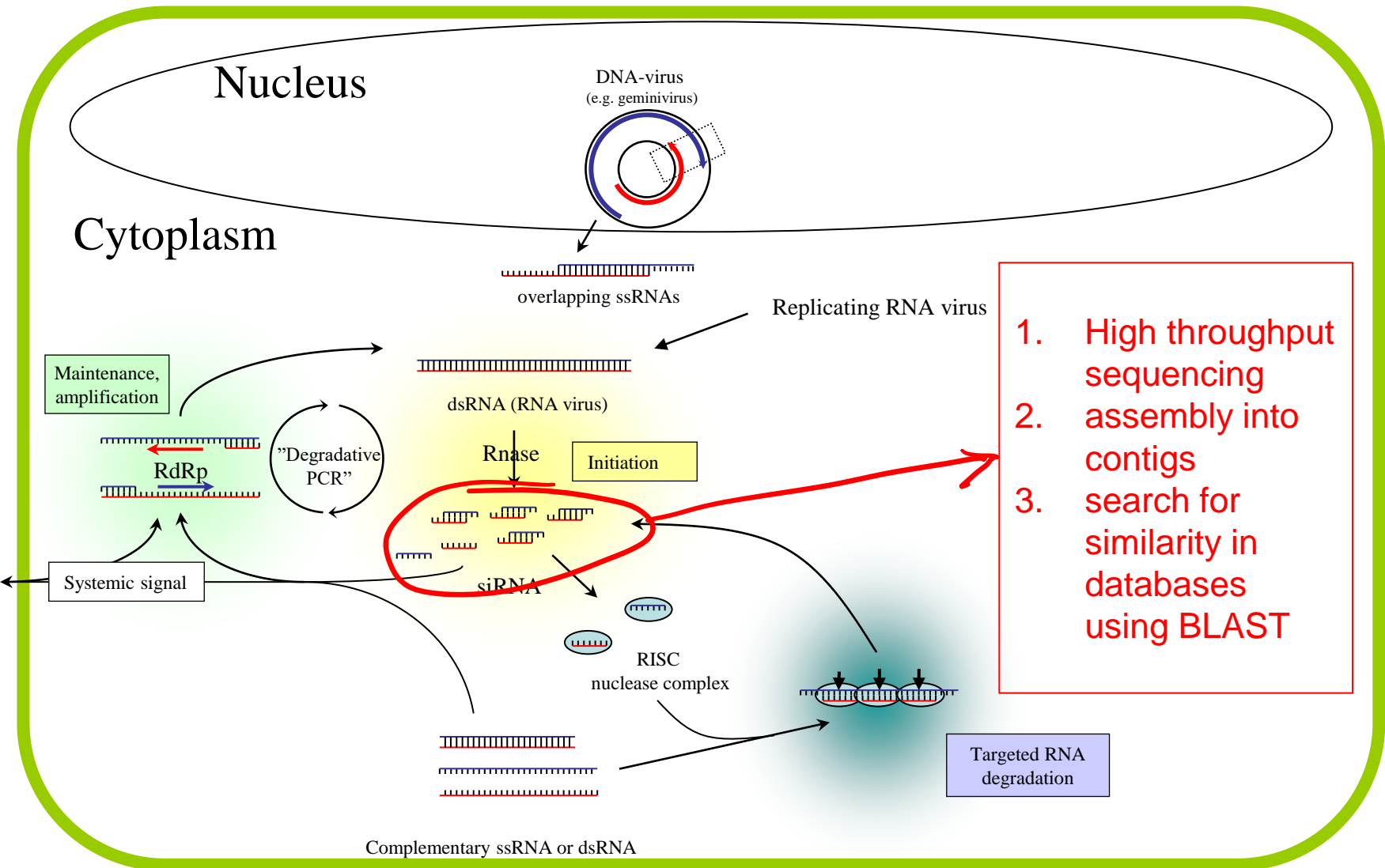
Predicting risk



RNA sequencing and assembly for virus identification and discovery

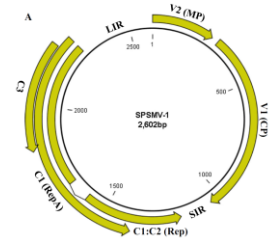
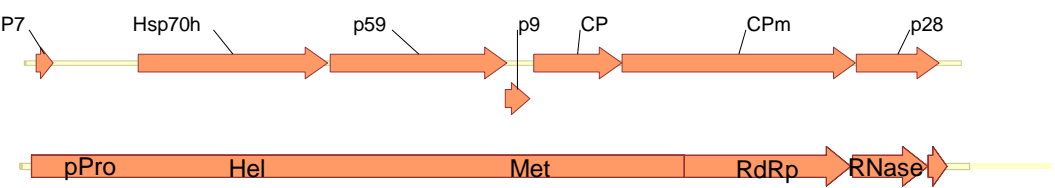


RNA sequencing and assembly for virus identification and discovery

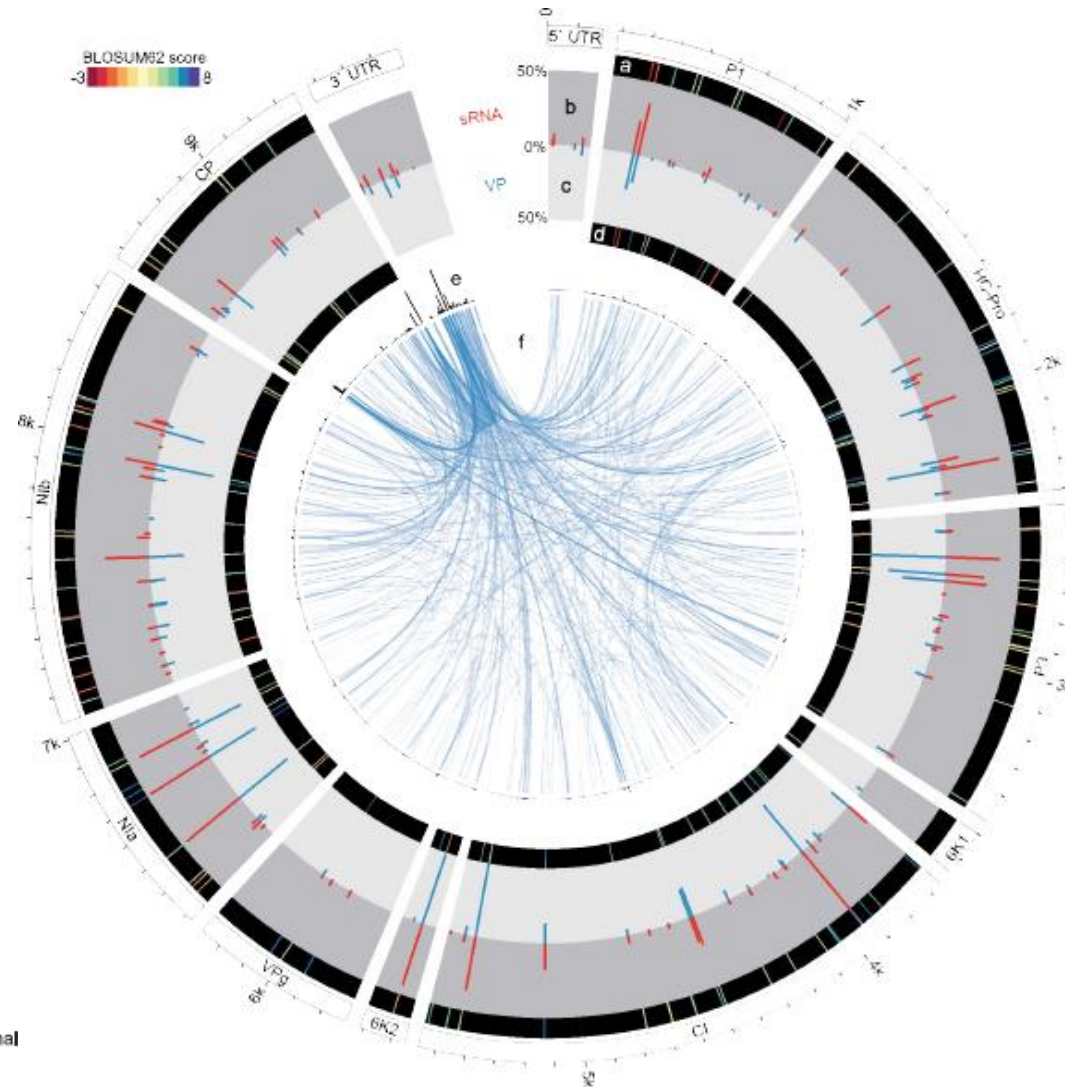
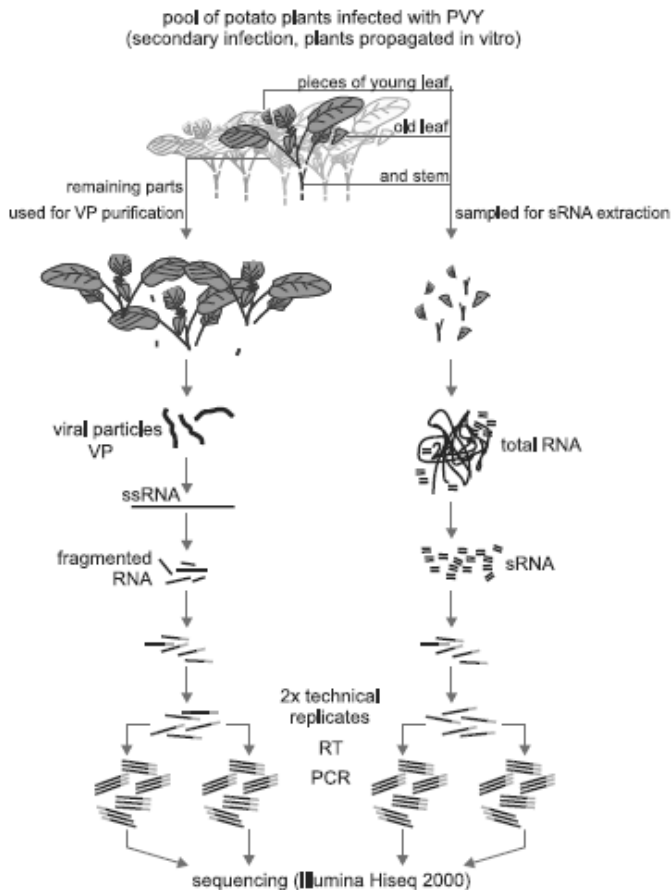


Number of contigs assembled by Velvet using 21–24 nt sRNA, or only 22 nt sRNA sequences, with virus specific hits as identified using Translated Nucleotide Blast (Blastx).

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	(≤ 949)	71	78
		SPCSV	0	-	0	0
		Badnavirus	38	(≤ 256)	62	55
		Mastrevirus	5	(≤ 210)	6	5
SPCSV	1,271,382	Total contigs	283		1675	285
		SPFMV	0	-	0	0
		SPCSV	10	(≤ 70)	64	12
		Badnavirus	44	(≤ 181)	63	44
		Mastrevirus	8	(≤ 260)	10	6
SPFMV + SPCSV	1,067,577	Total contigs	221		1363	581
		SPFMV	20	(≤ 1600)	43	51
		SPCSV	2	(≤ 81)	41	12
		Badnavirus	38	(≤ 266)	63	51
		Mastrevirus	5	(≤ 210)	8	5



SNP landscape highly similar between siRNA and RNA from virus particles



1

Consensus genome
sequence reconstruction

2

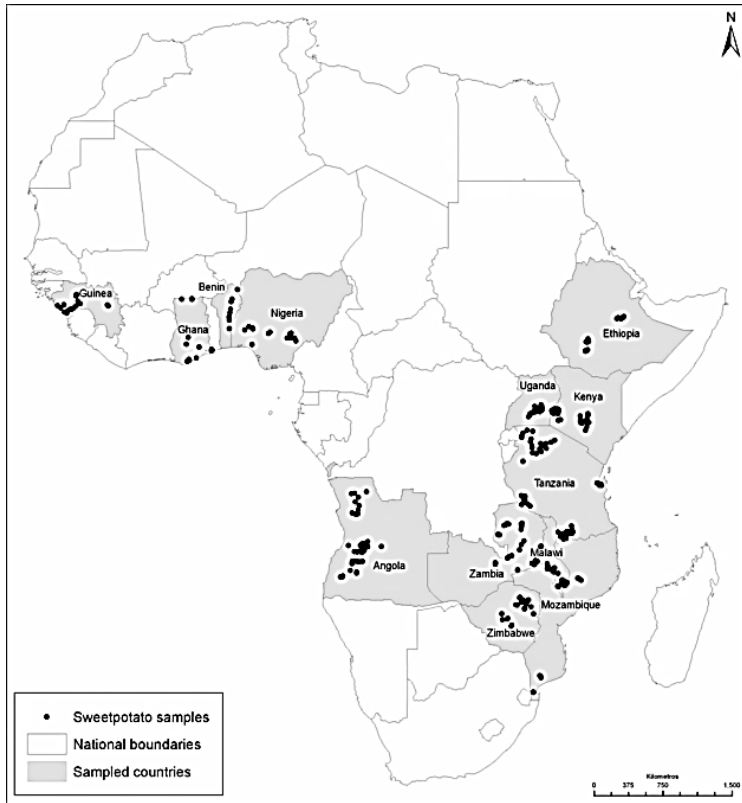
Analysis of mutational cloud

3

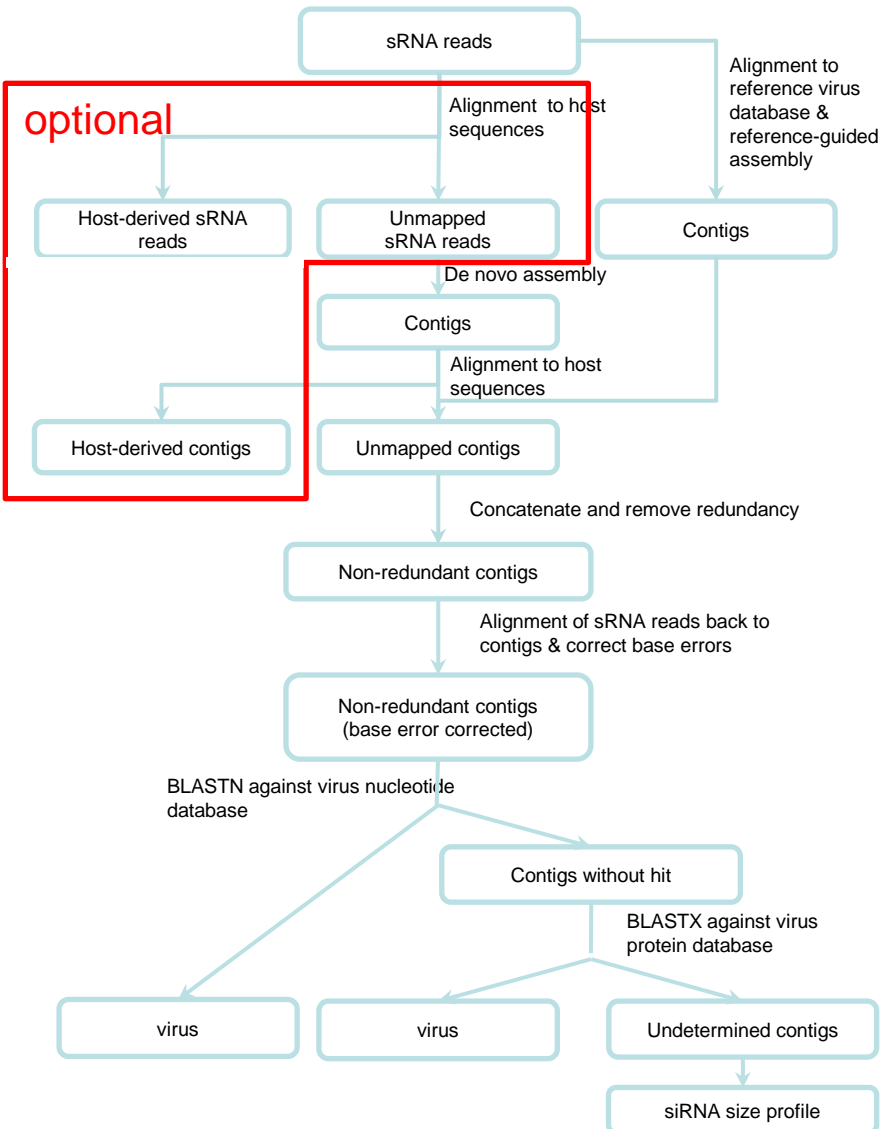
Recombination anal

The African sweetpotato virome

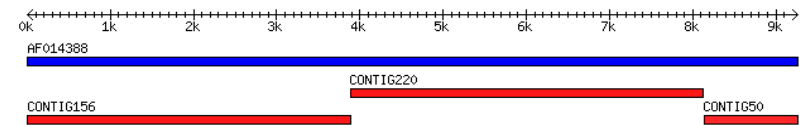
~2000 samples and bioinformatics pipeline for virus identification using siRNA assembly and genome subtraction



Small RNA sequence analysis pipeline: VirusDetect_v1.6



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.



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

Alignment:

```

Query: 1   tgaggttgaatatgctcgtattgttcgcaagtaatgggtgaagatgtagctattcaaaag 60
           ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 8144 tgaggttgaagatgctcgtattgttcgcaagtaatgggtgaagattagctattcaaaag 8203

Query: 61   aaacgatgctcaacatgggtgttcaccaatgacctagacactcataagattgactcaaa 120
           ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 8204 aaacgatgctcaacatgggtgttcaccaatgacctagacactcataagattgactcaaa 8263
  
```

http://bioinfo.bti.cornell.edu/virome/index



Home Participants Sampling Data

Home Participants Sampling Data Publication Link

Field Map Virus Map

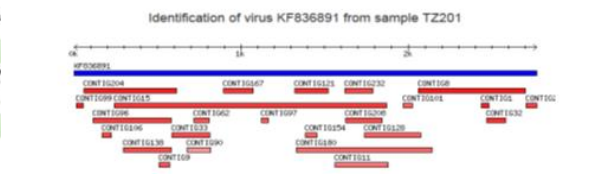
Samples in field TZF111

Sample	Date(DD/MM/YYYY)	Age(month)	Sample Image	Field Image
TZ201	28/03/2012	3	IMG_0289.JPG	IMG_0292.JPG
TZ202	28/03/2012	3	IMG_0294.JPG	IMG_0296.JPG
TZ203	28/03/2012	3	IMG_0297.JPG	IMG_0300.JPG
TZ204	28/03/2012	3	IMG_0301.JPG	IMG_0304.JPG
TZ205	28/03/2012	3	IMG_0305.JPG	IMG_0307.JPG



Field (TZF111) information

Filed No.: TZF111
 Region: Mwanza



Order	Query ID	Query Start	Query End	Subject Start	Subject End	Identity	E value	Strand
1	CONTIG1	15	59	2439	2483	4445(97%)	2e-11	1
2	CONTIG3	3	639	2060	2098	629(63/96%)	0.0	1



Pan-African Sweet Potato Virome
 Home

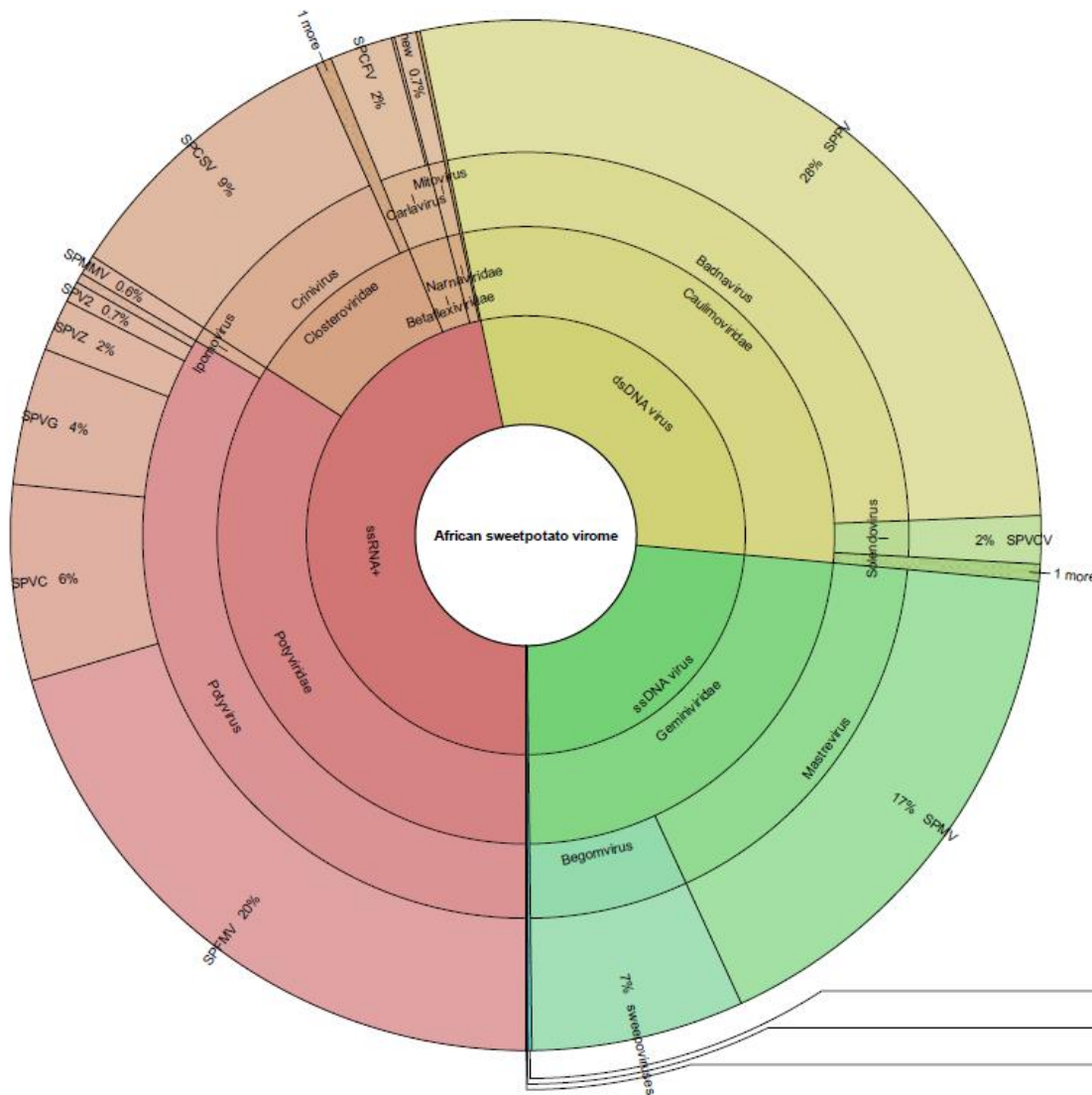
Reference	GC	Coverage(%)	Depth	Depth(100x)	Genus	Description
J090174	438	383(90.8)	4	74.0	Sedavirus	Sweetpotato sedavirus
FJ80944	7901	2782(94.3)	23	28.9	Sedavirus	Sweetpotato sedavirus
FJ80943	8052	2311(95.8)	20	92.0	Sedavirus	Sweetpotato sedavirus
KF83682	3068	1838(91.8)	21	92.8	Sedavirus	Sweetpotato sedavirus complete genome
KU76910	2831	914(92.4)	10	108.3	Sedavirus	Sweetpotato sedavirus complete genome China Guangxi 2011, complete genome



Terms of Use

Results: the viruses

3193 viruses from 1168 samples



New viruses:
Potyviruses: 3
Ampeloviruses: 3
Mitoviruses: many
Nepovirus
Soymovirus
Emaravirus
Begomoviruses
Alphasatellites



Badnaviruses of Sweet Potato: Symptomless Coinhabitants on a Global Scale

Jan F. Kreuze^{1*}, Ana Perez², Marco Galvez Gargurevich¹ and Wilmer J. Cuellar¹

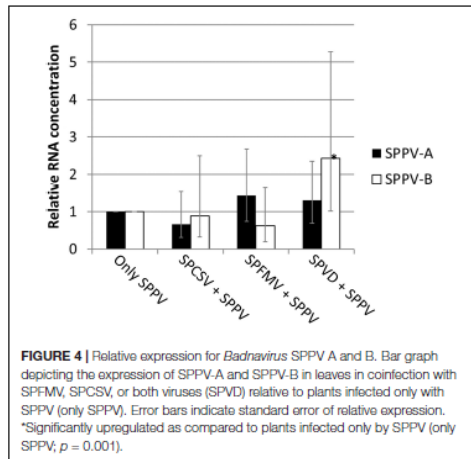
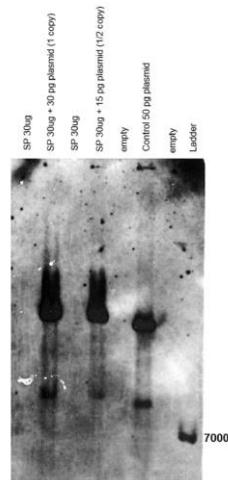
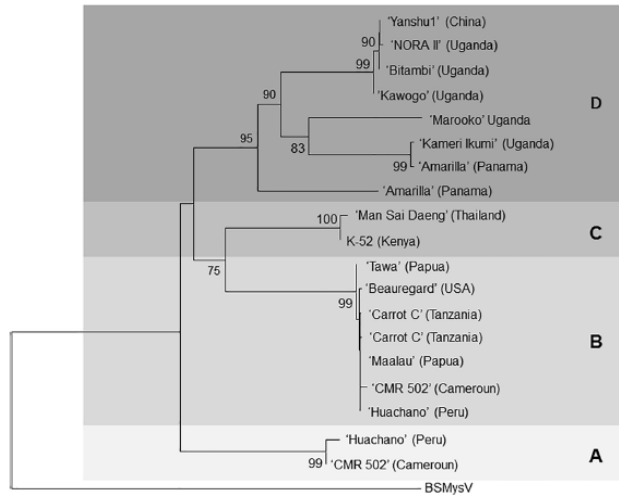
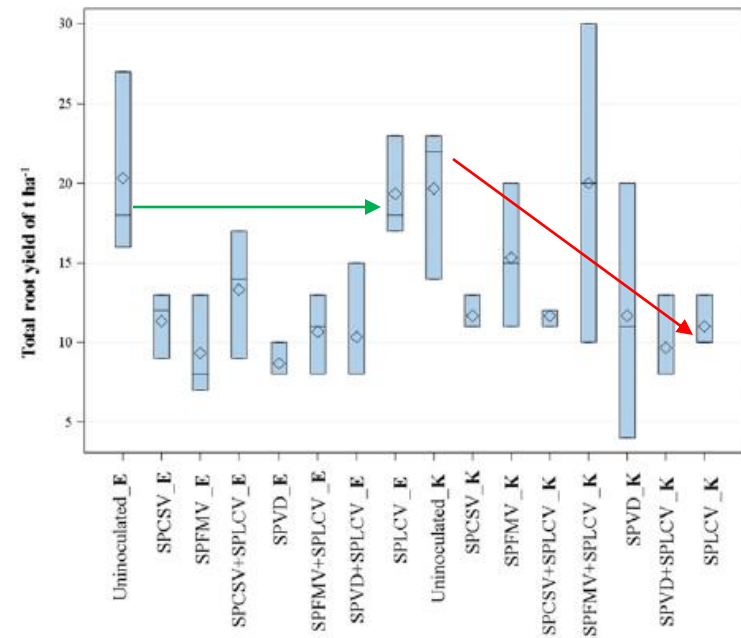


FIGURE 4 | Relative expression for *Badnavirus* SPPV A and B. Bar graph depicting the expression of SPPV-A and SPPV-B in leaves in coinfection with SPPMV, SPCSV, or both viruses (SPVD) relative to plants infected only with SPPV (only SPPV). Error bars indicate standard error of relative expression. *Significantly upregulated as compared to plants infected only by SPPV (only SPPV; $p = 0.001$).

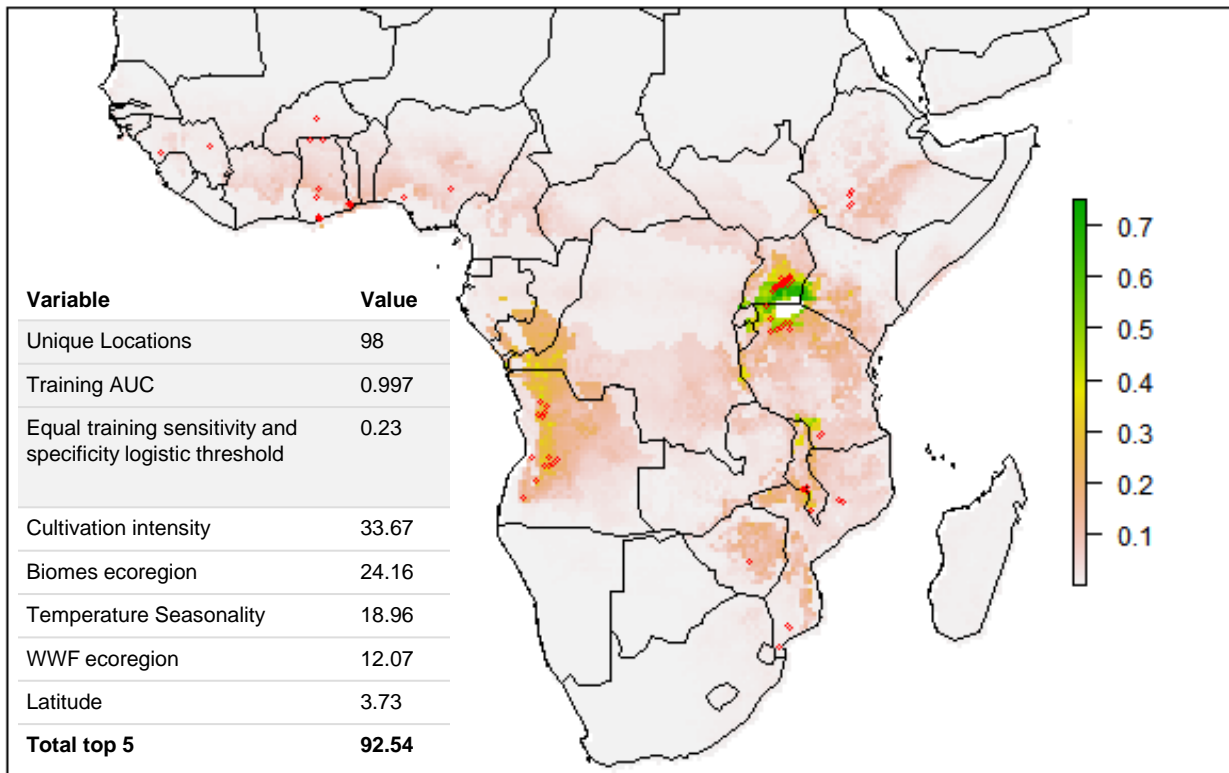
Storage Root Yield of Sweetpotato as Influenced by *Sweetpotato leaf curl virus* and Its Interaction With *Sweetpotato feathery mottle virus* and *Sweetpotato chlorotic stunt virus* in Kenya

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

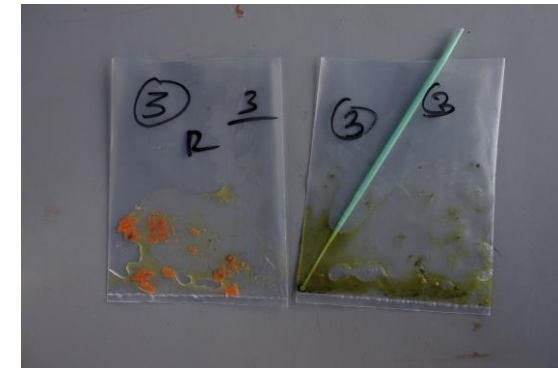
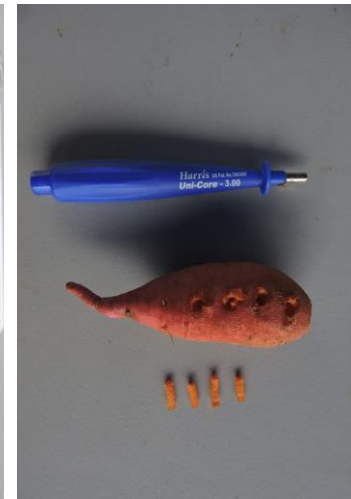
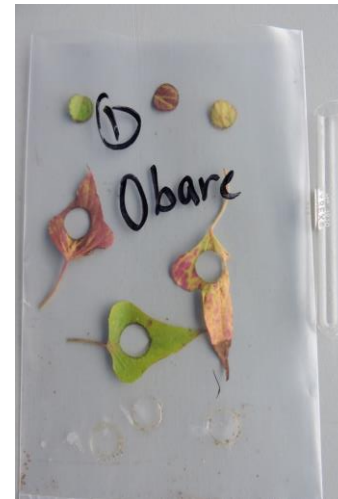
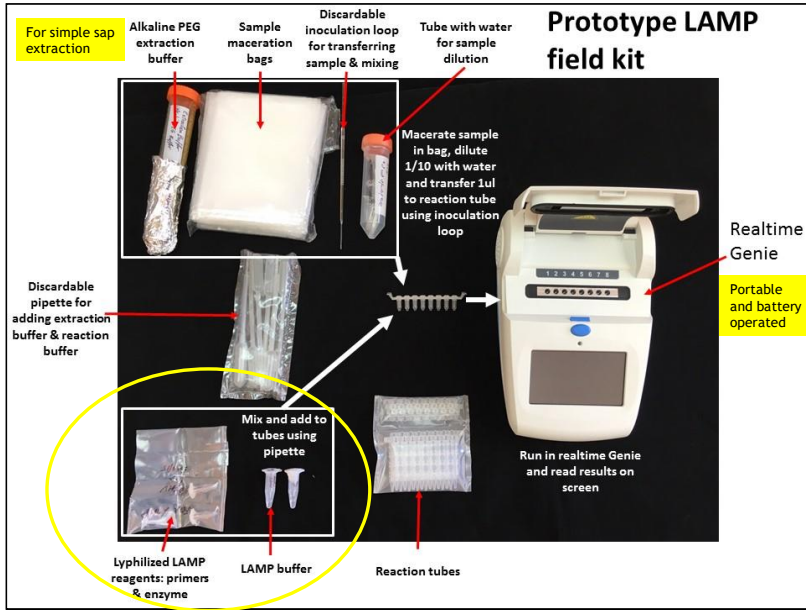


Can we make predictions about likely occurrence beyond evaluated areas?

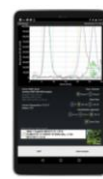
Example for begomoviruses: Niche analysis using MaxEnt modeling and bioclimatic-ecosystem variables



Field LAMP kit



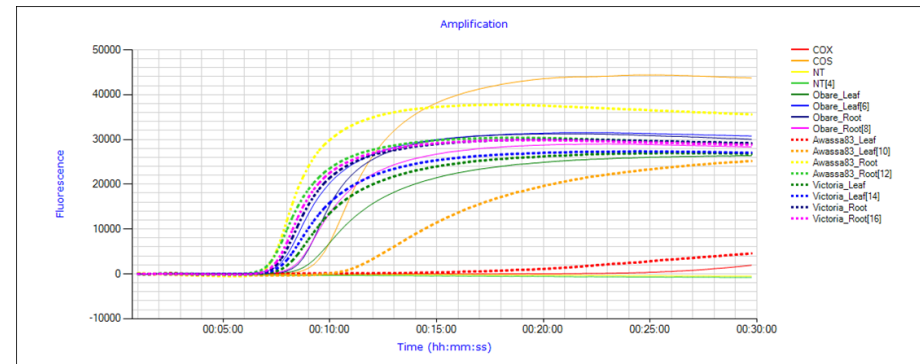
Lyophilized reagents



BioRanger

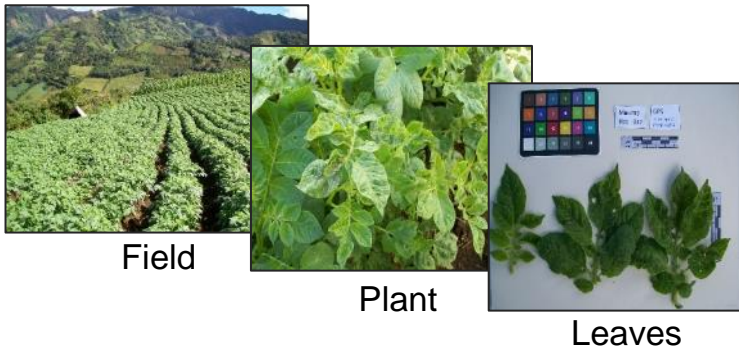


Easy to interpret

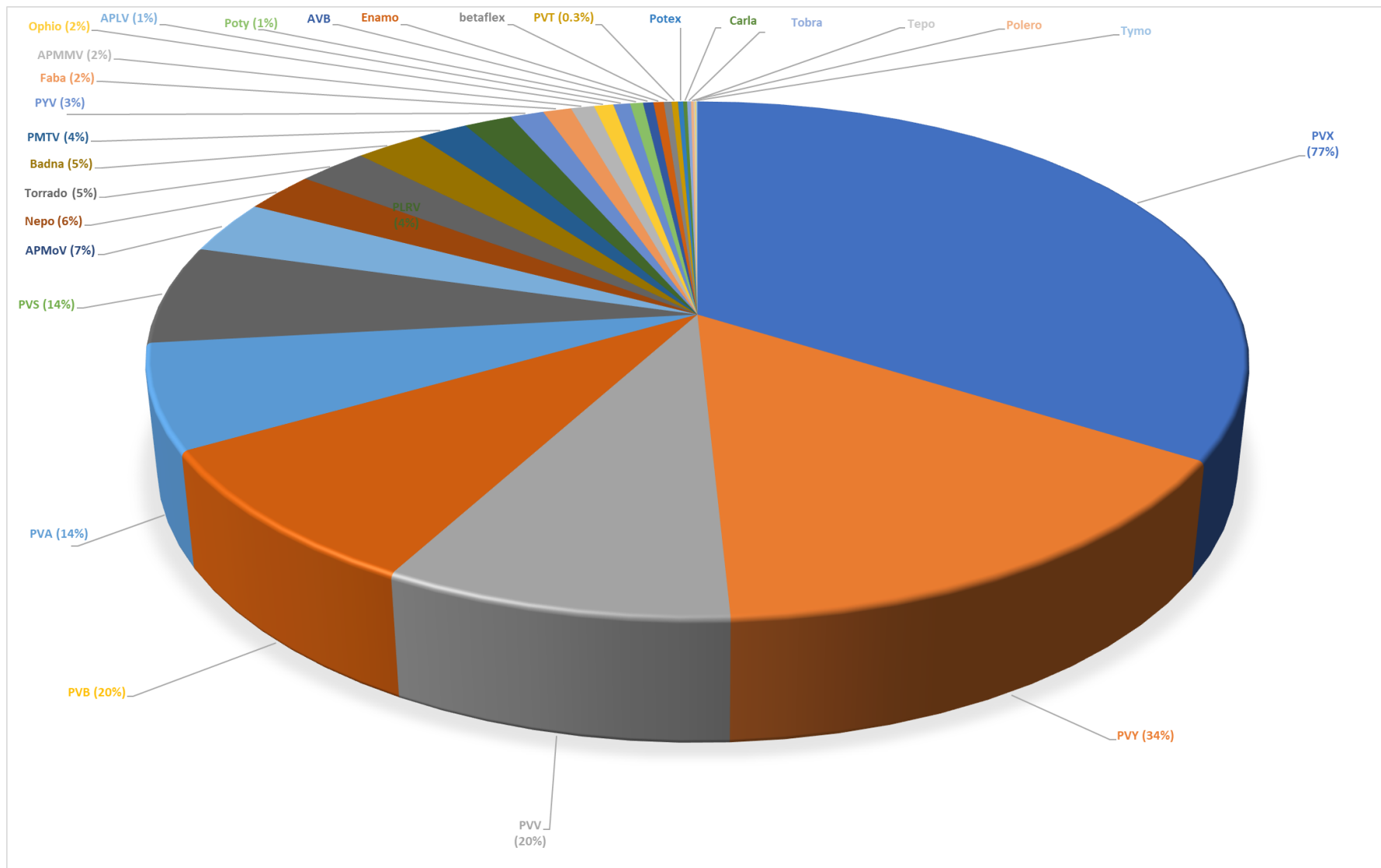


The Peruvian potato virome

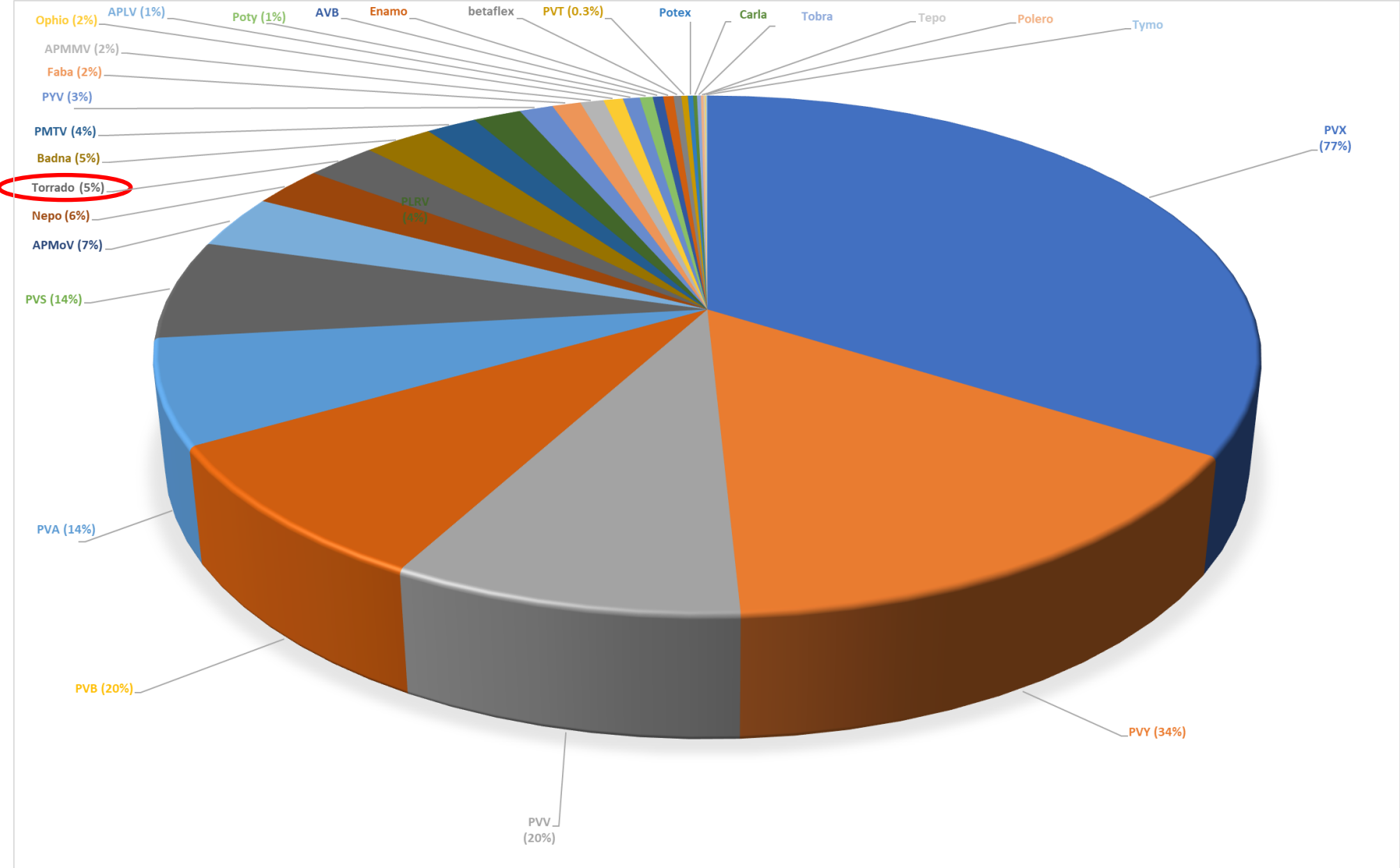
- Sample collection:
994 geo-referenced potato leaf samples (Cajamarca, Huanuco, Junin, Huancavelica, Cusco)



General results



Are any of the new viruses of relevance?

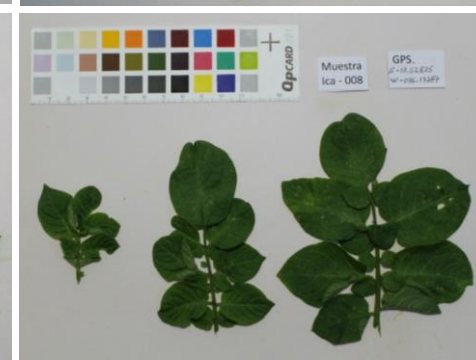
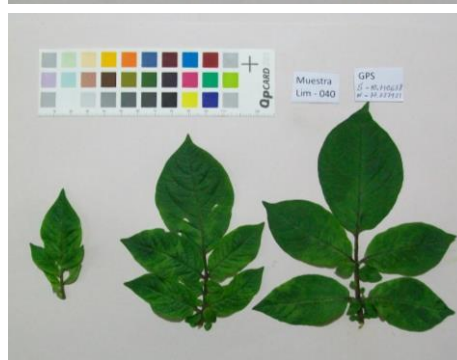
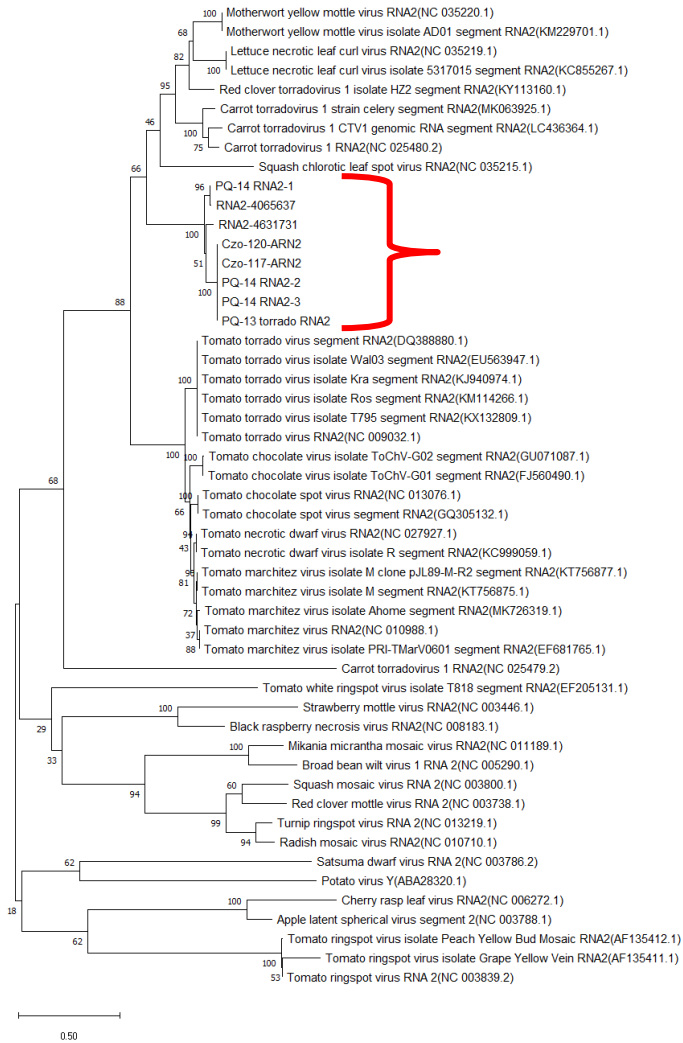


Rugose stunting disease of potato: the history of the emergence and decline of a viral disease

- 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 altogether 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





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