



Plant Health
Initiative

Global Plant Virus Management: Diagnostics, Surveillance, and Modelling

Jan Kreuzer, Wilmer J. Cuellar, Lava Kumar, B.M. Prasanna,
Aman Omondi

Alliance



AfricaRice



International Center for Tropical Agriculture
Since 1967 Science to cultivate change



International Maize and Wheat Improvement Center



INTERNATIONAL
POTATO CENTER



Science for resilient livelihoods in dry areas



Transforming African Agriculture

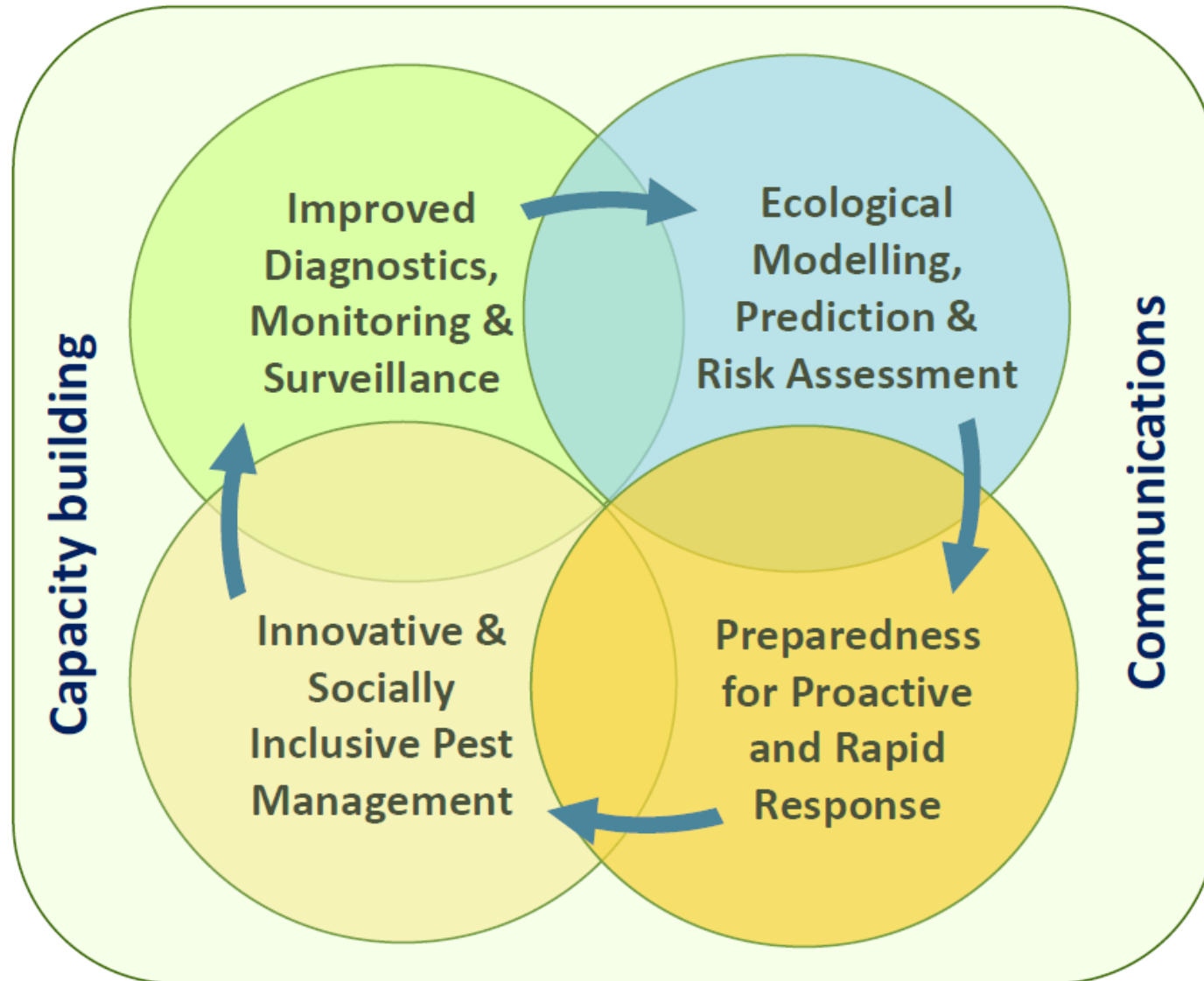


Plant Health Management under Changing Climates

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 crop losses, their epidemiology and cheap rapid accurate diagnostics

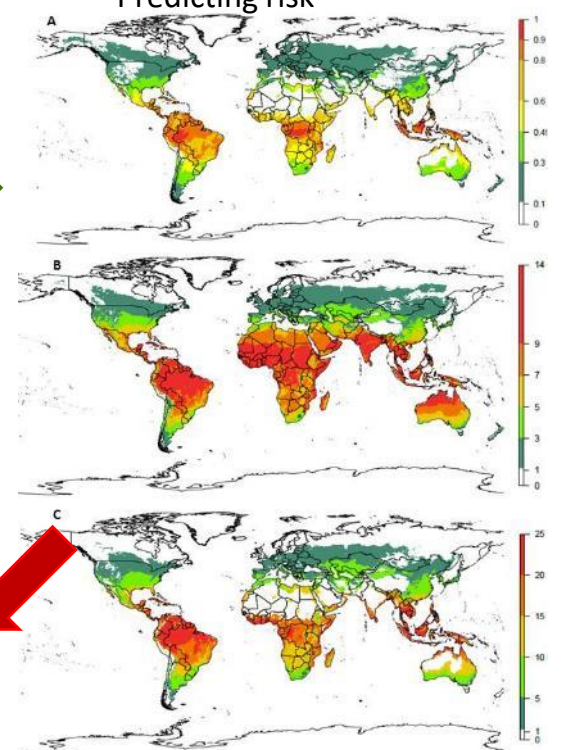
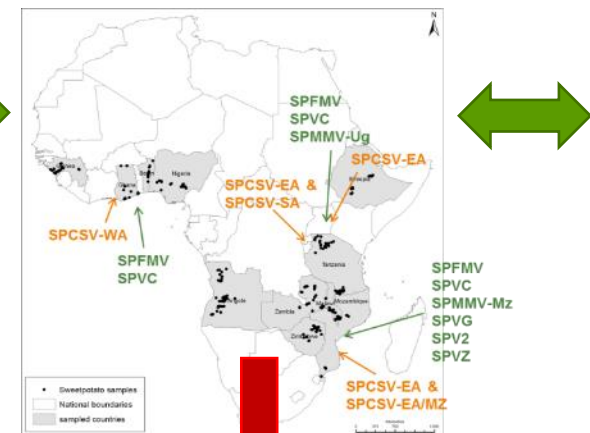
Appropriate diagnostics technologies

Characterization & surveillance of (new & emerging) pathogens

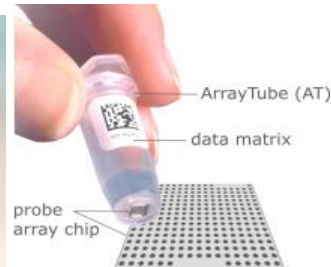
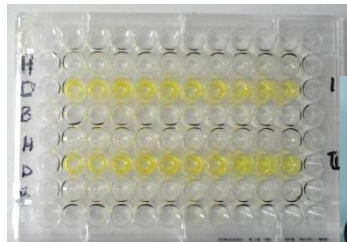
Predicting risk



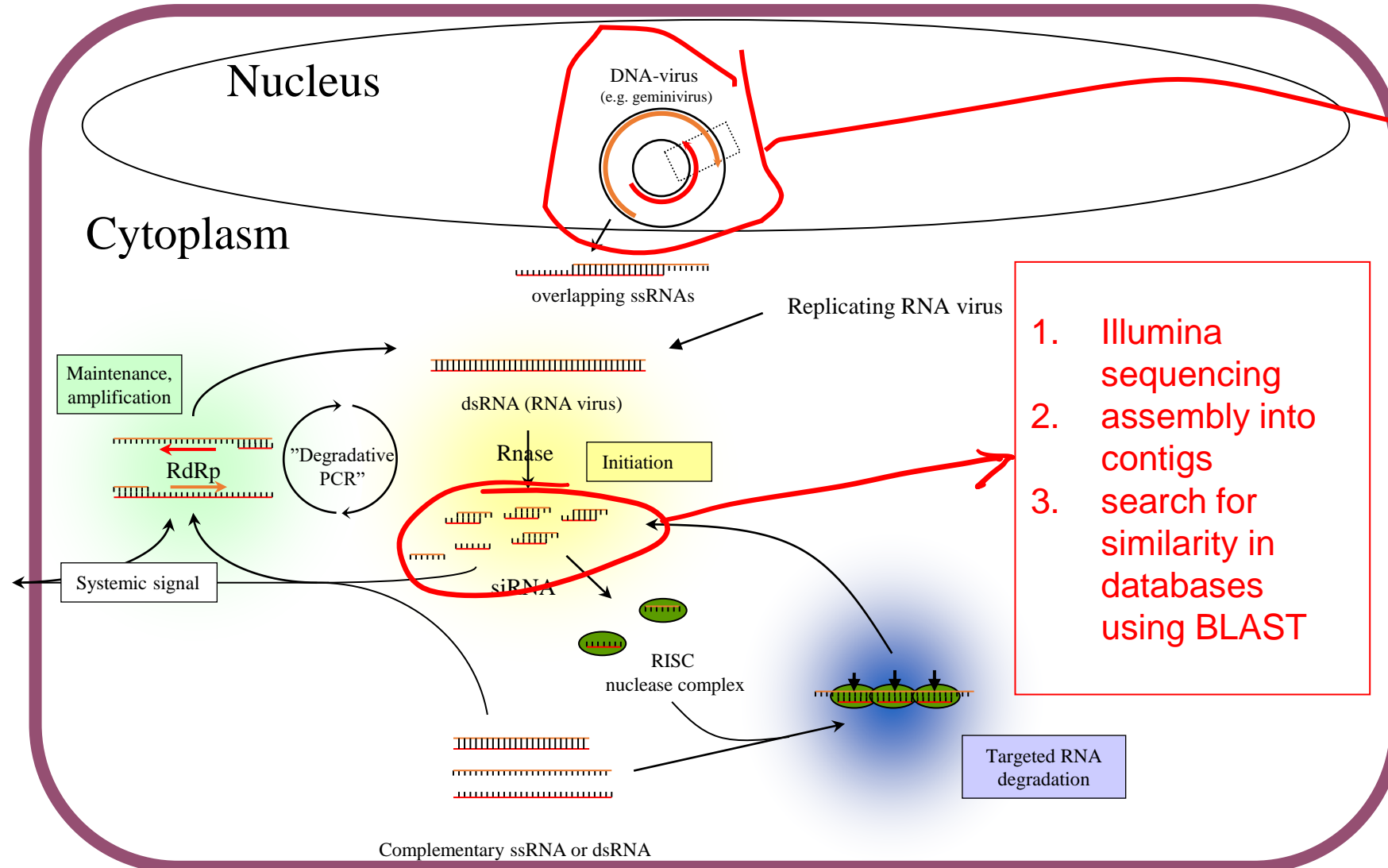
Figure 2 Components of prototype field LAMP kit for detection of sweetpotato viruses.



disease management
germplasm exchange
crop improvement



Surveillance/monitoring using HTS

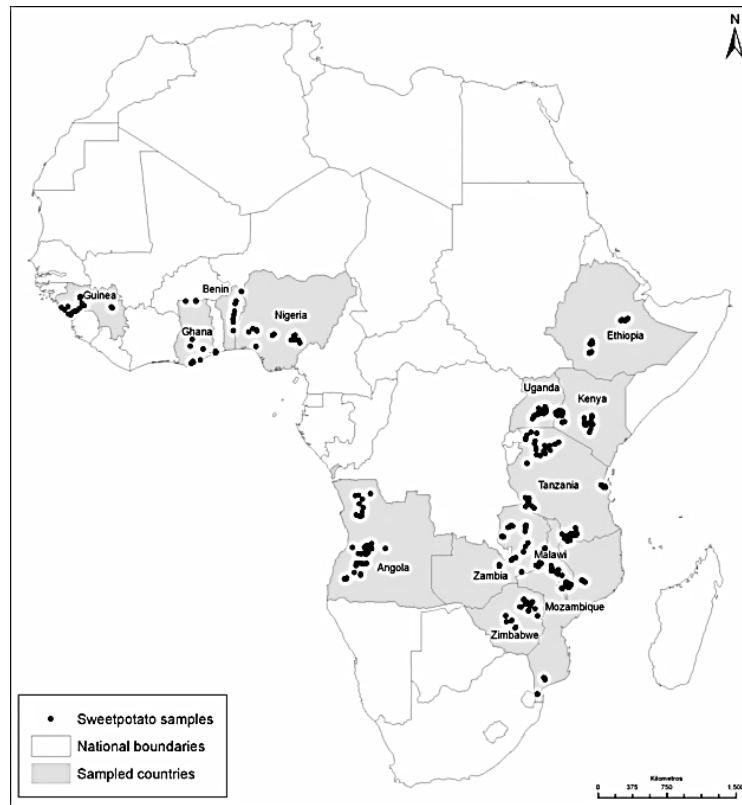


1. Rolling circle amplification
2. Nanopore sequencing

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

The African sweetpotato virome

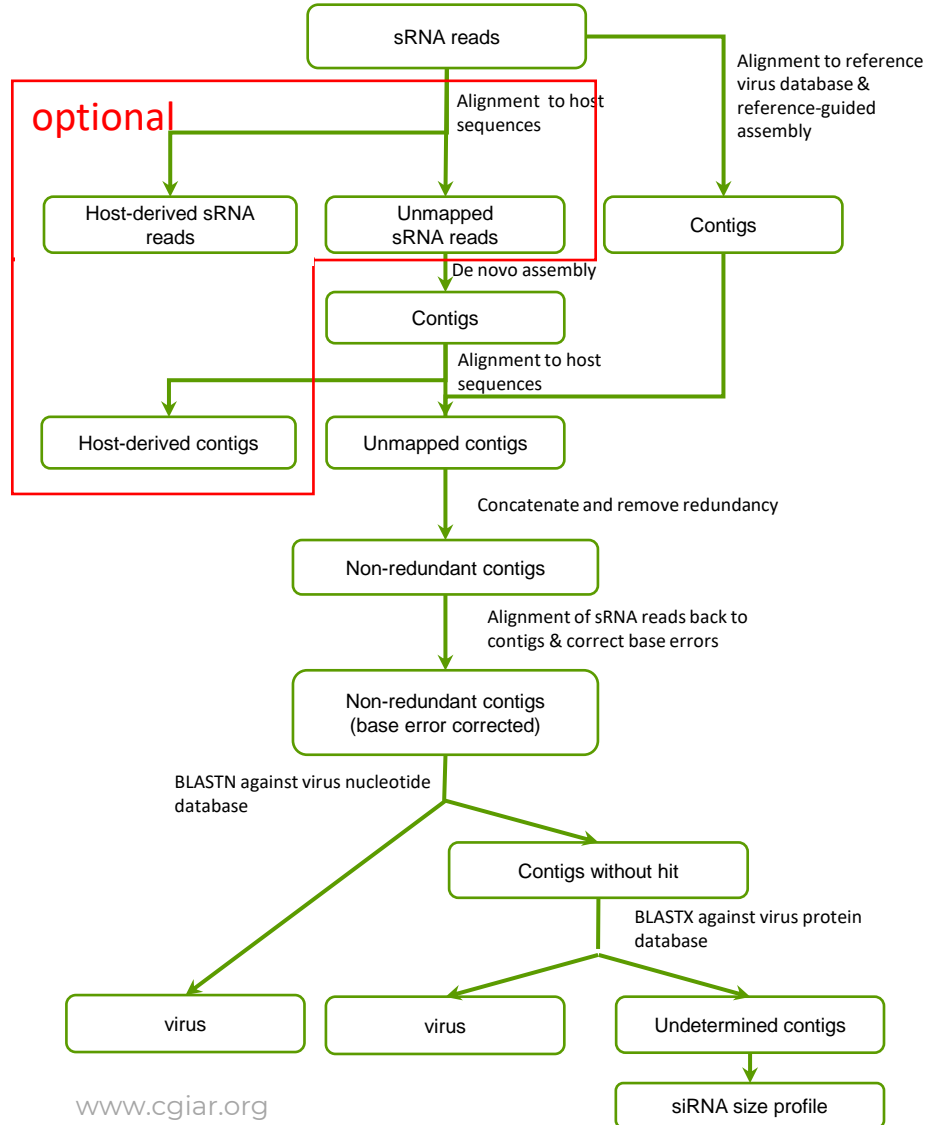
~2000 samples and bioinformatics pipeline for virus identification using siRNA assembly and genome subtraction



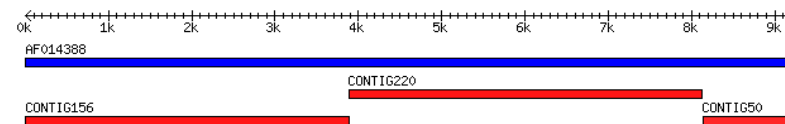
Small RNA sequence analysis pipeline: VirusDetect



Plant Health Initiative



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 bimavirus 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 bimavirus 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.



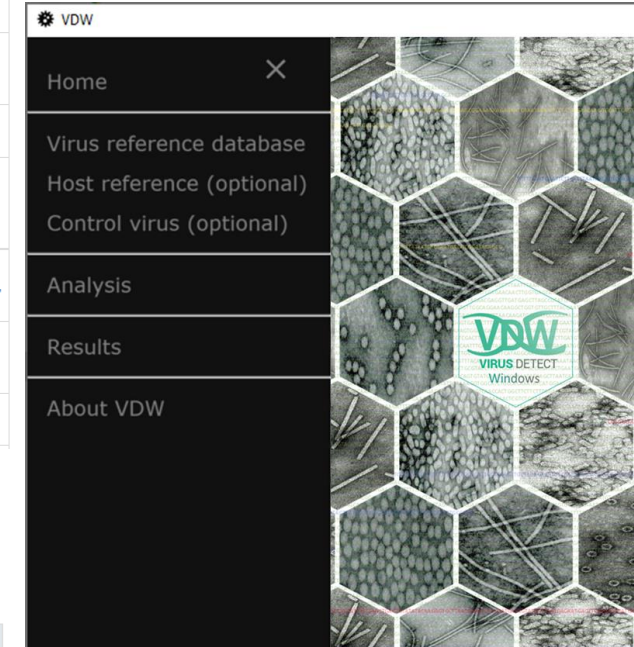
Order	Query ID	Query Start	Query End	Subject Start	Subject End	Identity	E value	Strand
1	CONTIG50	1	1121	8144	9264	1086/1121(96%)	0.0	1

Alignment:

```

Query: 1  tgaggtgaatatgctcgtattgttgcgcaagtaatgggtgaagatgtagctattcaaag 60
          |||
Sbjct: 8144 tgaggtgaagatgctcgtattgttgcgcaagtaatgggtgaagattagctattcaaag 8203

Query: 61  aaacgatgctcaacatgggtttcatccaatgacatagacactcataagattgactcaaa 120
          |||
Sbjct: 8204 aaacgatgctcaacatgggtttcatccaatgactatagacactcataagattgactcaaa 8263
    
```



Pan-African Sweet Potato Virome

Home Participants Sampling Data

Field Map Virus Map

Samples in field TZF111

Sample	Date(DD/MM/YYYY)	Age(month)	Sample Image	Leaf Image
TZ201	28/03/2012	3	IMG_0289.JPG	IMG_0293.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

Sample TZ201 processing & analyzing results

Sample Cleaning

sample ID	Raw Reads	Clean Reads	Nucleic	Unclassified/RNA	RNA seq/RNA-seq
TZ201	2810854	1330068	63.17	2237936923	24400009239

Length Distribution

Identification of virus KF836891 from sample TZ201

Order	Query ID	Query Start	Query End	Subject Start	Subject End	Identity	E value	Strand
1	CONF101	15	59	2439	2483	4445(97%)	2e-11	1
2	CONF108	3	639	2060	2096	629(63/96%)	0.0	1

```

Alignment:
Query: 15  gagatgagatgtragagaccctctgatgtttcttggctaa 59
Subject: 2439 gagatgagatgtragagaccctctgatgtttcttggctaa 2483

Alignment:
Query: 3    gaattgaaaagagagaccattaccctgaaggaggaggaaaacctctcttcaattt 82
Subject: 2060 gaattgaaaagagagaccattaccctgaaggaggaggaaaacctctcttcaattt 2119

Query: 83  ctctgatattgatattgaaaattaaatctttggggttttcccattatttt 122
Subject: 2120 ctctgatattgatattgaaaattaaatctttggggttttcccattatttt 2179

Query: 123  aaatatttttttaaaaacatttttttttttttttttttttttttttttttttttttt 331
                
```

Virus Identification

Reference	Coverage(%)	Identity	Depth	Depth(min)	Depth	Description
J02511	439	382(96.8)	4	74.0	76.0	Sorghana virus (Sorghana)
FJ285844	7961	2762(94.8)	23	88.9	91.3	Sorghana virus (Sorghana)
FJ285843	8082	2511(95.6)	20	82.0	84.4	Sorghana virus (Sorghana)
KF836892	3048	1588(91.6)	21	82.8	84.1	Sorghana virus (Sorghana) contig: contig_038
KF836891	2831	916(92.4)	10	108.3	108.0	Sorghana virus (Sorghana) contig: contig_039

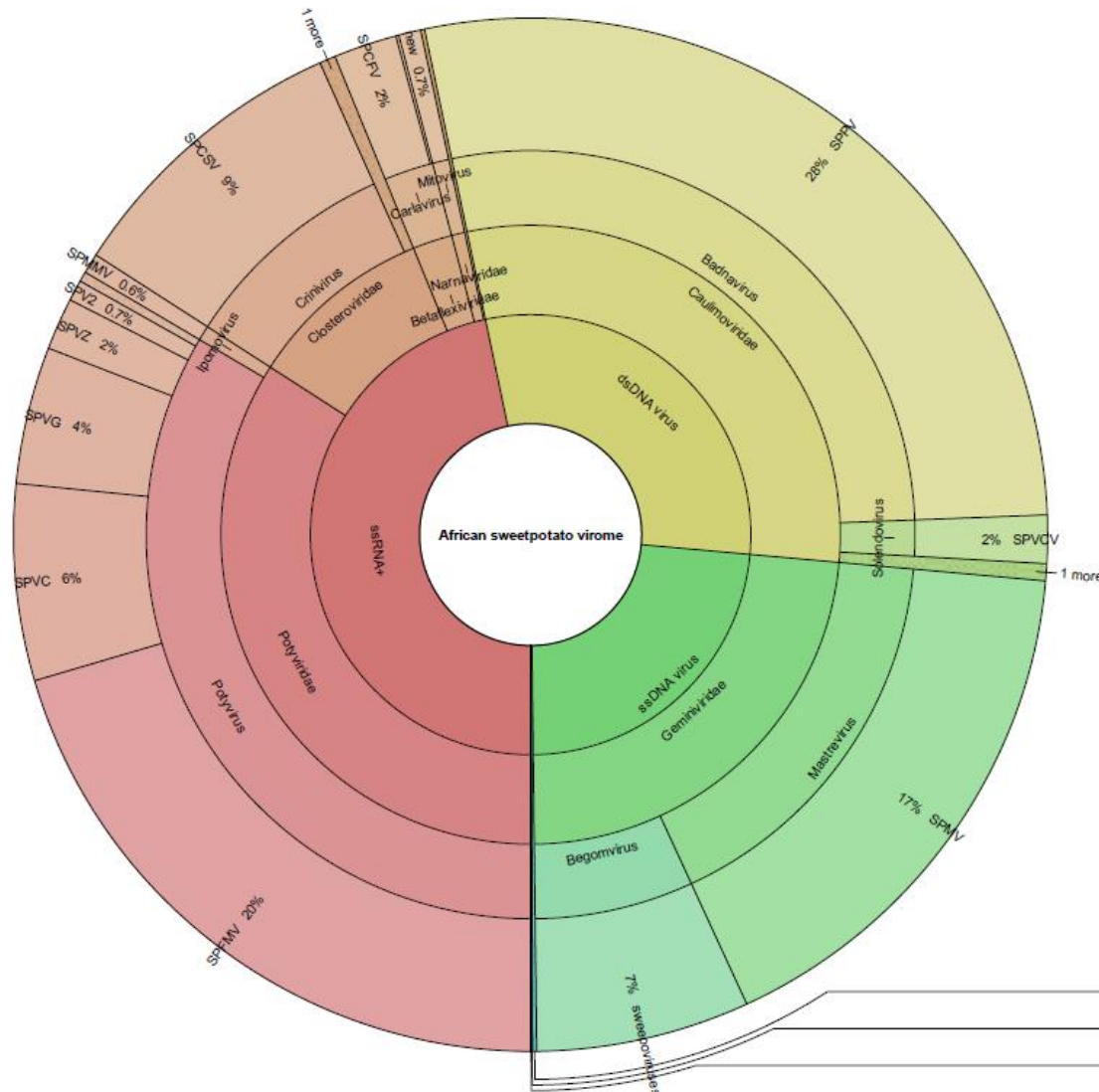
www.cgiar.org

Knowledge Portal

Boyce Thompson Institute for Plant Research (BTI)

Results: the viruses

3193 viruses from 1168 samples



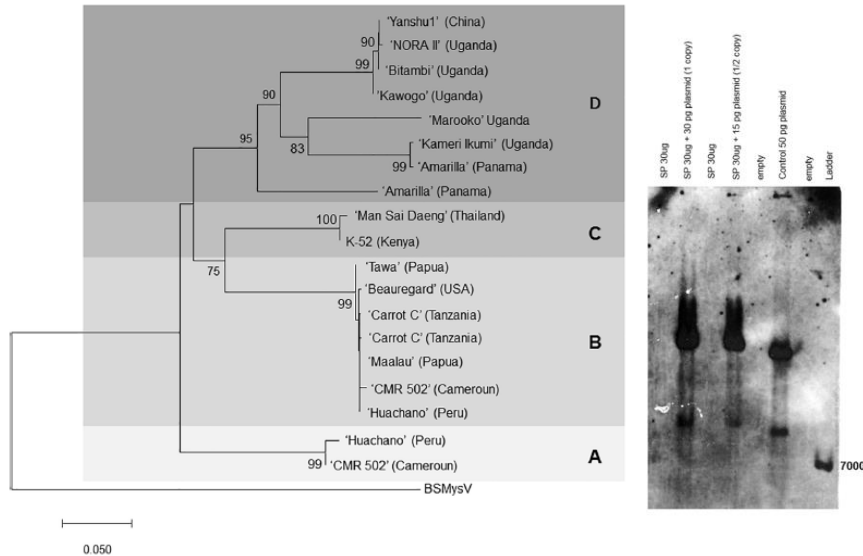
New viruses:
 Potyviruses: 3
 Ampeloviruses: 3
 Mitoviruses: many
 Nepovirus
 Soymovirus
 Emaravirus
 Begomoviruses
 Alphasatellites

Are all viruses relevant?



Badnaviruses of Sweet Potato: Symptomless Coinhabitants on a Global Scale

Jan F. Kreuze^{1*}, Ana Perez², Marco Galvez Gargurevich³ and Wilmer J. Cuellar⁴



<https://doi.org/10.3389/fpls.2020.00313>

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,†}

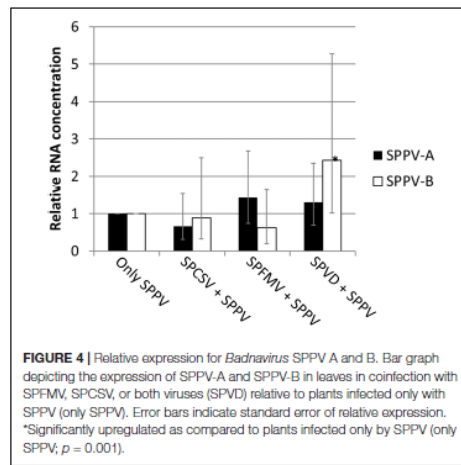
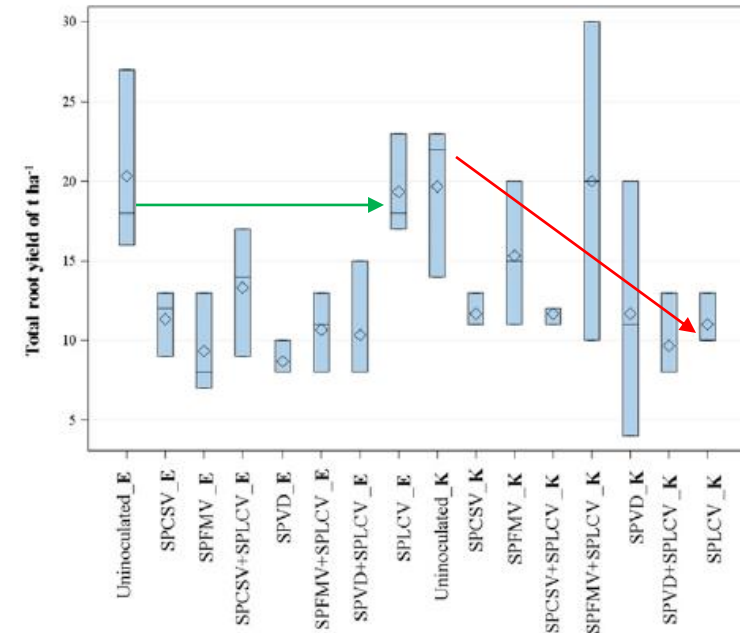
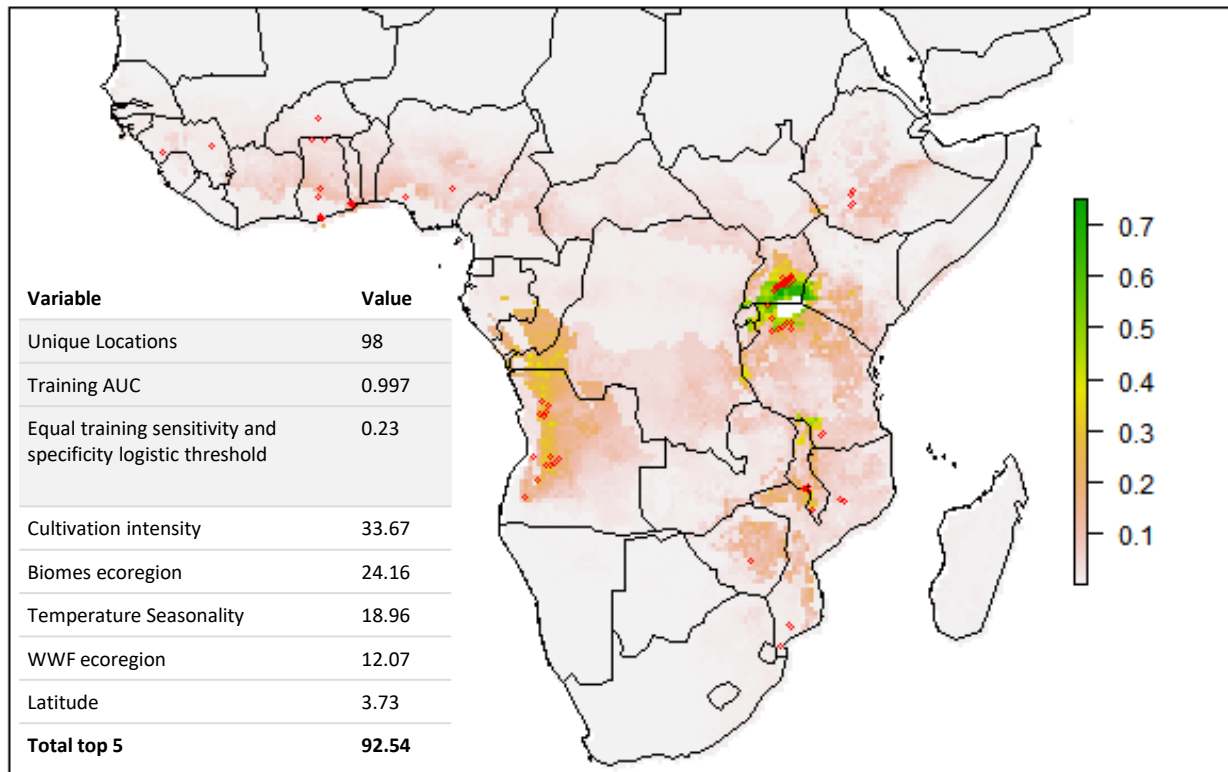


FIGURE 4 | Relative expression for *Badnavirus* SPPV-A and B. Bar graph depicting the expression of SPPV-A and SPPV-B in leaves in coinfection with SPPMV, SPCSV, or both viruses (SPVD) relative to plants infected only with SPPV (only SPPV). Error bars indicate standard error of relative expression. *Significantly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

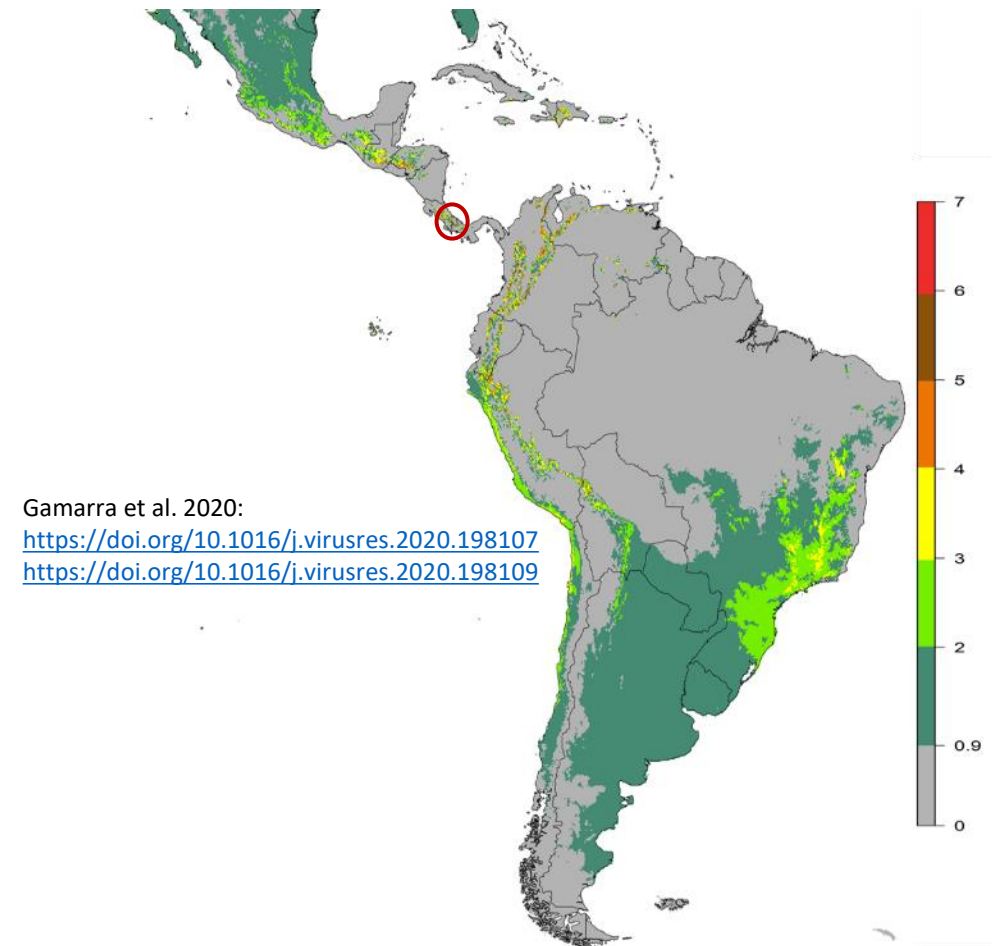
<https://doi.org/10.1094/PDIS-06-19-1196-RE>

Can we make predictions about likely occurrence beyond evaluated areas?

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



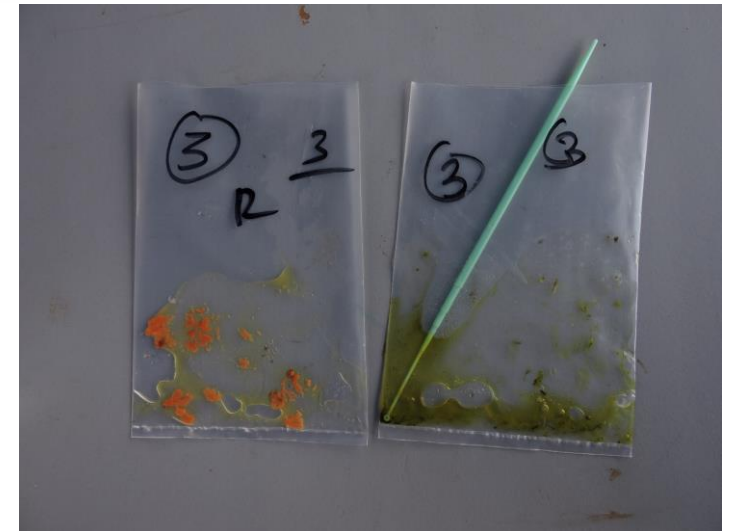
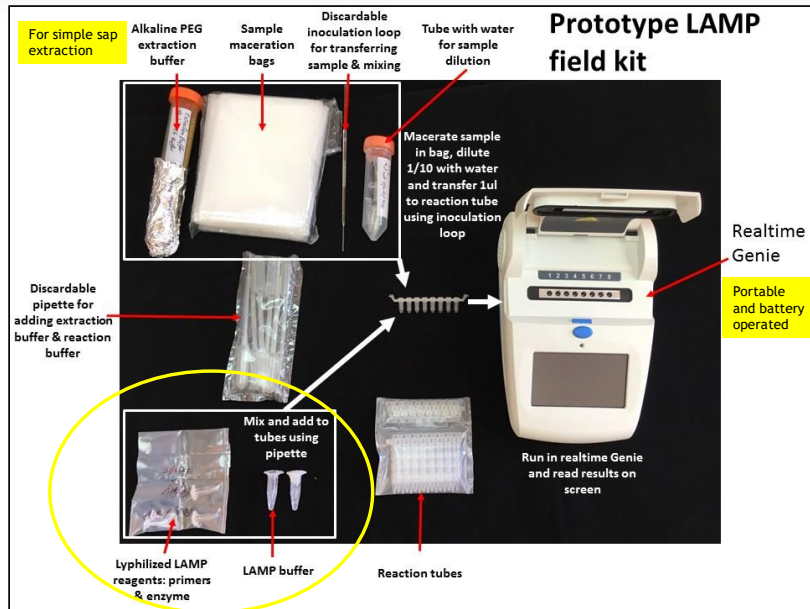
Example for PYVV: phenology modeling for surveillance



Field LAMP kit



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Wanjala et al., 2020: <https://doi.org/10.1094/PDIS-06-19-1196-RE>



Lyophilized reagents



BioRanger

Easy to interpret



www.cgiar.org



Njere et al., 2018: <https://doi.org/10.1007/s00705-018-3706-0>

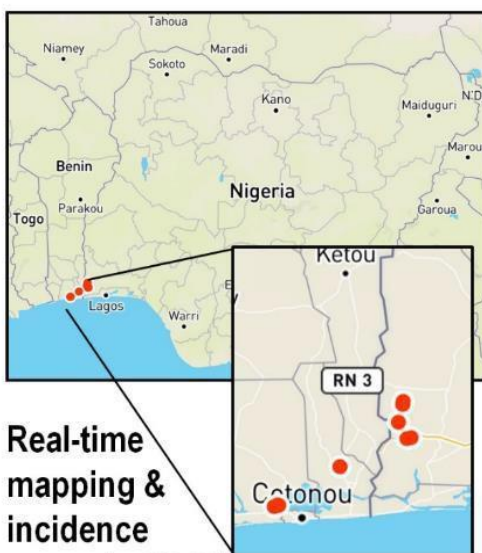
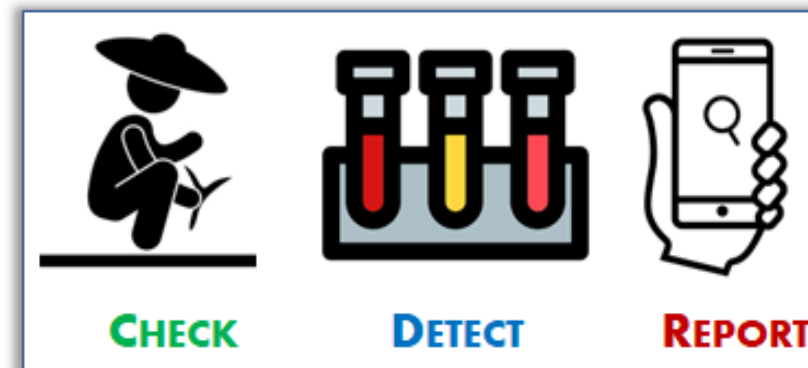
BBTV surveillance system



Step-1. Sample preparation (1 min)



Step-2. Sample loading into premixed BBTv RPA reagent mix (1 min)



Real-time mapping & incidence report in CDS base



Step - 4. Incidence reporting through CDS App



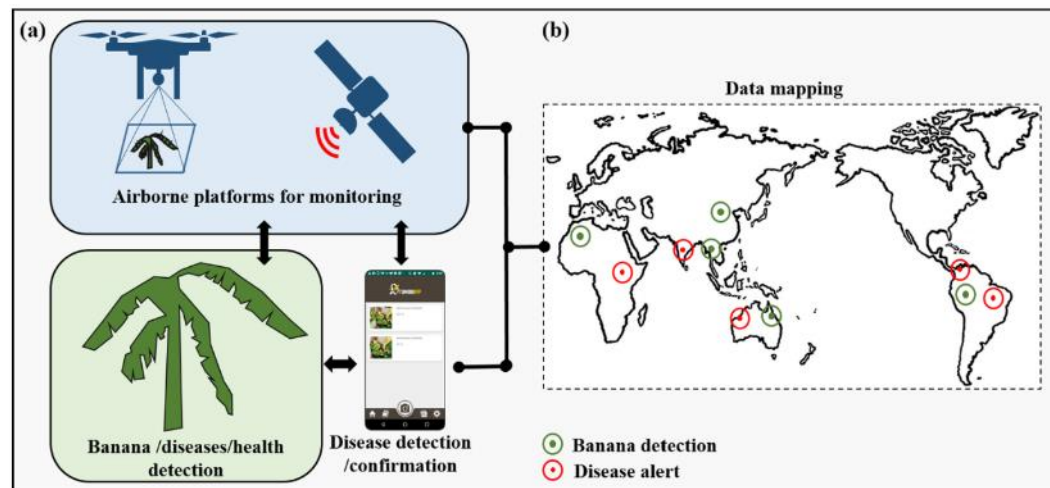
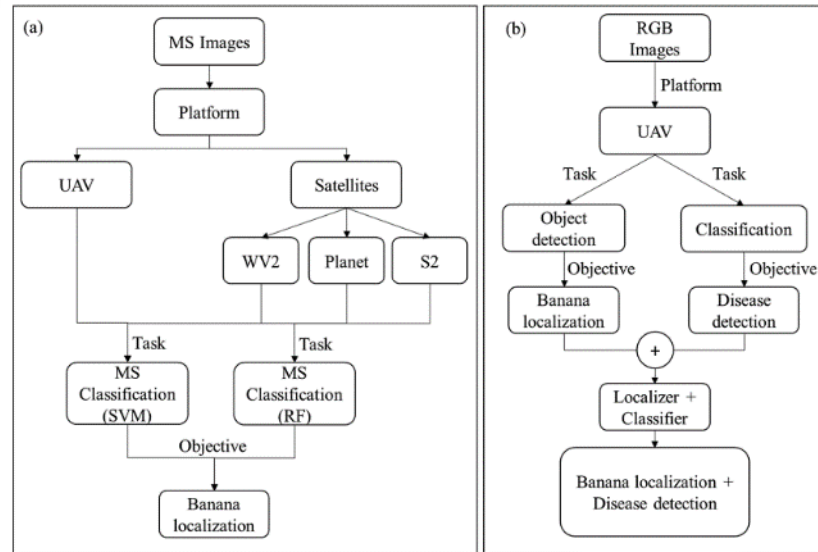
Step - 3. Reaction incubation and real time detection (10 – 15 min)



Real-time

- Reporting
- Mapping
- Diagnosis
- Control

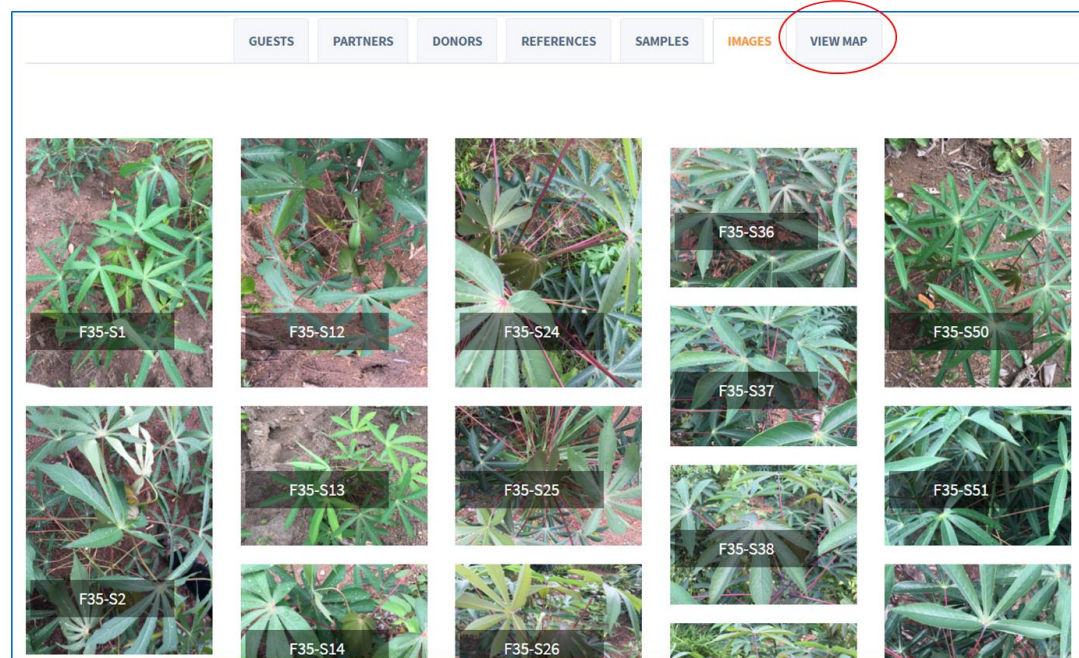
Remote Sensing for Disease (risk) Mapping



	Actual BBTD	Actual BXW	Actual Cluster	Actual Healthy
Predicted BBTD	99.4% 173/174			0.6% 1
Predicted BXW	1.8% 5	92.8% 256/276		5.4% 15
Predicted Cluster	1.1% 1		93.3% 84/90	5.6% 5
Predicted Healthy	1.2% 4	4.6% 15	3.4% 11	90.8% 297/327
	BBTD	BXW	Cluster	Healthy

CMD in SEA: dev. standard protocols

Field

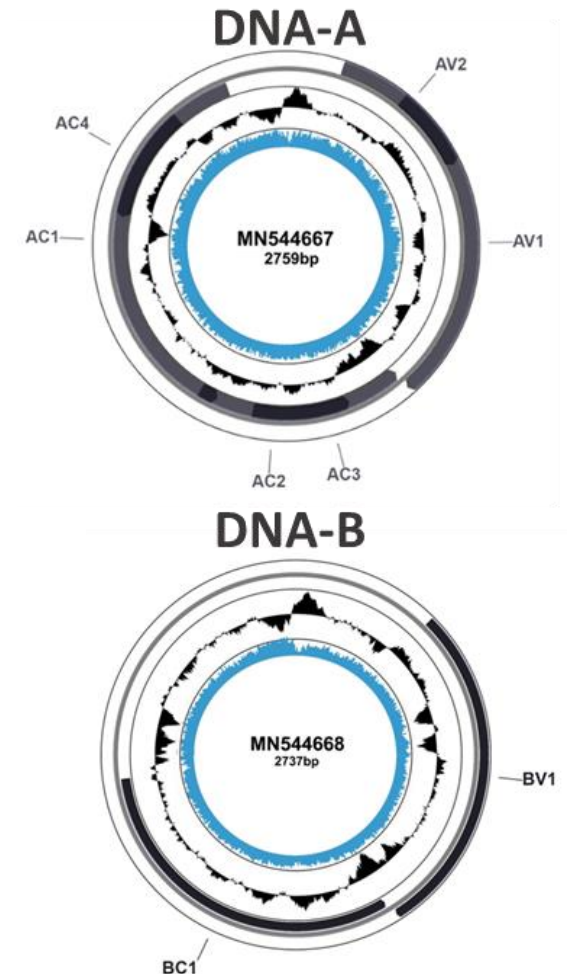


<https://pestdisplace.org>

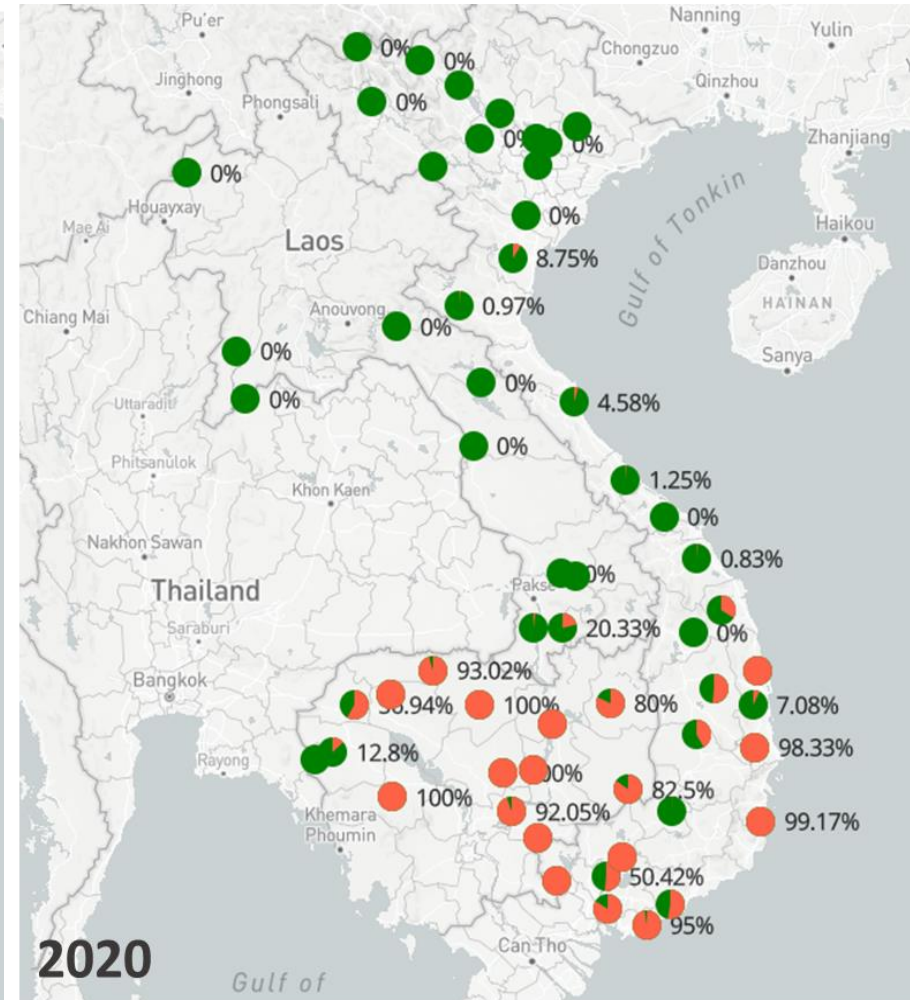
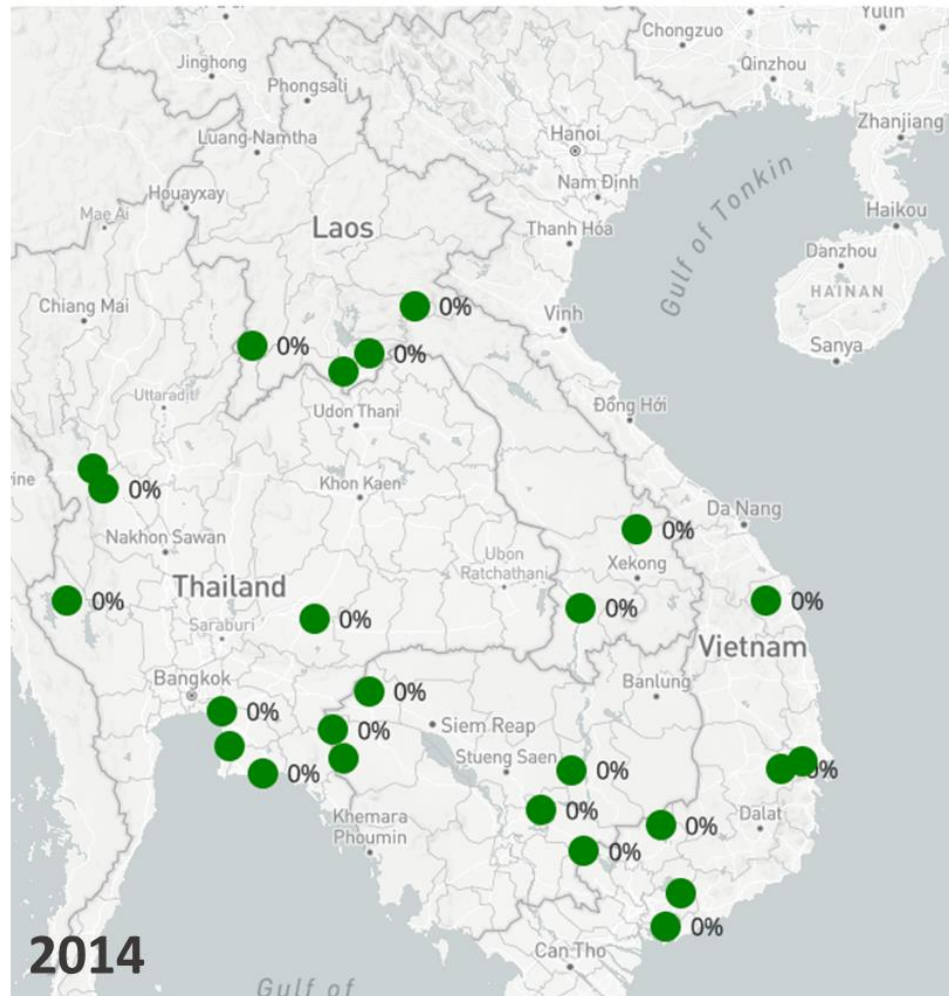
Cuellar et al., 2021 <https://hdl.handle.net/20.500.11766/67115>

RCA and sequencing of circular DNA: Only 1 (out of 12) species of cassava-infecting begomovirus is present in SEA: Sri Lankan cassava mosaic virus (SLCMV)

Lab



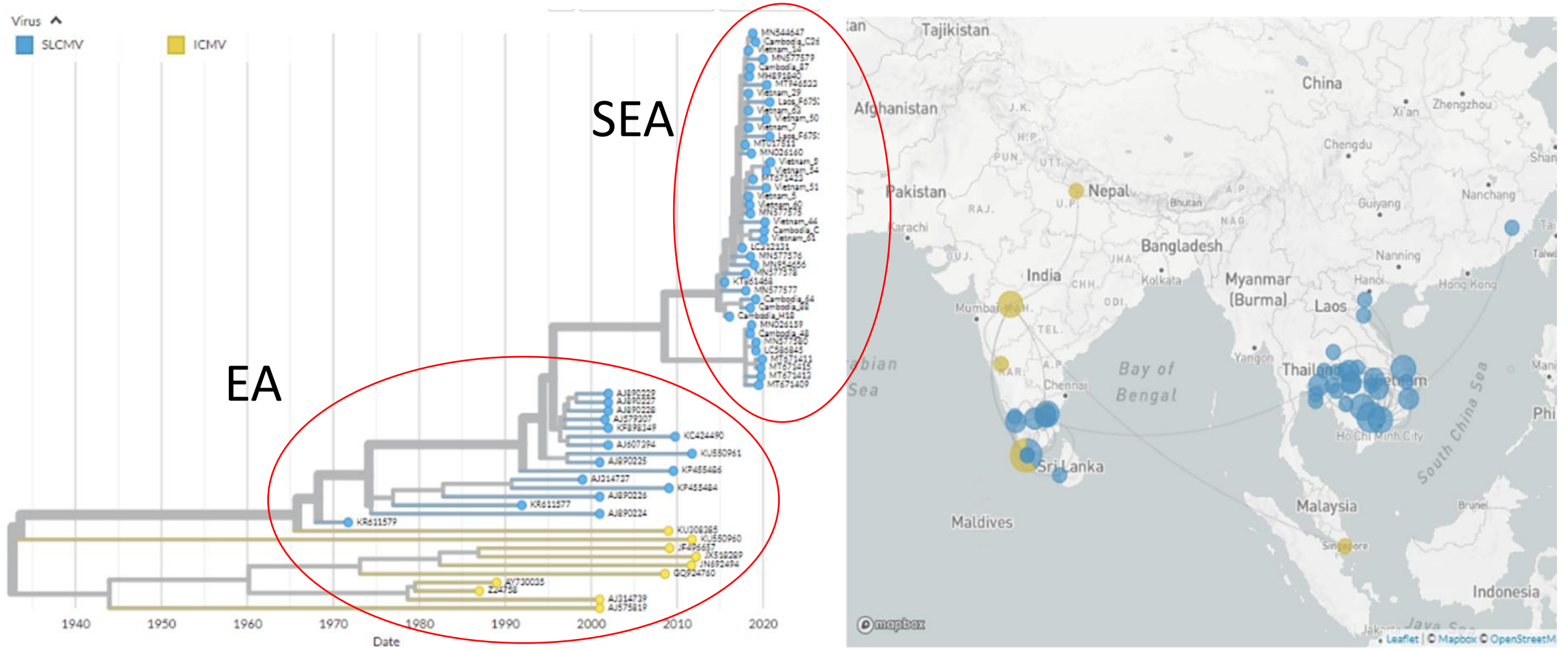
Cassava Mosaic Disease in SEA



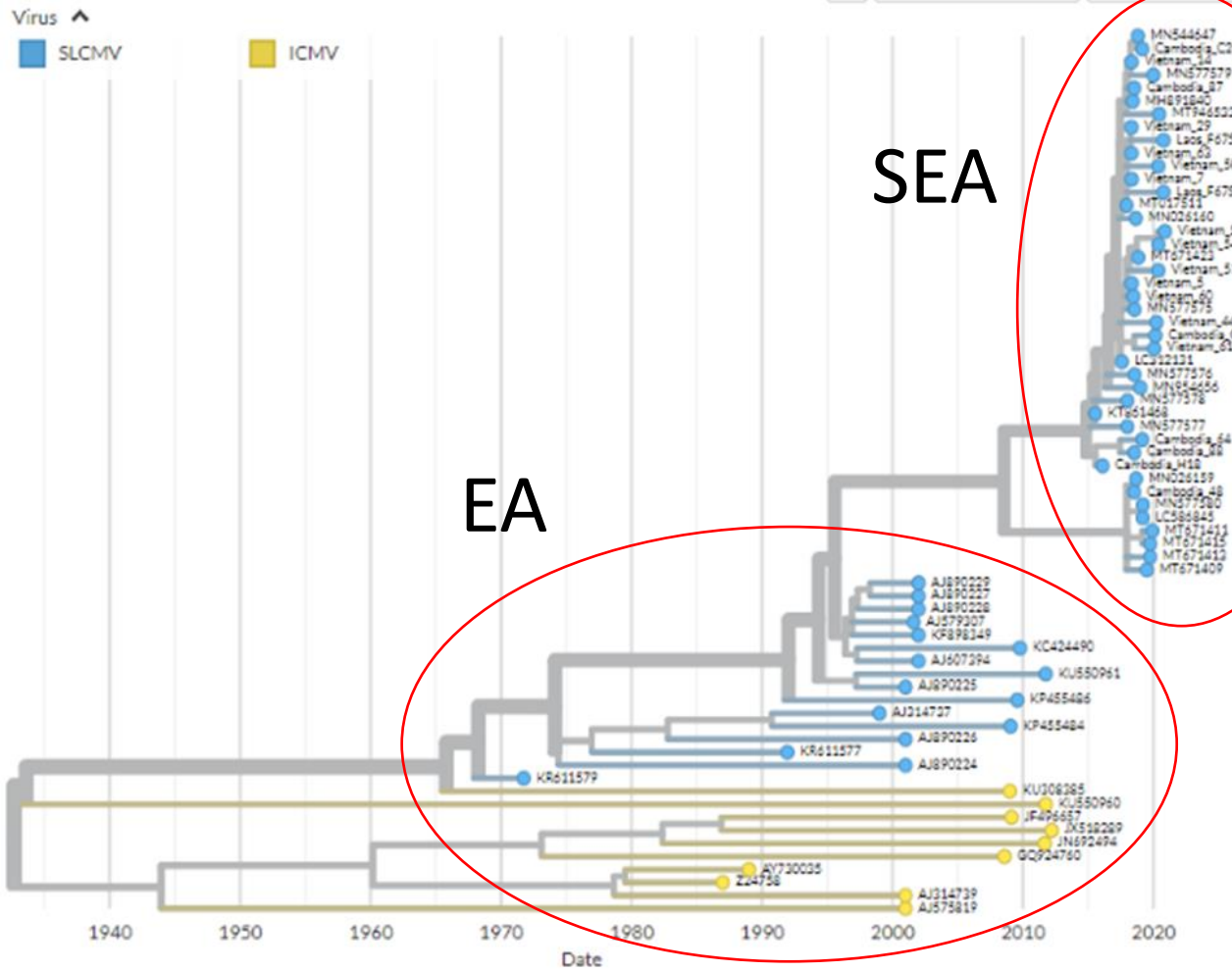
>30,000 observations

CMD in:
Thailand
Cambodia
Vietnam
Lao PDR
China

Tracking SLCMV genome evolution



<https://nextstrain.org/community/pestdisplace/CMDASIA1?c=virus&r=location>



C-terminus of the Rep protein

Sis1	SEPLYSGSN	*	} SEA
MN544647	SEPLYSGSN	*	
Ome1	SEPLYSGSN	*	
LC312131	SEPLYSGSN	*	
MH891840	SEPLYSGSN	*	
Tay2	SEPLYSGSN	*	
Ome2	SEPLYSGSN	*	
Tay1	SEPLYSGSN	*	
KT861468	SEPLYSGSN	QGP TQGS *	
Pra1	SEPLYSGSN	QGP TQGS *	
AJ579307	R EPLYSGSN	QGP TQGS *	} EA
AJ890225	SEPLYSGSN	QGP TQGC *	
AJ890224	SEPLYSGSN	QGP TQGS *	
AJ890228	SEPLYSGSN	QGP TQGS *	
AJ314737	SEPLYSGSN	QGP TQSS *	

These extra 7 amino acids are involved in virulence

The Peruvian potato virome

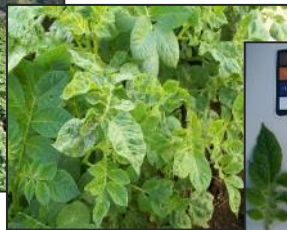


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- Sample collection:
994 geo-referenced potato leaf samples



Field



Plant



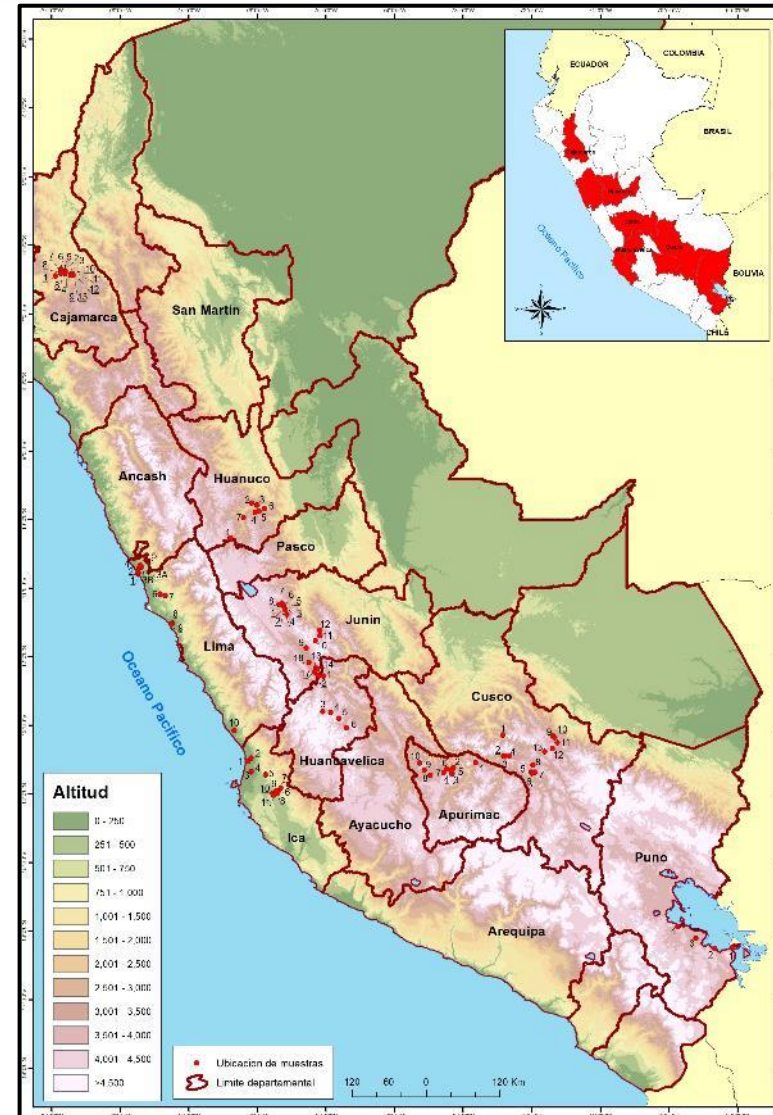
Leaves



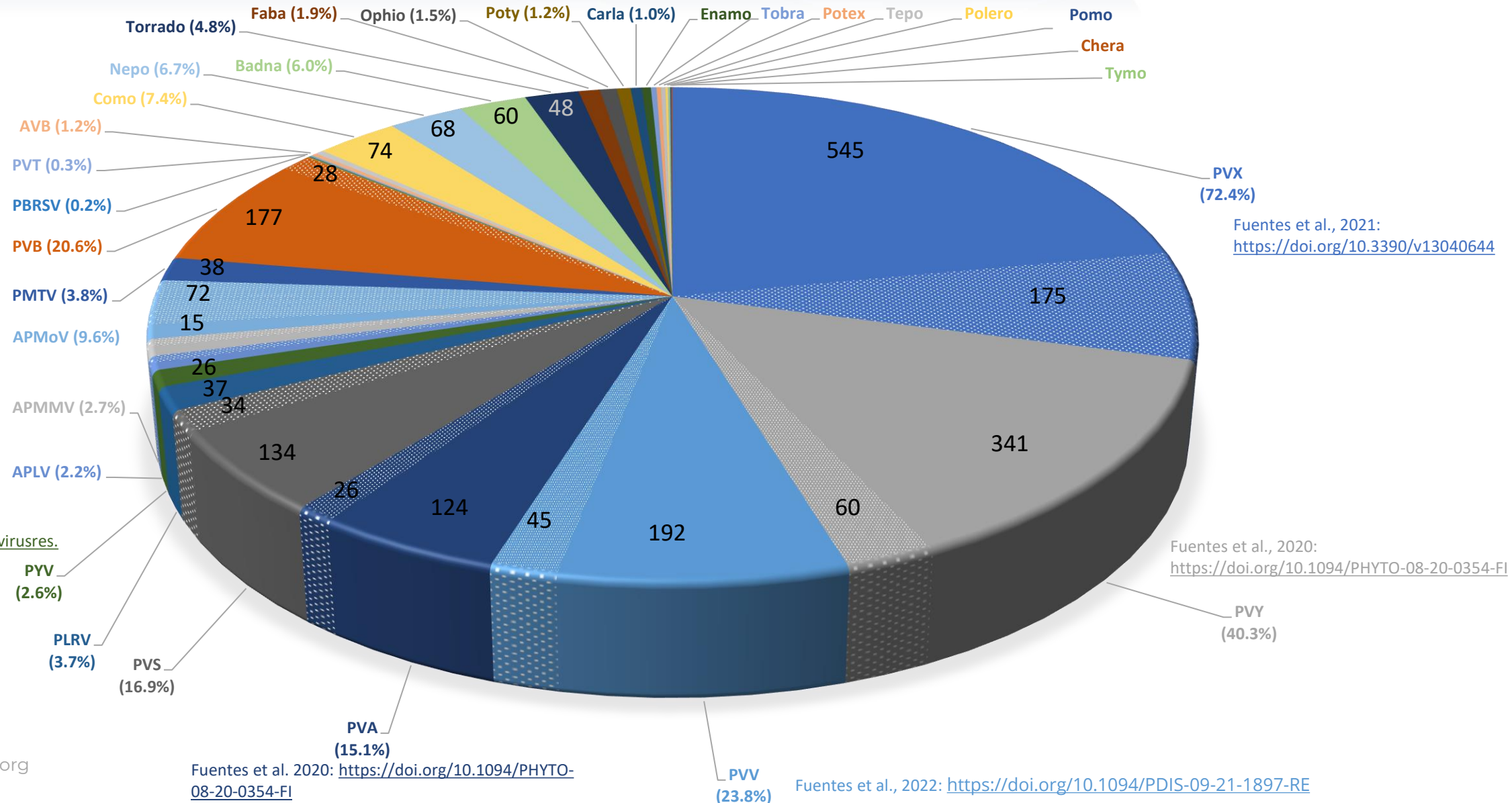
Drying



Weighting



General results



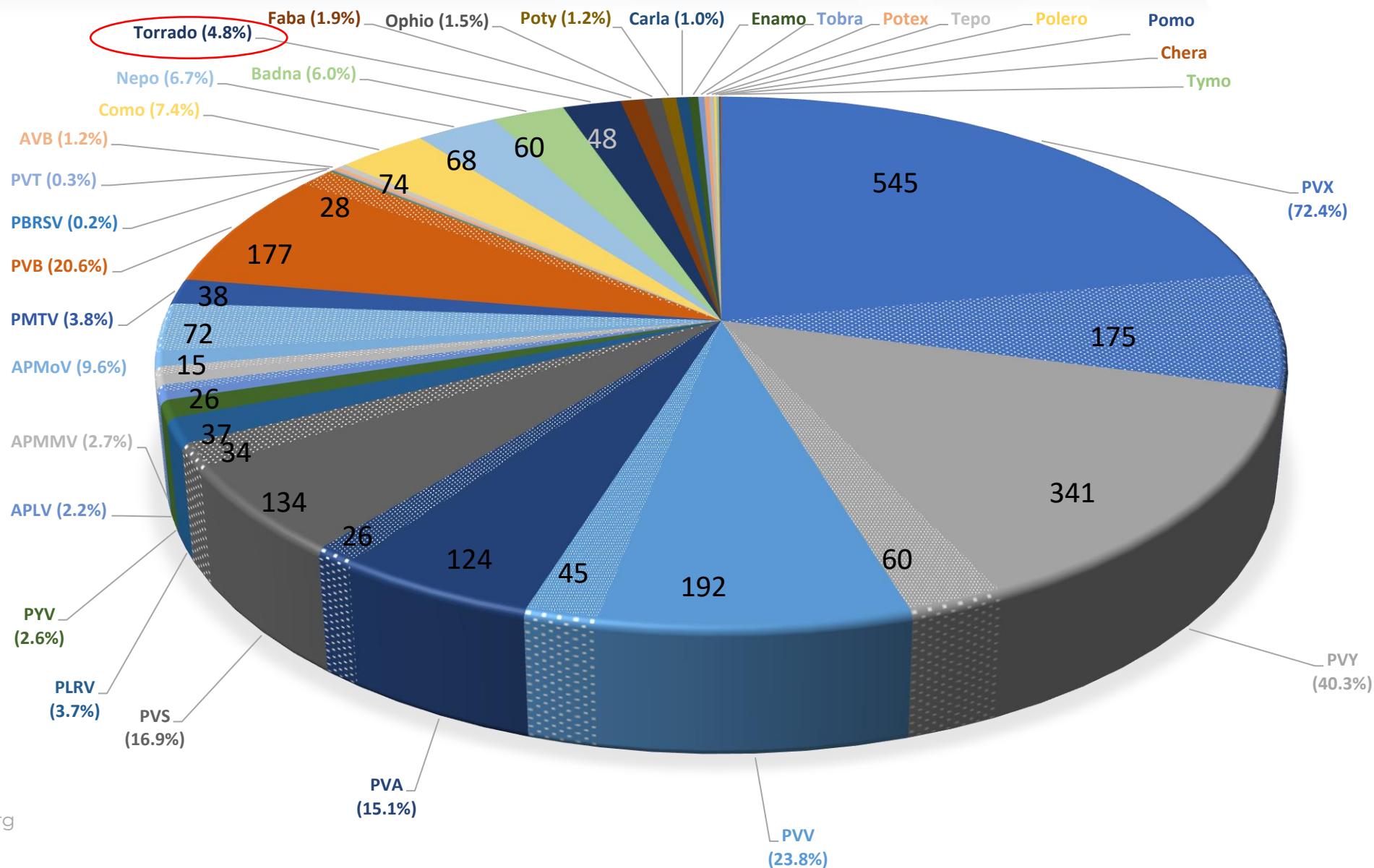
Silvestre et al., 2020: <https://doi.org/10.1016/j.virusres.2020.197944>

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, two dsRNA sizes, transmitted by South American potato psyllid (*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



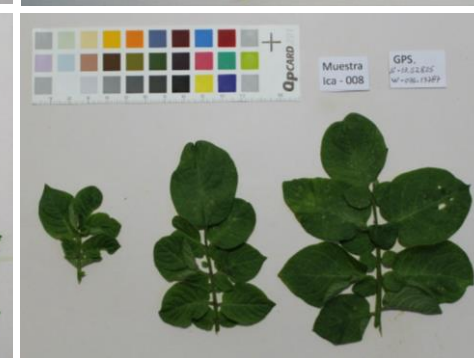
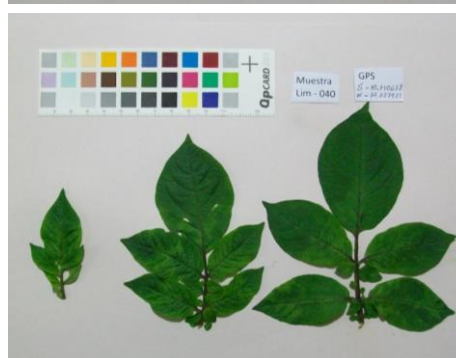
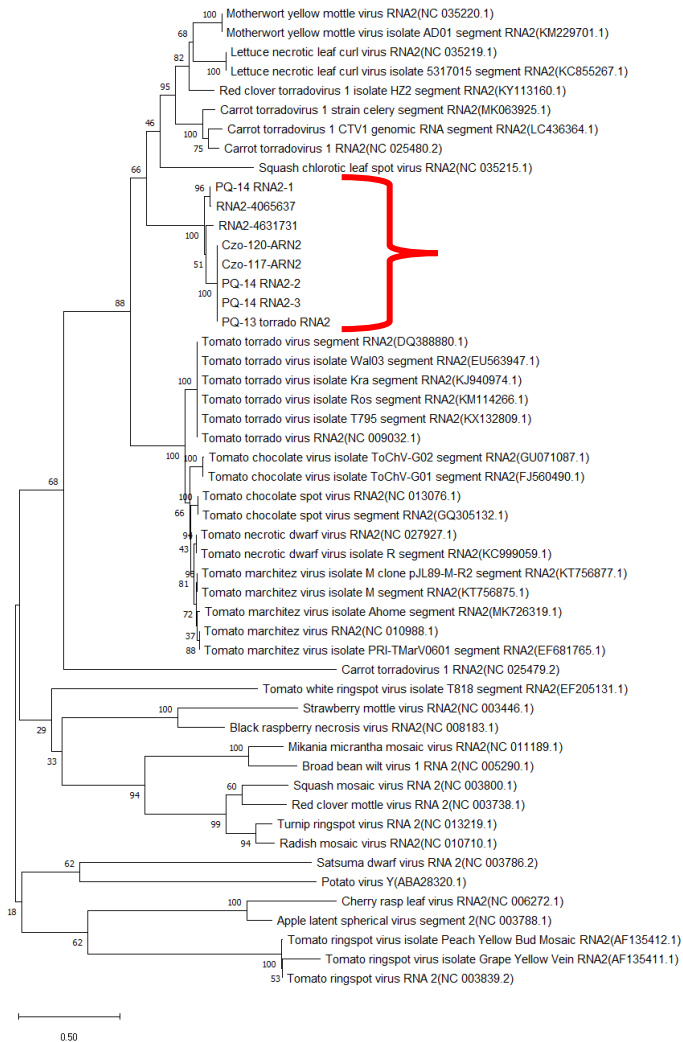
Are any of the new viruses of relevance?



Potato rugose stunting virus is a torradovirus and present in low frequencies in potatoes throughout Peru



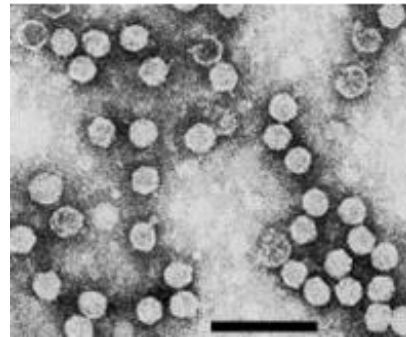
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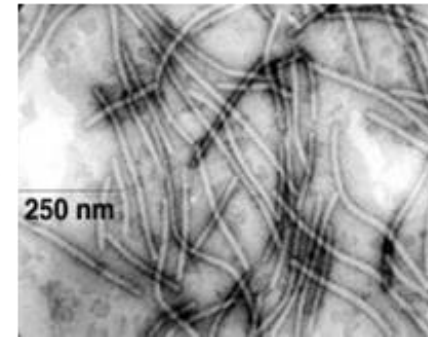
Maize Lethal Necrosis (MLN) in Africa



MCMV



Potyvirus (SCMV)



+

=

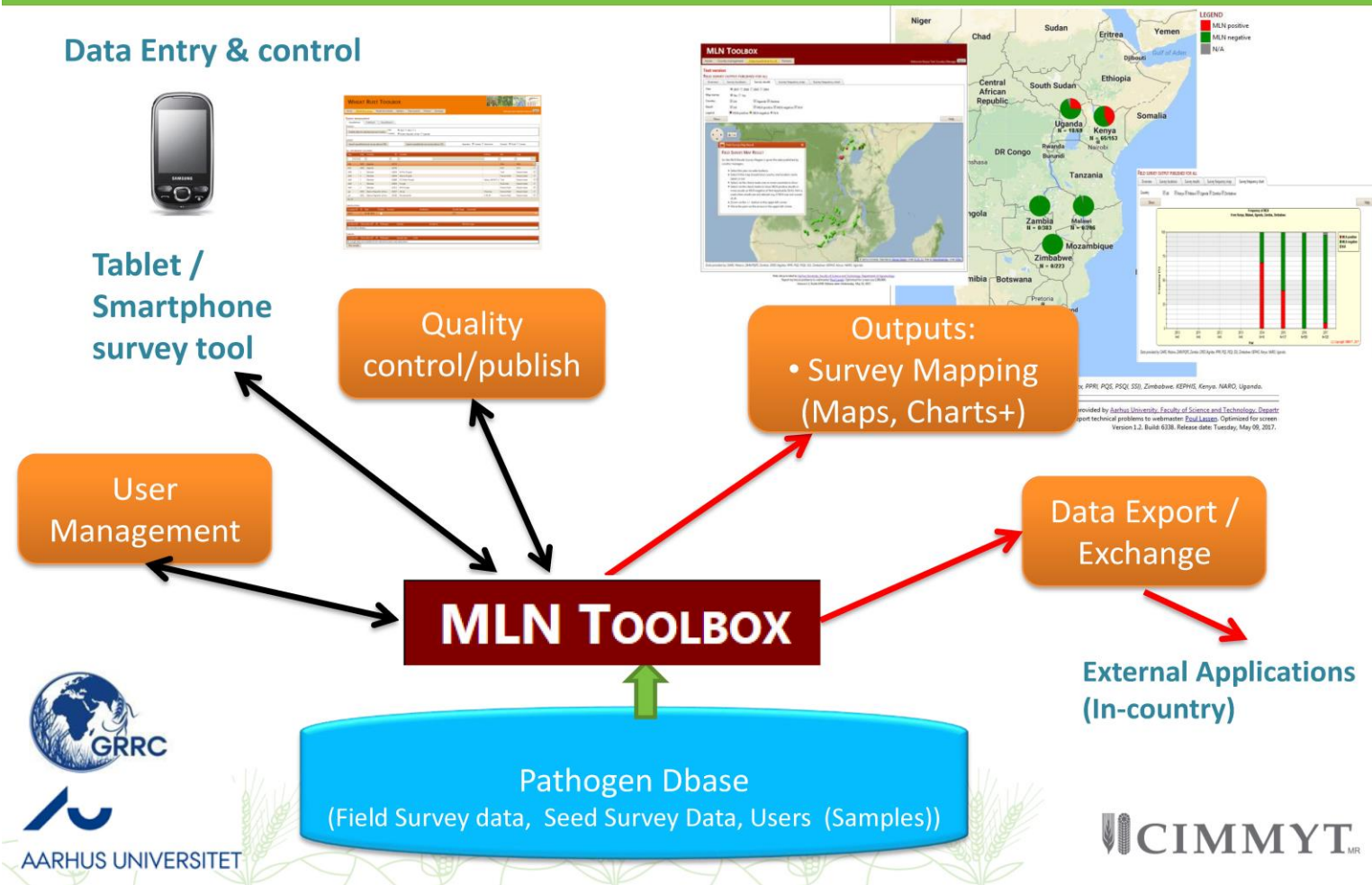
MLN



- MLN first appeared in Kenya in 2011 and was then reported in several countries in Uganda (2012), Tanzania (2012), Rwanda (2013), D.R. Congo (2013) and Ethiopia (2014).
- Losses to maize production in farmers' fields due to MLN in the impacted countries ranged from 25% to 100%.

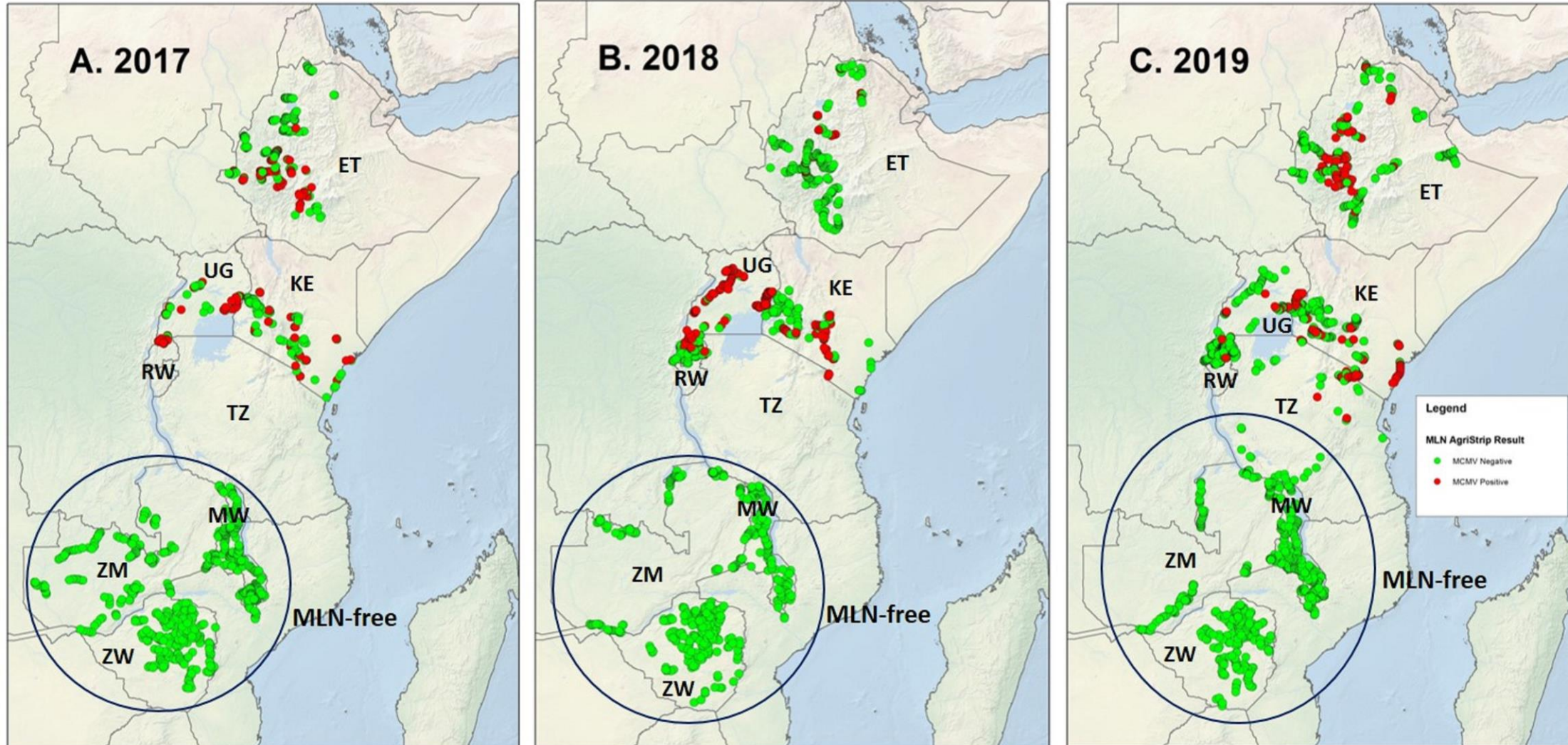
MLN Diagnostics and Surveillance

Data Management: Maize MLN Toolbox



- **MLN Diagnostics and Surveillance:** Coordinated by CIMMYT and implemented by NPPOs in five countries in eastern Africa (Ethiopia, Kenya, Uganda, Rwanda, and Tanzania) and three countries in southern Africa (Malawi, Zambia, and Zimbabwe).
- **MLN Toolbox** in partnership with NPPOs and Aarhus University.

Tracking MLN Dynamics over the Years

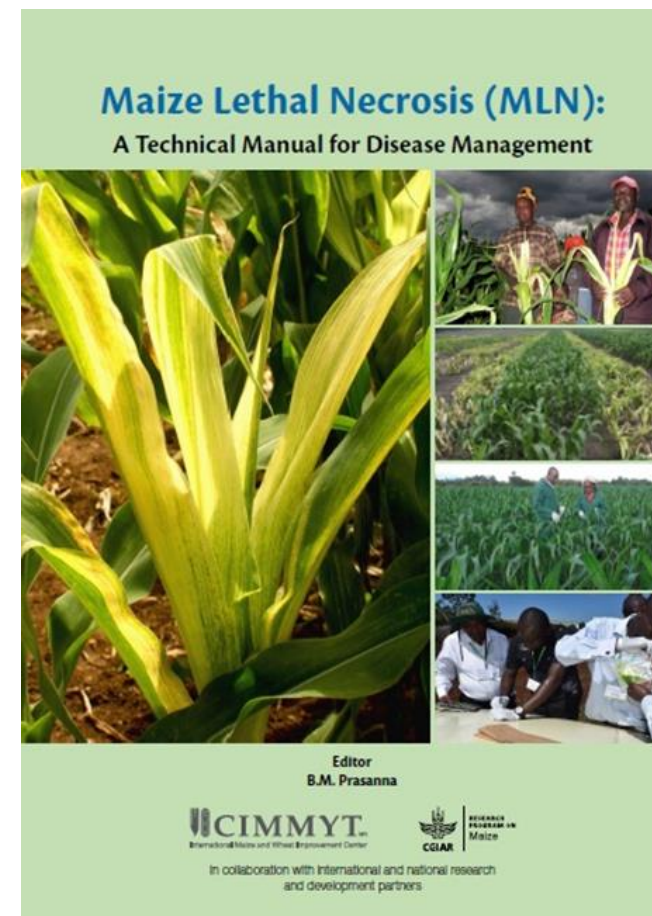
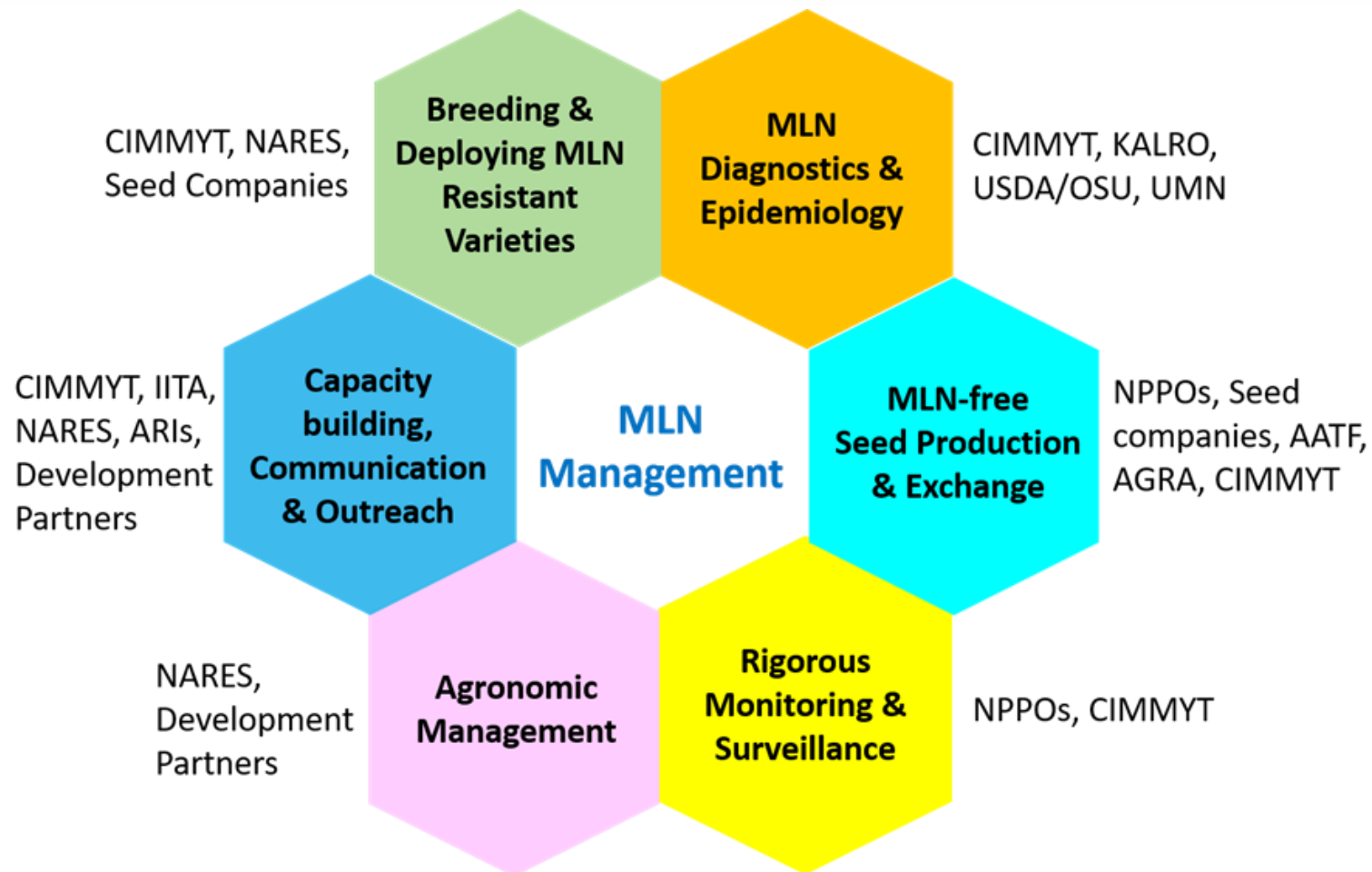


MLN Management in Africa

Success through Integration of Various Components



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No scope for complacency as MLN causing viruses are still prevalent in eastern Africa and are not eradicated..

The way forward

Many different databases that are unlinked, sometimes even for same diseases

More data=more power to predict & prepare

Need to make databases interoperable and FAIR (Findable, Accessible, Interoperable and Reusable)

Requires development of Application Program Interface & specifications

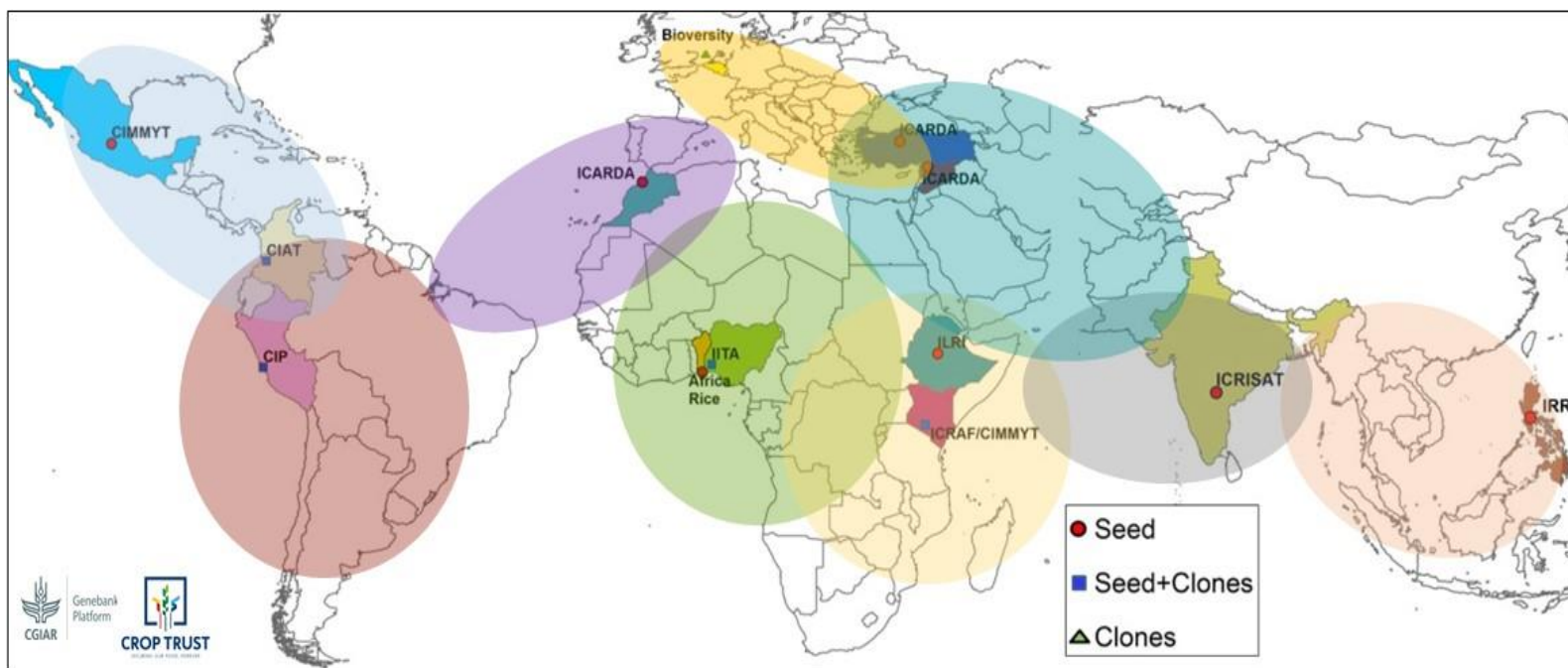


CGIAR Germplasm Health Program



Tools, technologies, strategies and policies for pest-free distribution of plant genetic resources

- **Global network committed to ensuring phytosanitary compliance and safe distribution of pest-free germplasm**
- **Prevention of transboundary spread of pathogens through germplasm**



A large, stylized graphic of a plant or tree, rendered in various shades of teal and blue. The graphic is positioned on the left side of the slide, curving from the bottom left towards the center. It features a central vertical stem with several horizontal branches or leaves, each with a distinct shape and color gradient.

Thank you!



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