What we have learned about viruses in SSA and Progress to date on developing new tools for **better & quicker virus assessment**

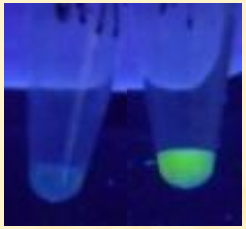
SWEETPOTATO ACTION FOR SECURITY AND HEALTH IN AFRICA

Developing sensitive & robust virus detection methods

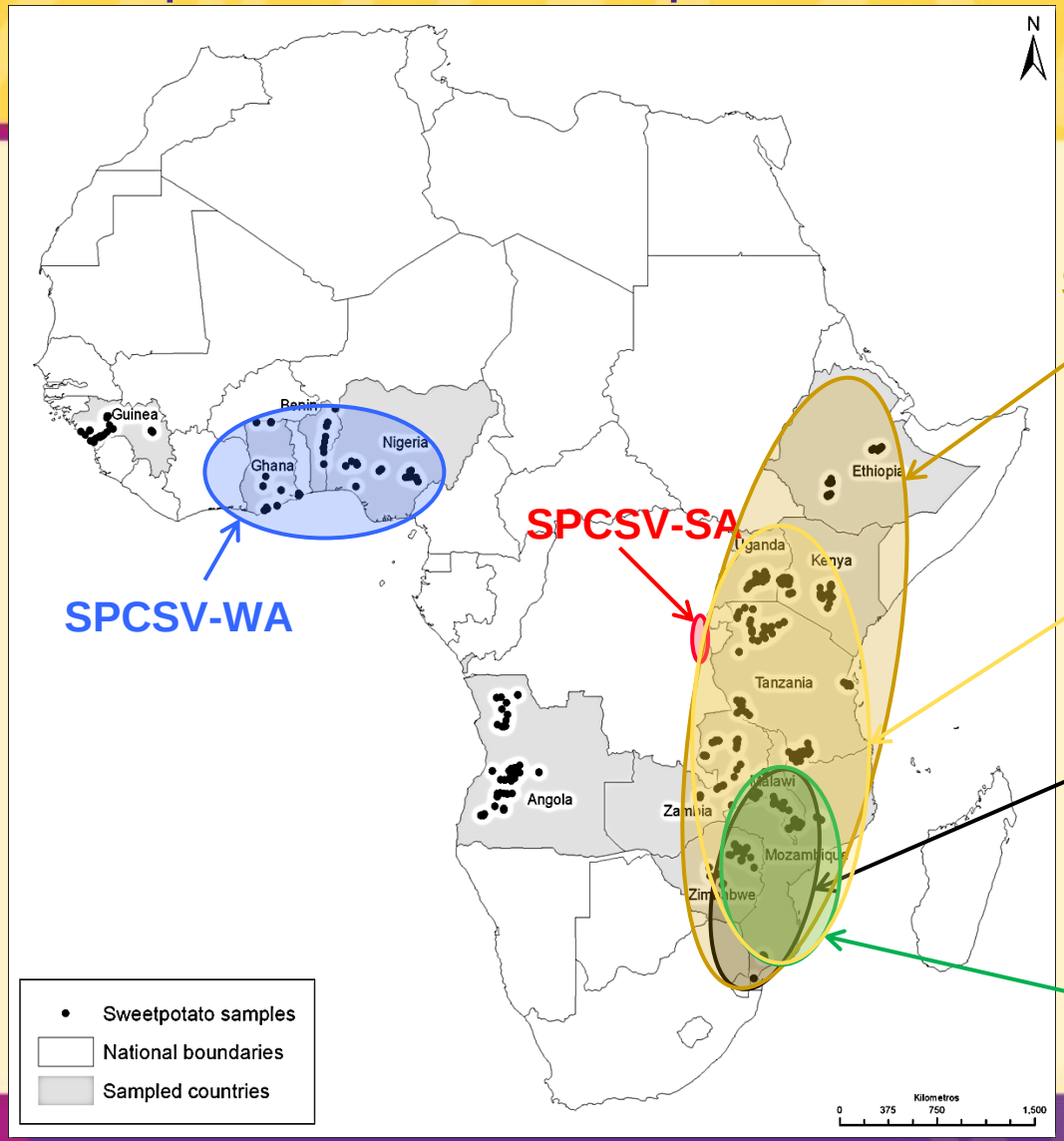
- Small RNA sequencing and assembly: towards universal viral diagnostics and sequencing

- Tube-arrays for sensitive detection of all viruses/pathogens of a crop at once (laboratory required)

- Field detection method with high sensitivity and ease of use -> LAMP



The pan African sweetpotato virome



SPCSV-EA

raw Illumina sRNA reads

remove barcode, adaptor, low quality and low complexity sequences

cleaned sRNA reads

align to draft sweetpotato genome and EST sequences

sweetpotato reads | unmapped reads

SPMMV

align to other plant genomes

sweetpotato reads | unmapped reads

sweetpotato miRNA, nat-siRNA, ta-siRNA

de novo assembly

contigs

blast against nr and nt database, compare against Interpro and pfam

SPVZ, SPVG, SPV2

virus genome contigs

align sRNA reads back to virus genome contigs

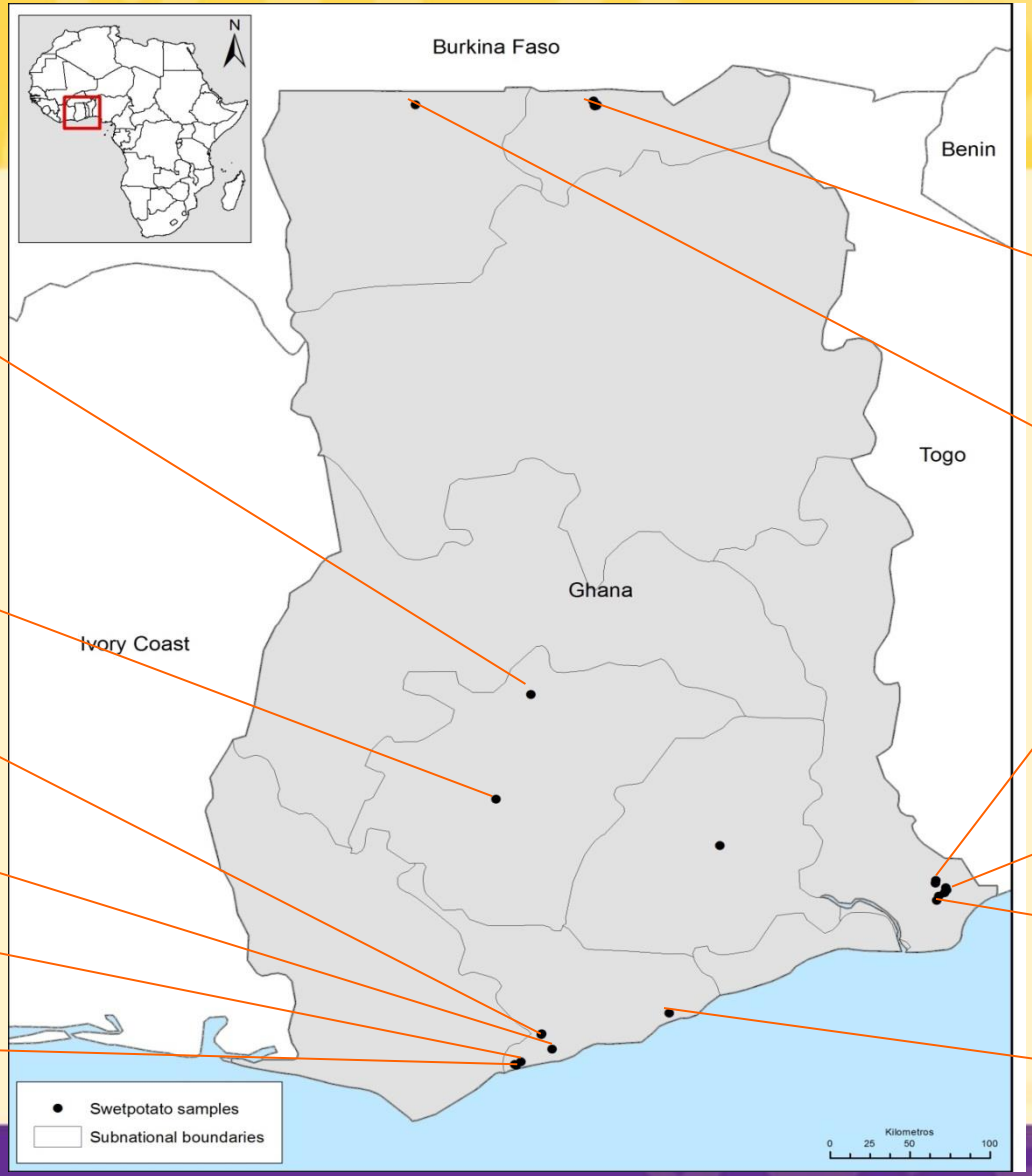
distribution of sRNA reads on virus genome contigs

design primers for PCR and Sanger sequencing

Fill gaps and detect virus variants

SPCSV-EA/MZ

SASHA
Sweetpotato Action for Security and Health in Africa



SPFMV
SPVC
SPVG
SPCSV-WA
SPLCV
SPPV

SPFMV
SPVC
SPCFV
SPLCV
SPPV

SPLCV

SPFMV
SPVC
SPVG
SPCSV-WA
SPLCV
SPPV

SPFMV
SPVC
SPCFV
SPLCV
SPPV

SPFMV
SPCV
SPCFV
SPCSV-WA
SPPV

SPFMV
SPCFV
SPCSV-WA
SPPV

SPFMV
SPVC
SPCSV-WA
SPLCV
SPPV

SPFMV
SPVCV
SPPV

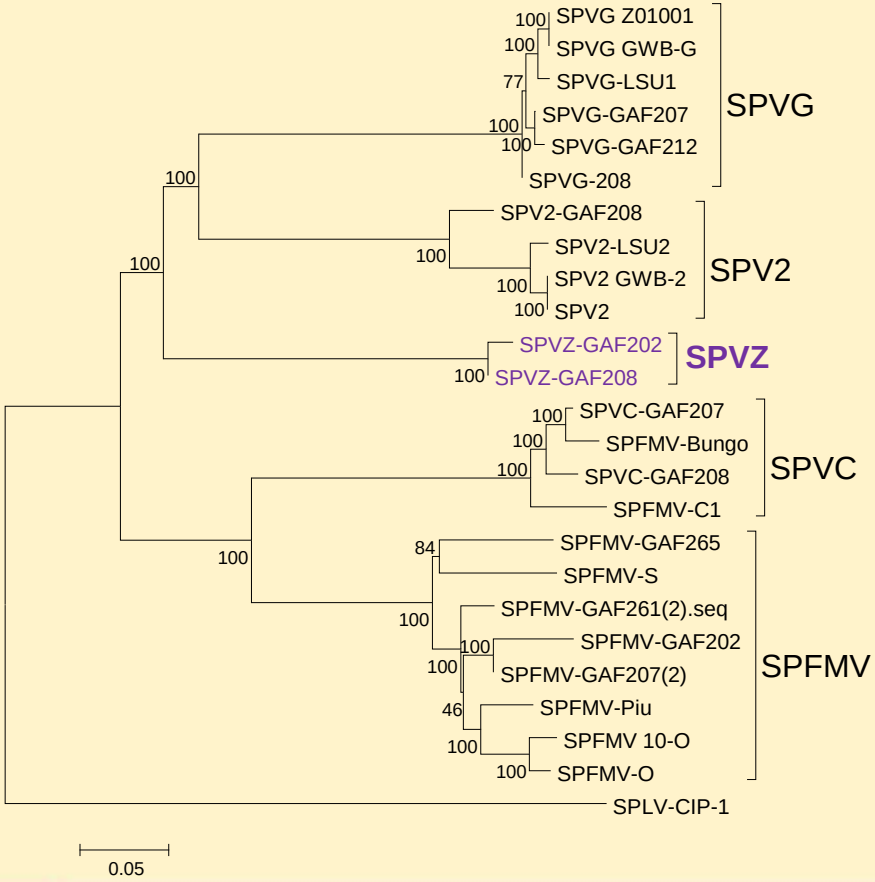
SPFMV
SPVC
SPLCV
SPCSV-WA
SPPV

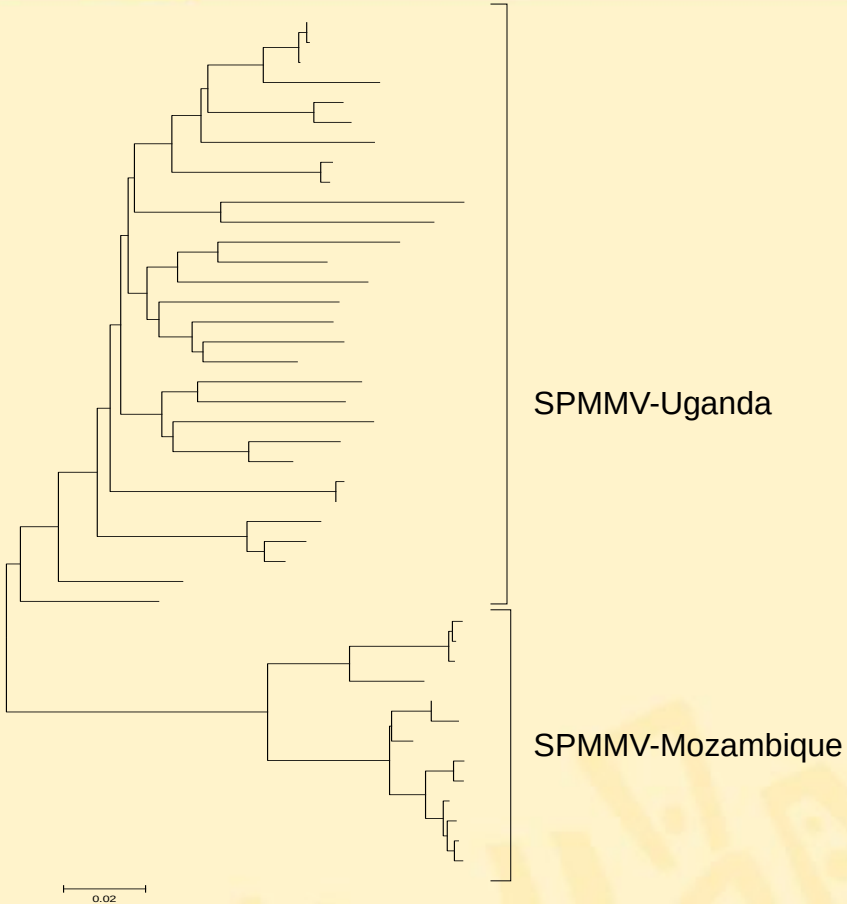
SPFMV
SPVC
SPLCV
SPCSV-WA
SPPV

Mozambique:

sample		Virus identified									
#	region	SPFMV	SPVC	SPVG	SPV2	SPMMV	SPCSV	SPCFV	SPLCV	SPPV	new
1	Angonia									X	
2	Angonia	X								X	
3	Angonia	X	X							X	
4	Angonia	X	X		X	X	X		X	X	
5	Angonia	X	X	X			X		X	X	SPVZ
7	Angonia	X					X		X	X	
8	Angonia	XX	XX	X						X	SPVZ
10	Angonia	X							X	X	
14	Gurue										
15	Gurue	XX	XX		X	XX	X			X	
17	Gurue	X	X		X	XX	X		X	X	
18	Gurue	XX	X	X	X	XX				X	SPVZ
19	Gurue	X					X			X	
20	Gurue	XX	X	X						X	SPVZ
21	Gurue	X									
22	Gurue	X		X			X		X		
24	Gurue									X	
25	Gurue	XX	X		X	XX	X			X	
27	Gurue	X	X		X	X	X			X	
28	Maputo	XX							X	X	
31	Maputo	XX			X	X	X			X	SPVZ
32	Maputo	XX	X	X					X	X	SPVZ
33	Maputo	X	X		X	X			X	X	
34	Maputo	X	XX			X	X		X	X	
35	Maputo	X					X			X	
36	Maputo	XX	XX	X		XX	XX			X	
37	Maputo	X	X	X					X	X	
39	Maputo	X	X				X			X	
41	Chokwe	XX	X	X		XX	X		X	X	
42	Chokwe	X	X	X		X	X		X	X	
44	Chokwe	XX	X	X		X		XX	X	X	
46	Chokwe	XX	X	X	X	XX	X		X	X	SPVZ, a
49	Chokwe	X				XX	X		X	X	
51	Chokwe	X	X	X		XX	X		X	X	SPVZ,a
55	Chokwe	XX				X	X		X	X	
66	TANZANIA	X					X		X	X	

Variability: potyviridae

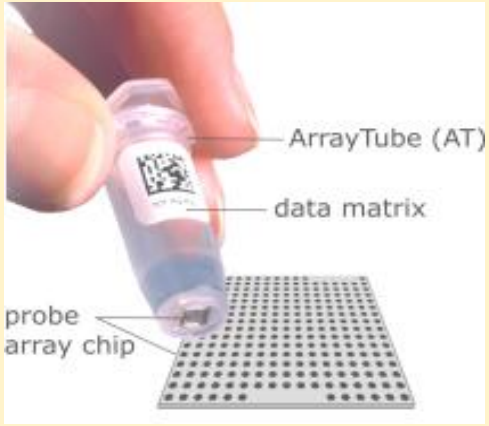


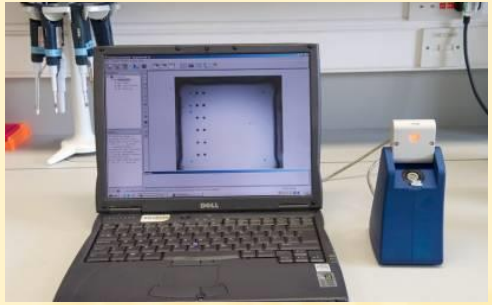


ClonDiag arrays for sweetpotato viruses:

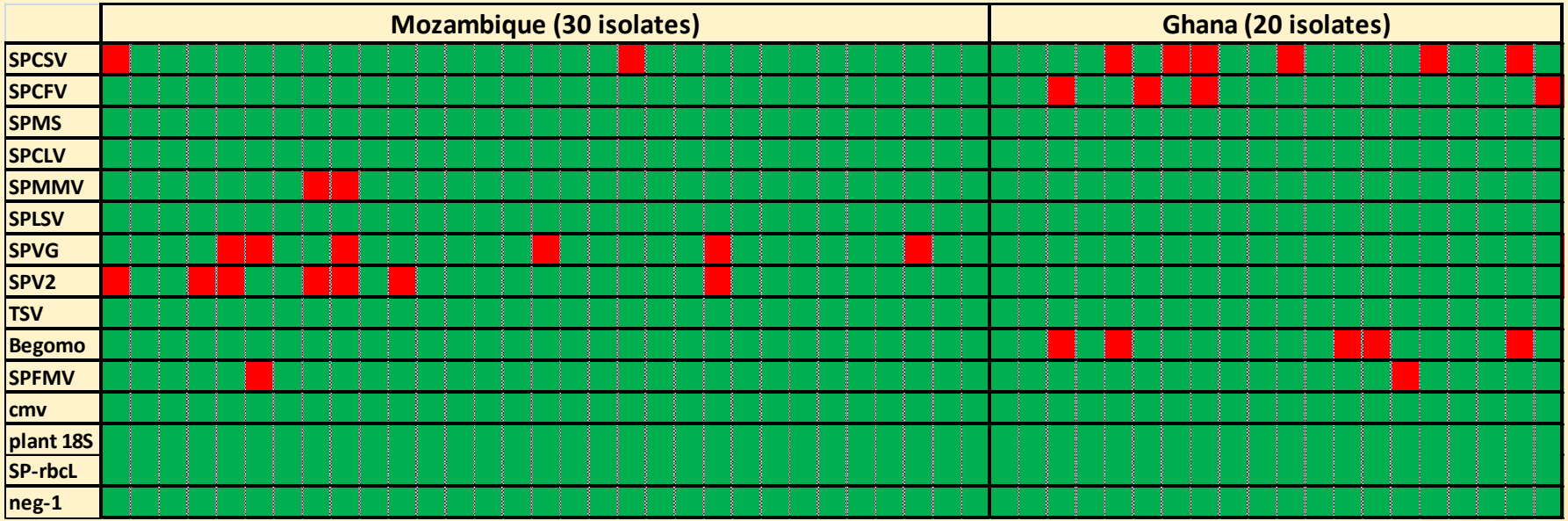
- Mini microarray embedded in tube

  Up to 80 features
- One step labelling (biotin amp)
- Cheap scanner
- Manipulations in tube
- Benefit: many (all) viruses in one assay, sensitive (similar to PCR)





Comparison of 2nd iteration sweetpotato virus array and **small RNA sequencing**

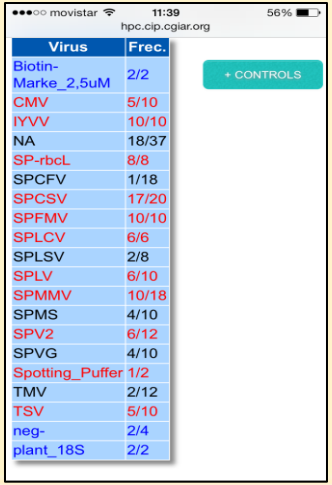


Green=agreement
Red = disagreement

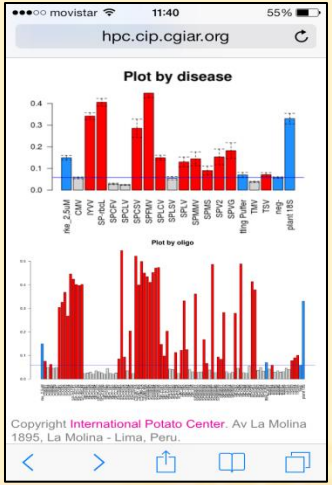
Tube-array smartphone app for results analysis

- Image recognition and signal intensity analysis code ready, based on HTML5 standard
- First prototype version available (works through web-browser, all platforms):
- http://hpc.cip.cgiar.org/clondiag/step/A.php

  Evaluate against dedicated analysis software

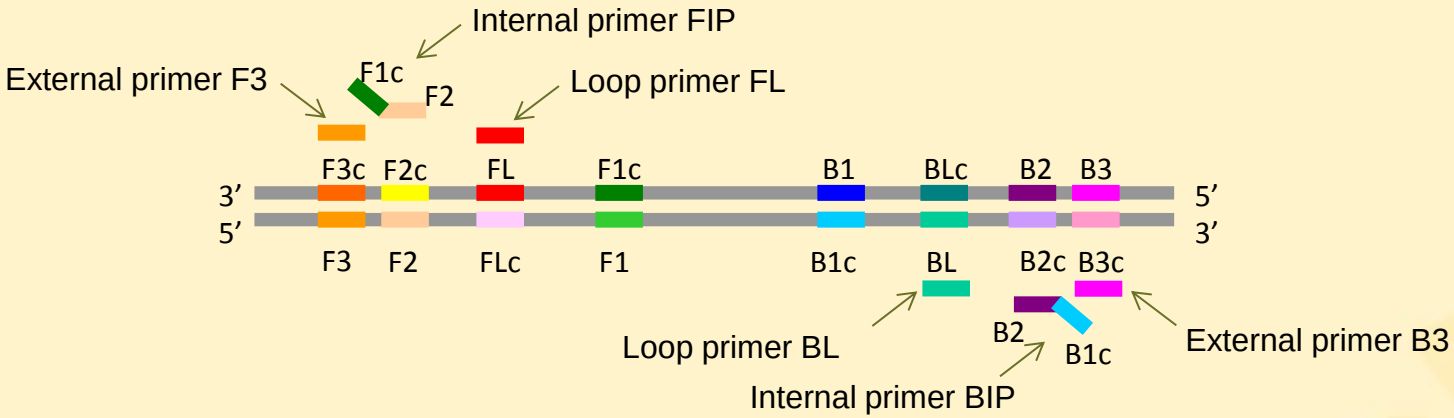


Virus	Frec.
Biotin-Marke_2,5uM	2/2
CMV	5/10
IYVV	10/10
NA	18/37
SP-rbcL	8/8
SPCFV	1/18
SPCSV	17/20
SPFMV	10/10
SPLCV	6/6
SPLSV	2/8
SPLV	6/10
SPMMV	10/18
SPMS	4/10
SPV2	6/12
SPVG	4/10
Spotting_Puffer	1/2
TMV	2/12
TSV	5/10
neg-plant_18S	2/4
	2/2



Towards sensitive molecular field detection kits: **LAMP**

- Functions at a single temperature (no complicated and expensive thermal cycling equipment required)
- Enzymes more robust to contaminants & can be lyophilized (robustness for field conditions)
- 20-60 minute reaction time & more sensitive even than PCR



Overcoming many of the limitations of PCR-based methods
(cost and complexity of thermal cycling equipment)

LAMP: addressing the bottlenecks for field use

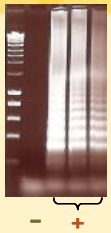
Sweetpotato Action for Security and Health in Africa

DNA/RNA extraction → LAMP

Developed simple field extraction method (FERA)

Lyophilized reagents; development reusable sodium acetate based heat pack for running reactions

agarose gel electrophoresis

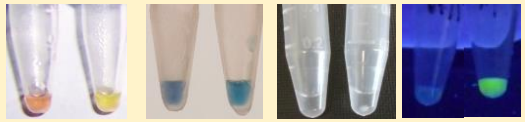


method development / optimisation

in the lab

Improving non-instrumented detection methods

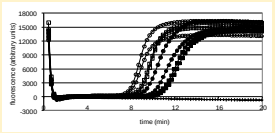
colour change/ precipitation



LFD detection

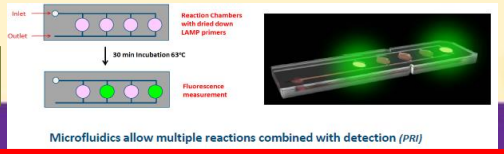
multiplex detection

real-time: Genie



real-time

microfluidics



in the field

Conclusions and recommendations for future research

- TubeArrays are performing well and may be a useful tool for distribution hubs
- Fast, sensitive and easy to use field based diagnostics is still a challenge, isothermal amplification most promising (and flexible) solution and should be further invested in
- NGS sequencing data will continue to contribute to improving and validating both LAMP and TubeArrays
- Virus variability & distribution studies are helping us understand possible causes of differences in variety performance among regions

- Cuellar, W.J., Galvez, M., Fuentes, S., Tugume, J., **Kreuze, J.F.** (2014) Synergistic interactions of begomoviruses with sweet potato chlorotic stunt virus (genus *Crinivirus*) in sweetpotato (*Ipomoea batatas* L.). **Molecular Plant Pathology**, *in press*
- Gibson R, Kreuze J. (2014) Degeneration in sweetpotato due to viruses, virus cleaned planting material and reversion: a review. ***Plant Pathology,*** *Doi: 10.1111/ppa.12273*
- Boonham, N., **Kreuze, J.**, Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., Mumford, R. (2014) Methods in virus diagnostics: From ELISA to next generation sequencing. ***Virus research*** *186: 20-31 DOI 10.1016/j.virusres.2013.12.007*
- De Souza, J., Fuentes, S., Savenkov, E.I., Cuellar, W, **Kreuze, J.F.** (2013) The complete nucleotide sequence of sweet potato C6 virus: a carlavirus lacking a cysteine-rich protein. ***Archives of Virology*** *158:1393-1396 DOI 10.1007/s00705-013-1614-x*
- Clark, C.A., Davis, J.A., Abad, J.A., Cuellar, W., Fuentes, S., **Kreuze, J.F.**, Gibson, R., Mukasa, S.B., Tairo, F., and Valkonen, J.P.T. (2012) Sweetpotato Viruses: 15 Years of Progress on Understanding and Managing Complex Diseases. ***Plant Disease***, *96: 168-185 DOI 10.1094/PDIS-07-11-0550*
- Cuellar, W.J., De Souza, J., Barrantes, I., Fuentes, S., **Kreuze, J.F.** (2011) Distinct cavemoviruses interact synergistically with sweet potato chlorotic stunt virus (genus *Crinivirus*) in cultivated sweet potato. ***Journal of General Virology***, 92: 1233-1243 *DOI 10.1099/vir.0.029975-0*
- Cuellar, W.J., Cruzado, R.K., Fuentes, S., Untiveros, M., Soto, M., **Kreuze, J.F.** (2011) Sequence characterization of a Peruvian isolate of Sweet potato chlorotic stunt virus: further variability and a model for p22 acquisition. ***Virus research***, 157: 111-115 *DOI 10.1016/j.virusres.2011.01.010*