Progress in Diagnostic Tools for Virus Detection & Potential Usefulness for Breeders in SSA

SASHA

Sweetpotato Action for Security and Health in Africa

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Nairobi
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Overview of Objectives in Phase 2

3.2 Improve and validate diagnostic methods for support of seed quality, germplasm management and exchange. We will seek to have a more efficient virus diagnostic and removal process (less time, less costly) in place at the Support Platform level and ultimately at the field level by:

3.2.1 Conducting validation experiments of the ClonDiag tube-array to see if it can replace the use of NCM-Elisa and grafting in virus diagnosis

3.2.3 Continuing to improve the design LAMP tool for detecting sweetpotato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV) in the field and validating its accuracy

3.1.4 Evaluating the effect of Begomoviruses on sweetpotato varietal yields, either singly or in combination with other major viruses

Workplan year 2/3

3.2.1 ClonDiag arrays

- Remove unspecific probes and re-design probes for false negative reactions for 4th iteration array, add SPC6V, perhaps group specific probes (FERA, Dec 2015) ✔
- Validate 4th iteration array in parallel with standard indexing (January-July 2016) ~
- Improve lens system, dealing with background specks, app improvements (August-December 2015) ~

3.2.3 Field testing LAMP

- Test stability of LAMP reagents in kit format (up to one year) by preparing aliquots and testing each month (July 2015-July 2016) ✔
- Bramwel will focus on begomovirus re-design & validate field use using lyophilized products and Genie June-December 2016) ~

3.1.4 Impact of begomoviruses on sweetpotato

- Isolate plants with SPFMV,SPCSV and/or begomovirus (October 2015) ~
- Evaluate effect of virus infection in green house conditions (December 2016) ~
- Obtain, harden and multiply virus free tissue culture plants from Naspot 1 & Gweri (Feb, 2016) ~
- Field trial to evaluate effect of different combinations on yield (March, 2017) ~

Key Achievements Year 2

- **3.2.1** Remove unspecific probes and re-design probes for false negative reactions for 4th iteration array, add SPC6V, perhaps group specific probes (FERA, Dec 2015)

  Validate 4th iteration array in parallel with standard indexing delayed due delivery issues with reagents (Now delivered)

  Remaining improvements image quality & apps delayed due to departure programmer (not essential for running the test)

Key Achievements year 1

3.2.3 Improve the design LAMP tool for detecting in the field and validating its accuracy

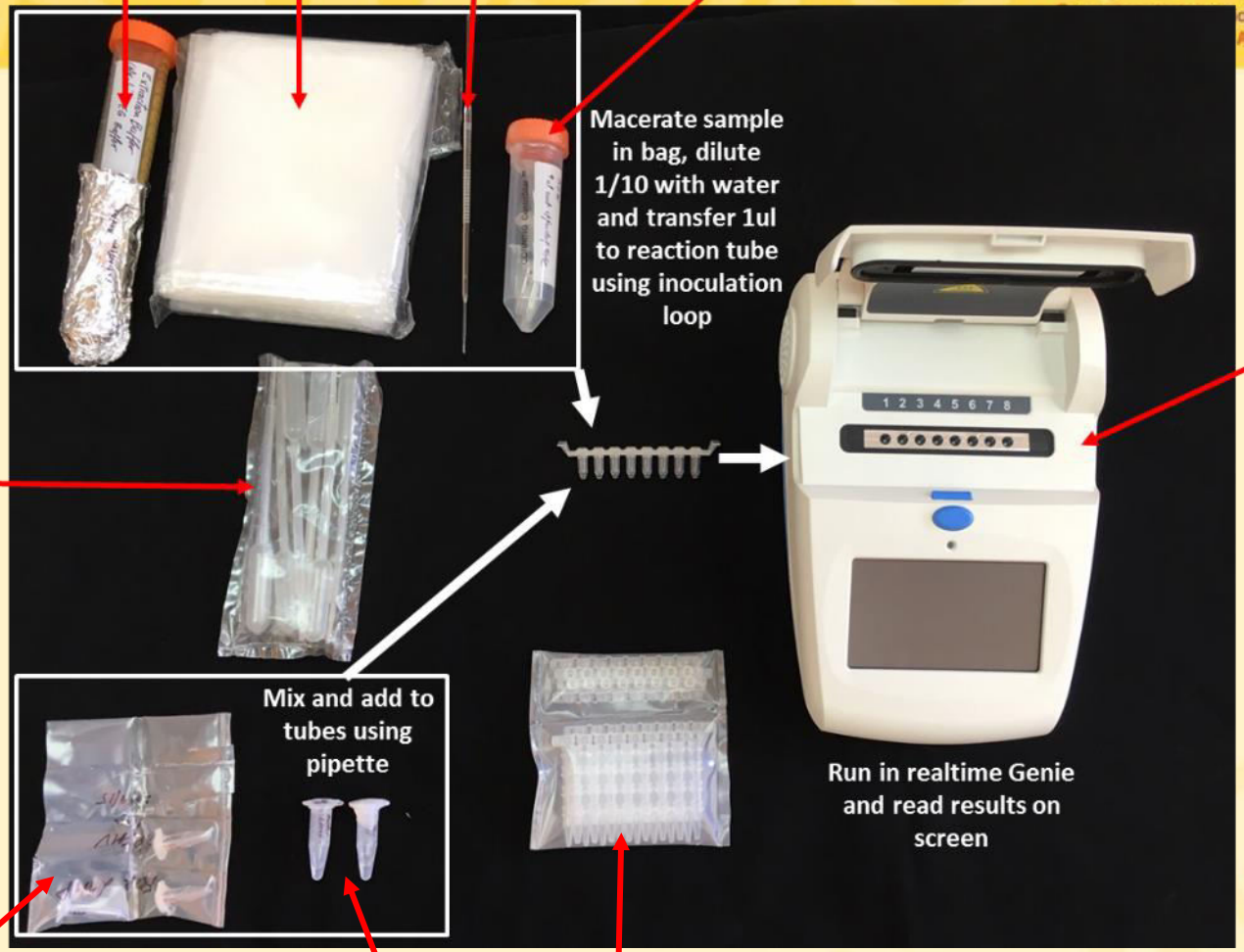
Components of field kits:

- Extraction buffer & bag for sample maceration
- Buffer, $MgSO_4$ & betaine separate as liquid at room-temperature
- All other components lyophilized together in individual extraction tubes
- Add buffer to tubes & include 1 ul of sample extract with inoculation loop

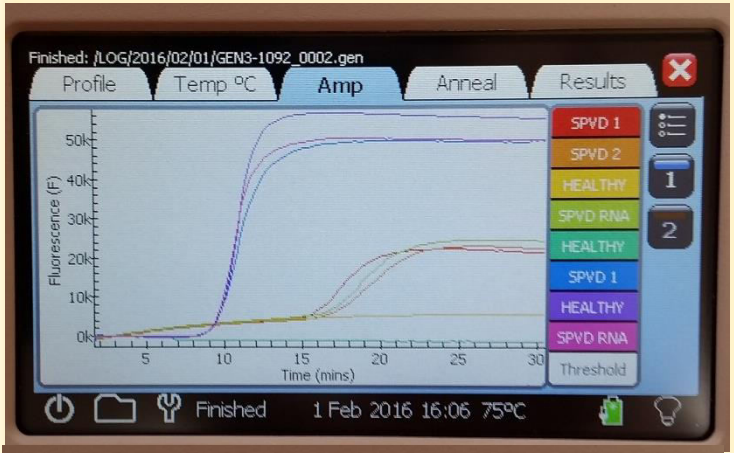
Current status:

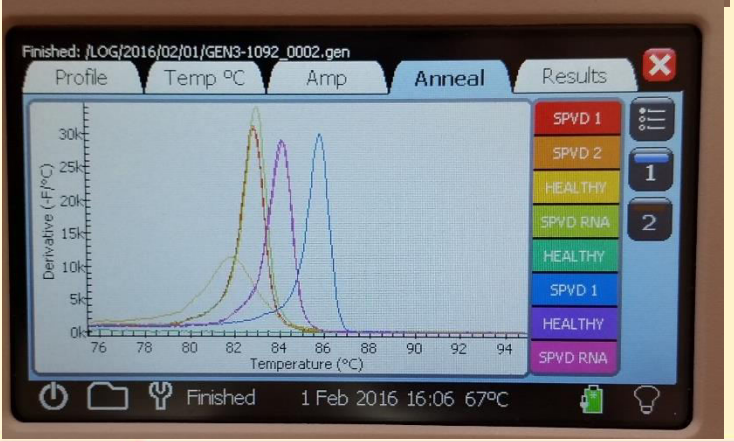
- Kit worked after 1 week of storage, but not anymore after 6 months
- Problem was inactivation of lyophilized enzyme, but also buffer (liquid component) gave reduced efficiency in amplification
- Checking new additives and modified storage conditions to increase stability
- Testing cheaper kit components and new (cheaper & more active) enzymes

Prototype LAMP field kit

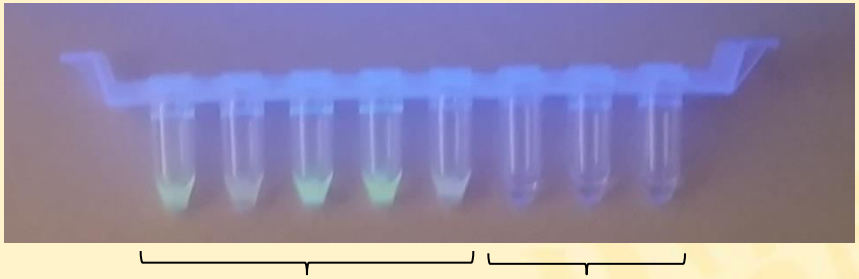


Genie output





Result using handheld UV lamp



Required improvements

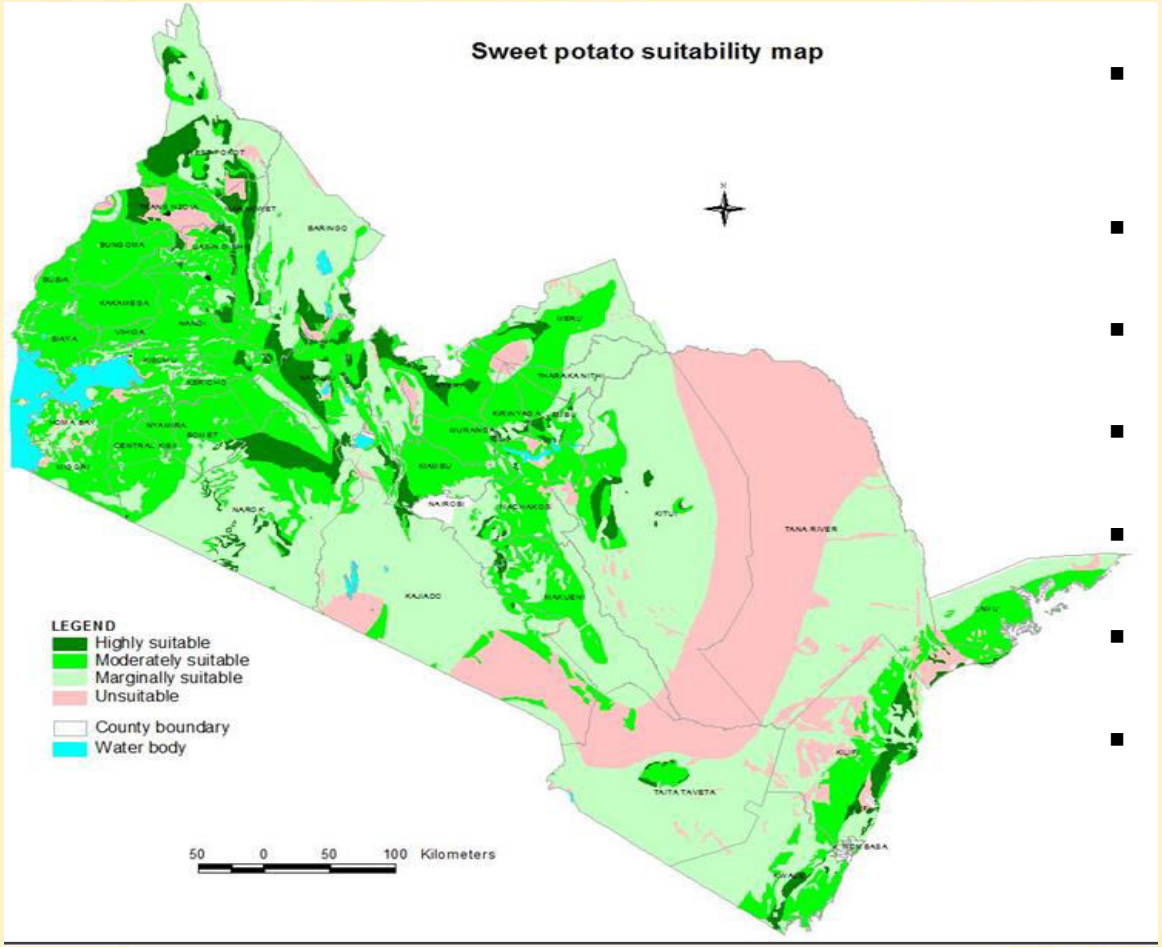
- Kit worked after 1 week of storage, but not anymore after 6 months
- Problem was inactivation of lyophilized enzyme, but also buffer (liquid component) gave reduced efficiency in amplification
- Checking new additives and modified storage conditions to increase stability
- Testing cheaper kit components and new (cheaper & more active) enzymes

Key Achievements year 2

3.1.4 Evaluating the effect of Begomoviruses on sweetpotato varietal yields

- Started indexing material at KEPHIS using I.setosa & materials with Begomovirus symptoms identified by symptoms (roll up/down)
- PCR detection for Begomovirus optimized
- Positive samples to be sequenced at BecA
- Conducted survey for sweetpotato viruses in major SP growing regions 3/5.
- Screen for all field material collected displaying Begomovirus symptoms by PCR
- Optimize conventional RT detection methods for other viruses (use to screen field collected samples together with grafting and NCM ELISA)

Country-wide survey to assess the incidence and distribution of begomoviruses in Kenya



- Survey Western, RV, Eastern and Coastal-25 Counties
- Oct-Dec, 2015; Jan-Feb,2016
- GPS location, photographs
- Vines established in screenhouse
- Graft inoculation
- NCM-ELISA
- Molecular tools:
  - PCR,
  - RT-PCR and
  - Q-PCR

Current status of samples collected

No.	Region	Sub-Counties visited	Samples for diagnostics	Samples for Next Generation Sequencing
1.	South Rift	10	87	30
2.	Western	21	330	40
3.	Nyanza	23	220	20
4.	Coast	8	61	12
5.	Eastern			
6.	Central			
Total		54	698	102

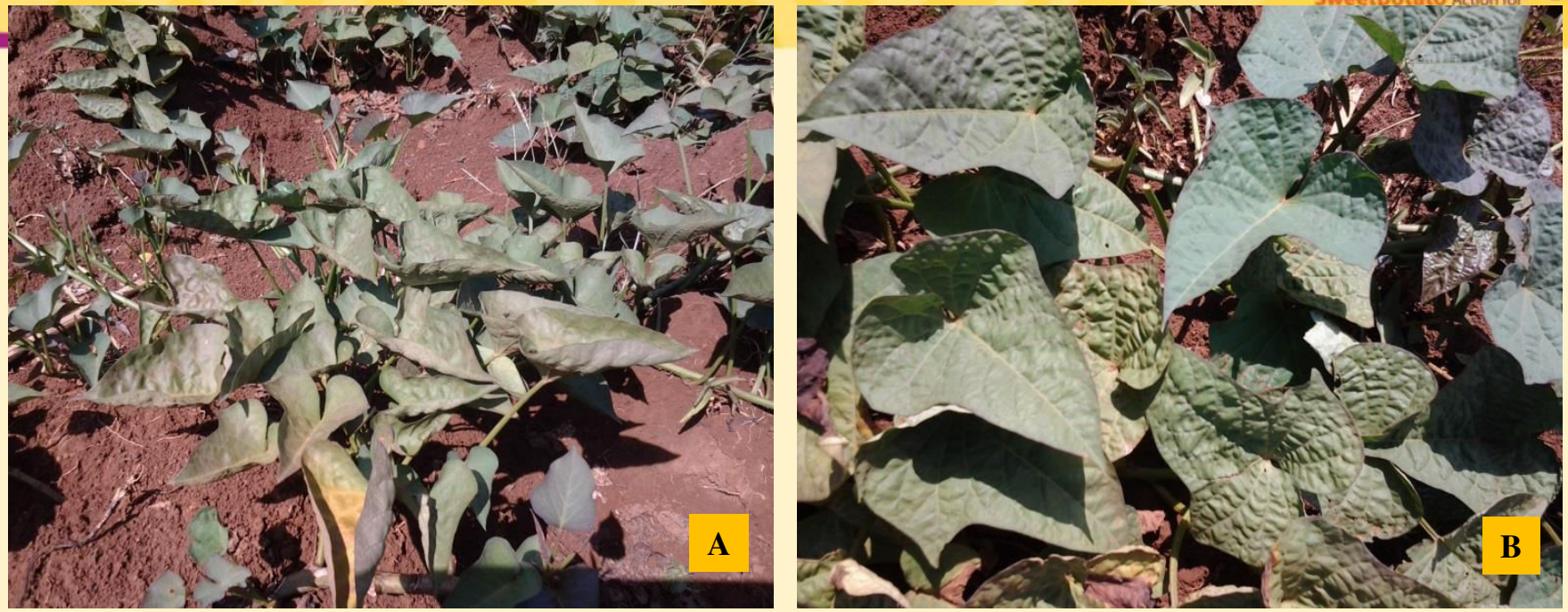


Figure 2: Field in Busia County showing sweetpotato begomovirus symptoms. Kampala variety showing roll - **A** up and **B** –rugosity

Figure : Field 3 in Baringo County. A and B two varieties showing roll up and C – variety showing sweetpotato symptoms (chlorosis, leaf deformation, leaf reduction and rugosity)

Figure : Sweetpotato field with mixed varieties with one variety severely affected by virus; showing stunted growth and chlorosis

Figure : Field 6 in Kericho County. Apparently clean (**R**) and infected vines (**L**).

Figure : Different symptom expression in sweetpotato varieties: **A** – vein clearing/rugosity, **B** – yellowing of veins, **C** – purple rings and **D** –roll up.

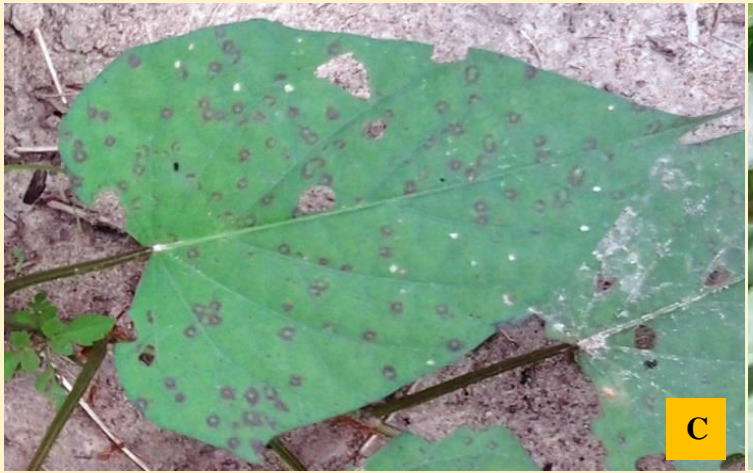


Figure : Comparing notes with farmers and advising on how to do negative/positive selection when selecting planting material

Begomovirus detection

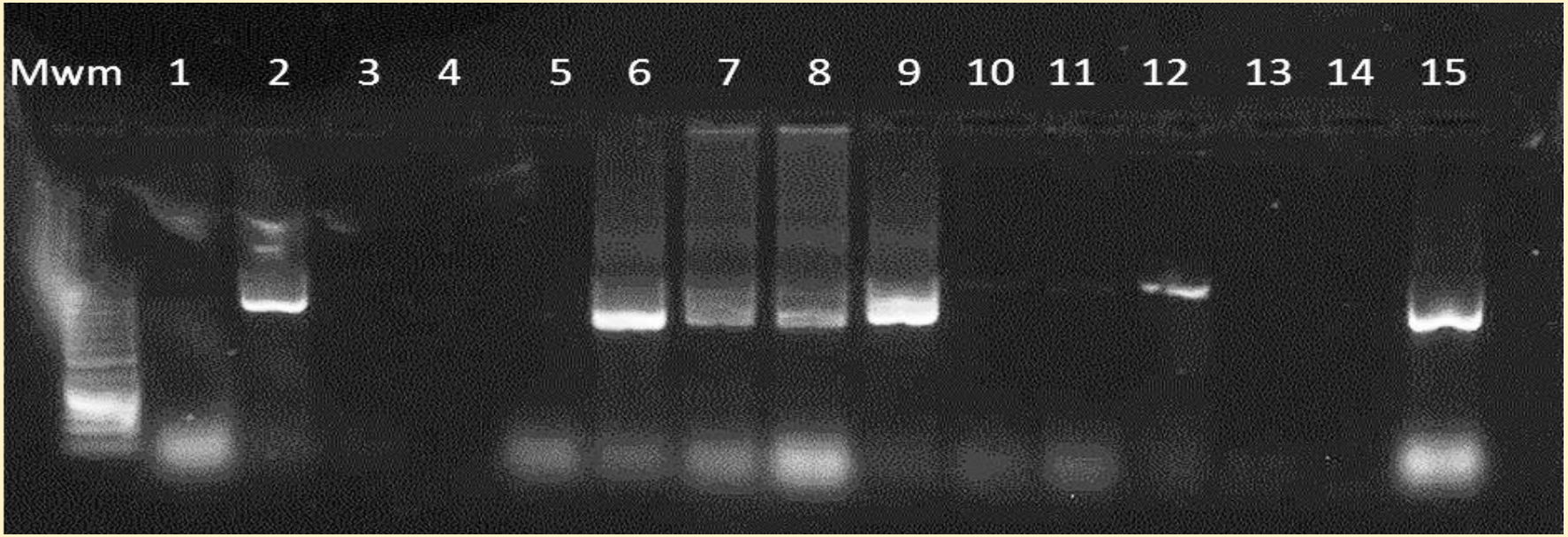


Fig: Mwm-100bp, **1-,2-,3-,4-,5-,6-,7-,8-,9-,10-,11-,12-,13-Non template,14-Lima -ve** and **15- Lima +ve Begomo**

Key Milestone Progress to Date

No.	Description	Due	Date	Current Status	Revised	Date	Comment concerning current status	Planned % of milestone as of Apr 2016	% progress of milestone as Apr 2016	% progress / planned Apr 2016
		Mon	Year		Mon	Year				
3.1.4	**Report evaluating the effect of Begomoviruses on yields**	6	2018	On track			Slight delay due to slow delivery of reagents for virus testing in Kenya.	50	50	100
3.2.1	**Availability of disease-free foundation material within 12 months of initiating clean-up**	6	2017	Started, delayed			Delayed due to pending delivery reagents to Kenya	60	55	92
3.2.3	**Validated portable LAMP tool for detecting SPFMV & SPCSV**	6	2016	Started, slightly delayed			New tests to improve storability of LAMP reagents required	80	80	100

African virome: distribution of viruses

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AFRICA		sequenced	infected	RNA viruses												DNA viruses							
				SPFMV	SPVG	SPVC	SPV2	SPVZ	Poty	SPMMV	SPCSV	SPCFV	SPC6V	CMV	mitovirus	Ampelovirus	viroid	Begomovirus	Alphasatellite	Mastrevirus	SPVCV	SPCV	Badnavirus		
West Africa	Guinea	35	94%	31%		3%									9%		3%	9%		71%	6%		54%		10
	Nigeria	95	75%	32%		2%			6%			11%			2%			8%		58%			32%		20
	Benin	27	96%	41%		7%														81%			22%		30
	Ghana1	44	100%	80%	5%	34%					39%	27%						75%		20%	2%		91%		40
	Burkina Faso1	4	100%	75%							75%							100%	50%	50%			75%		50
East Africa	Ethiopia	224	98%	41%	42%	21%					28%	2%	1%	1%	3%			22%		43%			92%		60
	Kenya	3	100%	100%		100%					67%									67%			100%		70
	Tanzania	171	95%	59%		11%				1%	37%		1%		2%			9%		32%		5%	75%		80
	Uganda	103	100%	76%		40%					52%	9%			4%			53%		48%		17%	82%		90
Southern Africa	Malawi	114	92%	70%	9%	20%	4%	3%			23%	23%			1%			2%		30%	1%	18%	65%		100
	Zimbabwe	147	97%	84%	12%	22%	5%	1%	1%		28%							1%		39%			81%		
	Angola	158	99%	35%	1%	1%		25%	2%	1%	13%			1%	2%	10%		14%		71%	8%	1%	91%		
	Mozambique1	44	98%	73%	30%	30%	20%	18%		39%	45%	2%						41%	5%	39%			73%		
Total:		1169	95%	56%	12%	17%	2%	4%	1%	2%	25%	5%	0%	0%	2%	1%	0%	18%	0%	46%	1%	4%	76%		

African virome: distribution of viruses

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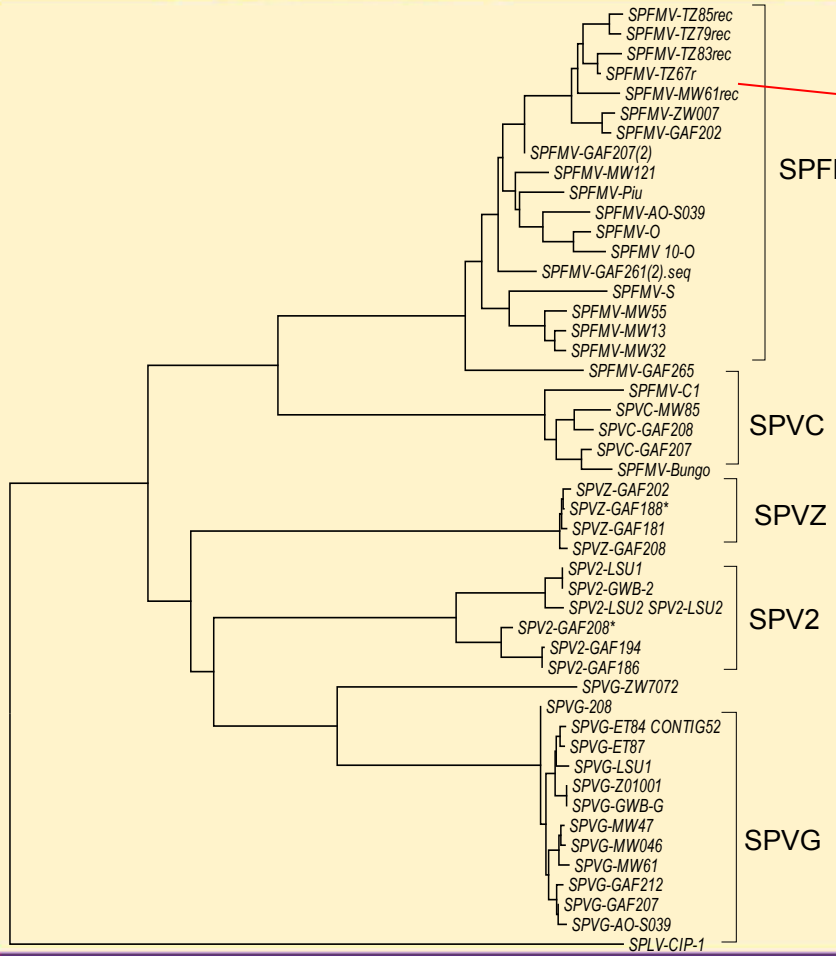
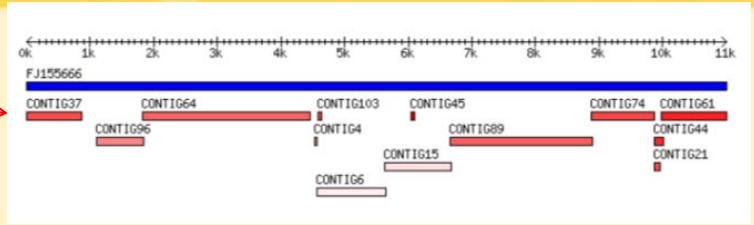
AFRICA		sequenced	infected	RNA viruses													DNA viruses					
				SPFMV	SPVG	SPVC	SPV2	SPVZ	Poty	SPMMV	SPCSV	SPCFV	SPC6V	CMV		Ampelovirus	viroid	Begomovirus	Alphasatellite		SPVCV	SPCV	
West Africa	Guinea	35	49%	31%		3%											3%	9%			6%		
	Nigeria	95	43%	32%		2%			6%			11%						8%					
	Benin	27	41%	41%		7%																
	Ghana1	44	100%	80%	5%	34%					39%	27%						75%			2%		
	Burkina Faso1	4	100%	75%							75%							100%	50%				
East Africa	Ethiopia	224	71%	41%	42%	21%					28%	2%	1%	1%				22%					
	Kenya	3	100%	100%		100%					67%												
	Tanzania	171	73%	59%		11%				1%	37%		1%					9%				5%	
	Uganda	103	95%	76%		40%					52%	9%						53%				17%	
Southern Africa	Malawi	114	82%	70%	9%	20%	4%	3%			23%	23%						2%			1%	18%	
	Zimbabwe	147	88%	84%	12%	22%	5%	1%	1%		28%							1%					
	Angola	158	66%	35%	1%	1%		25%	2%	1%	13%			1%		10%		14%			8%	1%	
	Mozambique1	44	93%	73%	30%	30%	20%	18%		39%	45%	2%						41%	5%				
Total:		1169	74%	56%	12%	17%	2%	4%	1%	2%	25%	5%	0%	0%		1%	0%	18%	0%		1%	4%	

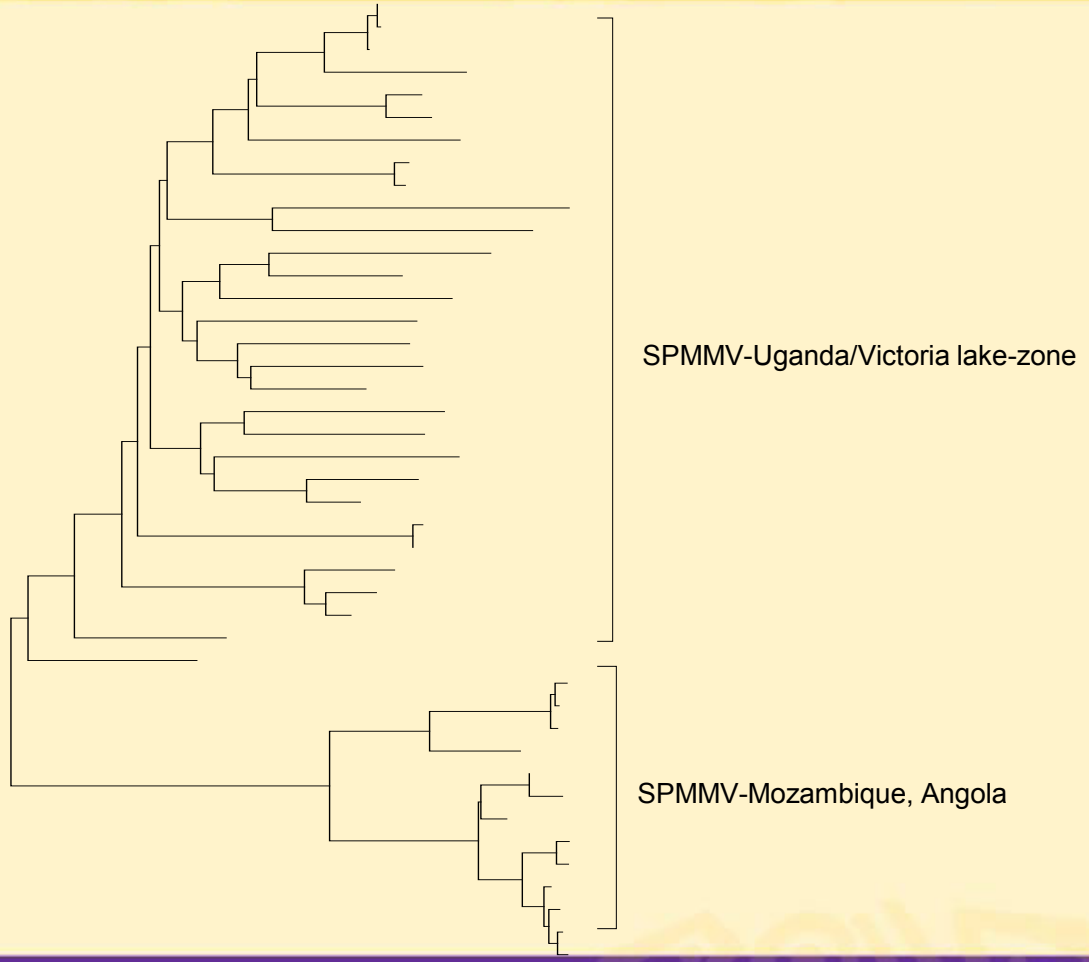
10 20 30 40 50 60 70 80 90 100

Variability: (sweet)potyviruses





Variability: Sweet potato mild mottle virus



Sweet potato chlorotic stunt virus

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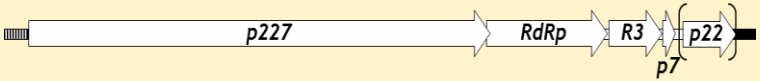
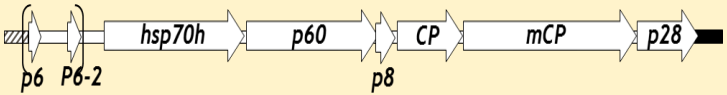
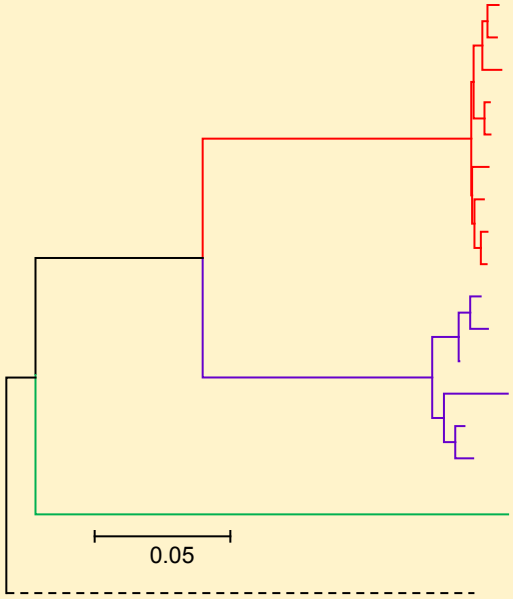
RNA1



RNA2



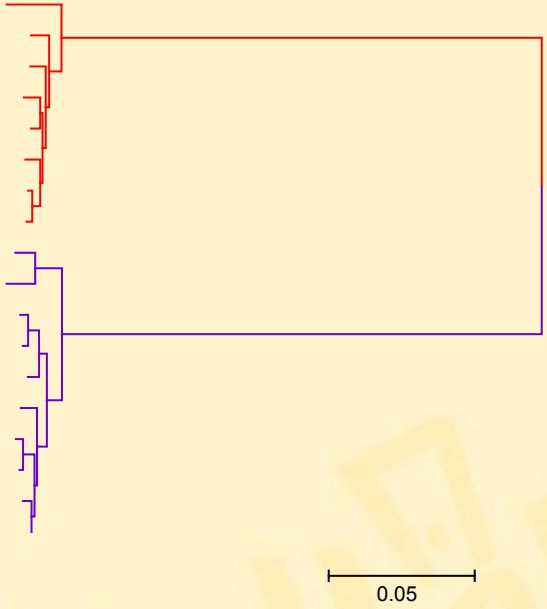


SPCSV-WA (west Africa, Angola & Zimbabwe)

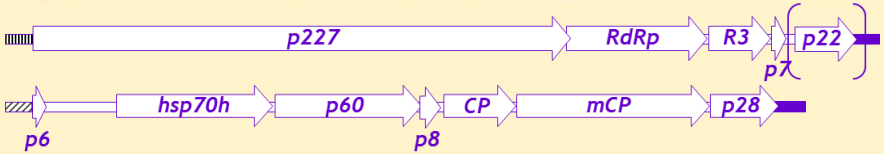
SPCSV-EA (East & Southern Africa)

SPCSV-MZ (Southern Africa)

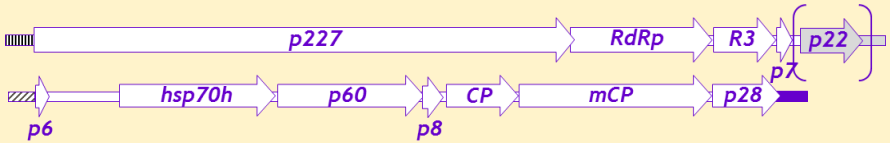
SPCSV-KML (uncommon, Uganda Tanzania; different virus?)



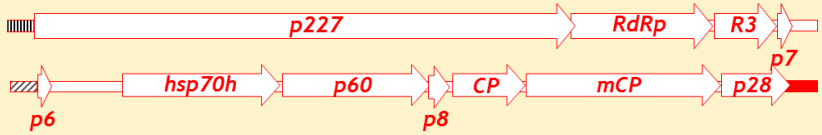
Sweet potato chlorotic stunt virus



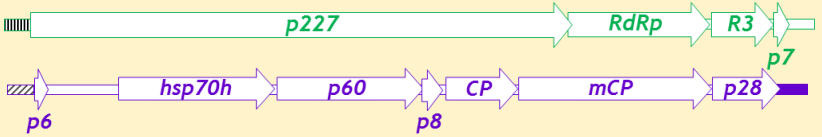
Ethiopia, Uganda, lake zone Tanzania, Mozambique



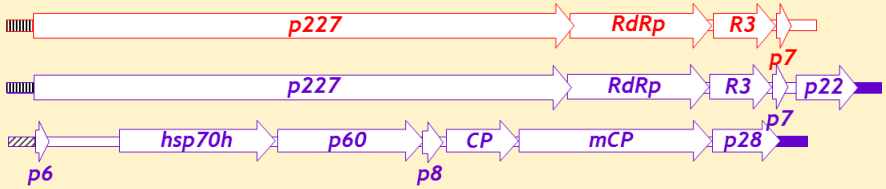
Southern Tanzania, Mozambique, Angola

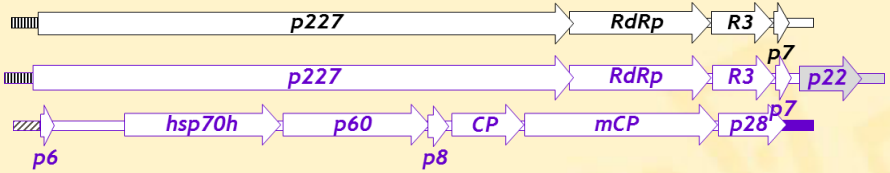


West Africa, Angola, Zimbabwe



Mozambique, Malawi, Angola, Zimbabwe





Potential usefulness for breeders

- Knowing the variation of viruses in your target regions will help you decide what to use for resistance screening, and why resistance may fail between regions
- LAMP can be run on real-time PCR machine and is semi-quantitative, easy extraction could enable higher throughput
- Faster and better diagnostics will enable more rapid exchange of breeding material

Papers or other communication outputs produced/in the pipeline

Untiveros M, Olspert A, Artola K, Firth AE, **Kreuze JF** and Valkonen JPT (2016) A novel sweet potato potyvirus ORF is expressed via polymerase slippage and suppresses RNA silencing. ***Molecular Plant Pathology*** in press, *DOI: 10.1111/mpp.12366*

This paper shows that potyviruses infecting sweetpotato are unique as compared to all others in encoding a large additional overlapping ORF (termed PISPO: Pretty Interesting Sweetpotato Potyvirus ORF), which is a suppressor of plant antiviral defense and expressed by transcriptional slippage.

VirusDetect: A novel bioinformatics pipeline for efficient virus discovery using deep sequencing of small RNAs. Resubmitted to ***Virology***

Perez, A., Galvez, M., Cuellar, W., Kreuze, J.F. Badnaviruses of sweetpotato: symptomless co-inhabitants on a global scale. **In preparation**, to be submitted third quarter of 2016

This paper will show that sweetpotato infecting Badnaviruses are highly variable and found infecting sweetpotato widespread throughout the world, but only occur in very low titers and are essentially symptomless.

Pending:

Sweetpotato viromes of Mozambique and Ghana

First report of Cotton leaf curl Gezira alpha-satellite in sweetpotato