

ClonDiag-arrays:

A possible replacement of biological indexing for detecting sweetpotato viruses?

SASHA

Sweetpotato Action for Security and Health in Africa

Jan Kreuze, Bramwel Wanjala, Segundo Fuentes, Ian Adams (FERA) Nairobi 26 Sept, 2017

Why?











Current procedure

5-6 months

3-5 months



Planting in pots and initial health testing

(2 months old plants)

Introduction to in vitro culture (duration: 3 months)



3 Initial multiplication and health testing

(duration: 5-6 months)

·Sweetpotato: NCM-ELISA for 10 viruses and PCR for Begomovirus, plus grafting on 1.setosa ·Potato: NASH for PVT and PSTVd: DAS-ELISA for 8 virus, Host Range test (with 11 Indicator plants) plus grafting on D. Stramonium.



(duration: 1 month)



Thermotherapy 32-34°C (Potato) 34-36°C (Sweetpotato)

(duration: 1 month)





Multiplication for virus testing

(duration: 1 month)



Meristem culture (duration:2-4 months)

Virus-free plants

Final diagnostic

(duration:4-5 months)

·Sweetpotato: NCM-ELISA for 10 viruses and PCR for Begomovirus, plus grafting on I.setosa ·Potato: NASH for PVT and PSTVd; DAS-ELISA for 8 virus, Host Range test (with 11 Indicator plants) plus grafting on D. Stramonium.

5-6 months



Sweetpotato Action for Security and Health in Africa

ClonDiag arrays: sensitive detection of multiple viruses



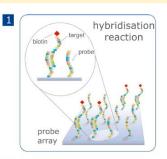
Used for clinical testing bacteria

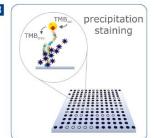
Multiplex PCR (sensitive) with labled nucleotides

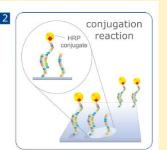
Hybridization to probes on array

→ ELISA

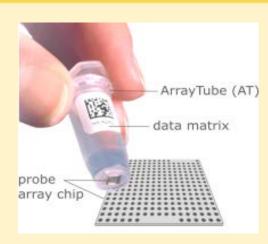
Probes and primers designed by FERA











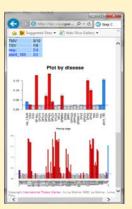


The evolution of a sweetpotato virus array

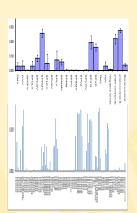


- 1st iteration: 10 viruses
- 2nd iteration: 14 viruses (tested samples Ghana-Mozambique)
- 3rd iteration: 19 viruses (tested on Pan African virome samples)
- 4th iteration: 19 viruses



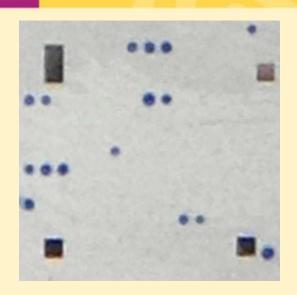






What does it look like?







Validation of the 4th iteration array

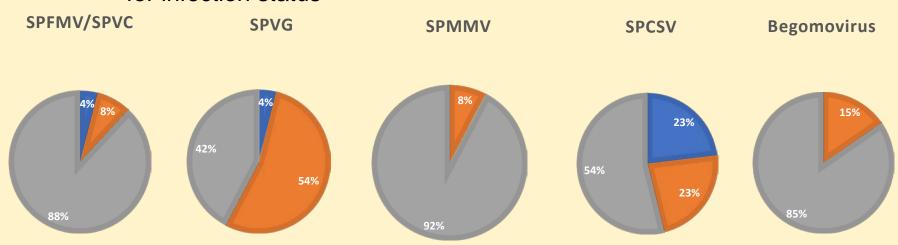


- 25 + 1 samples from greenhouse plants KEPHIS: leaves dried down, ground into powder and 50% sent to CIP-Lima for parallel analysis
- Processed at same time in CIP-Lima and KEPHIS, analyzed at KEPHIS with array reader & in Lima with smartphone
- Results compared

Validation of the 4th iteration array



KEPHIS array reader: 100% consistent with biological indexing for infection status



Array reader also detected SPV2, SPC6V, SPLV, SPCV, SPVCV, SPPV

Need to confirm conflict by complementary methods: PCR & NGS

Validation of the 4th iteration array

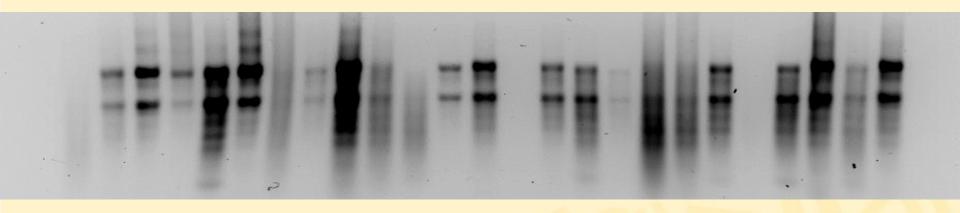


CIP smartphone app: 96% consistent for infection status

Repeatability same sample same lab: 100%

Sensitivity: loss of some detection between 1/25 and 1/50

Lima RNA quality was not similar between samples:



So, can we replace biological indexing?



- Cost of ClonDiag tube arrays around \$ 70 per sample in 2 days, vs \$153.71 per sample and 4-6 months for biological indexing
- Could be a good replacement for 1st round indexing

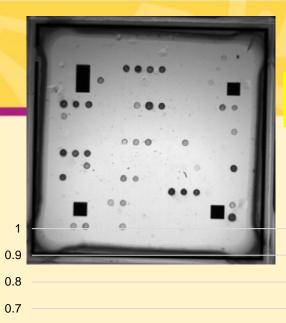
Complementary project funded by BMGF: Next generation phytosanitation



- Test Microfluidic format for running LAMP reactions (Quick-Chip)
- Optimize and validate small RNA sequencing based diagnostics
- Improve speed of virus elimination infected materials by improved tissue culture procedures and antiviral treatments.



Asante Sana



Ran on 12/06/2017

Repetitive testing

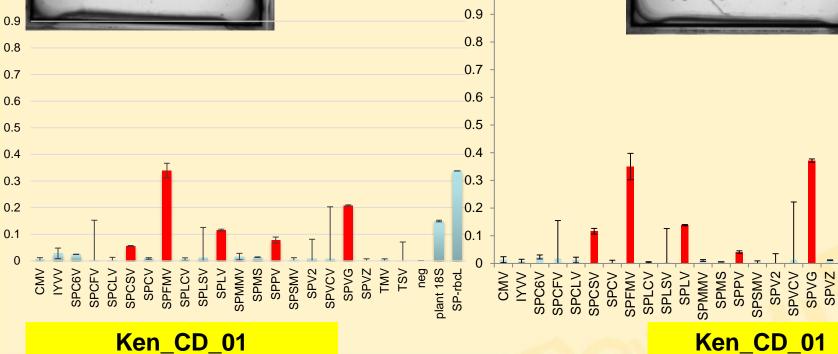


Ran on 16/03/2017

neg plant 18S

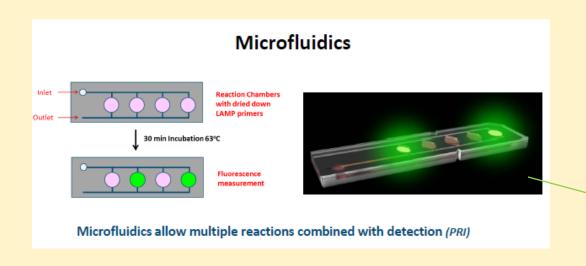
SP-rbcL

∠ML



phytosanitation project)





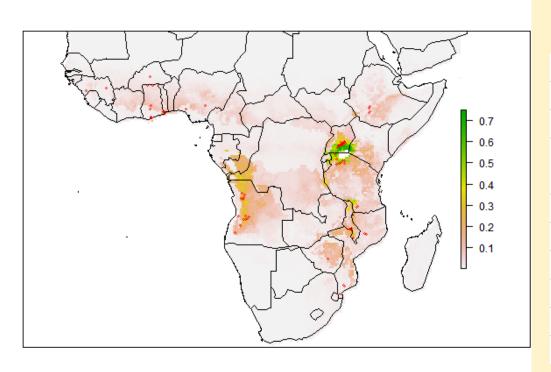


Progress virome analysis

Niche analysis using Maximum Entropy modeling and bioclimatic



variables



Example: begomoviruses, top 5 variables can explain 93% of occurrences

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