Best practices for clonal identity verification and health testing

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SWEETPOTATO ACTION FOR SECURITY AND HEALTH IN AFRICA

Outline

Introduction

Clonal identity

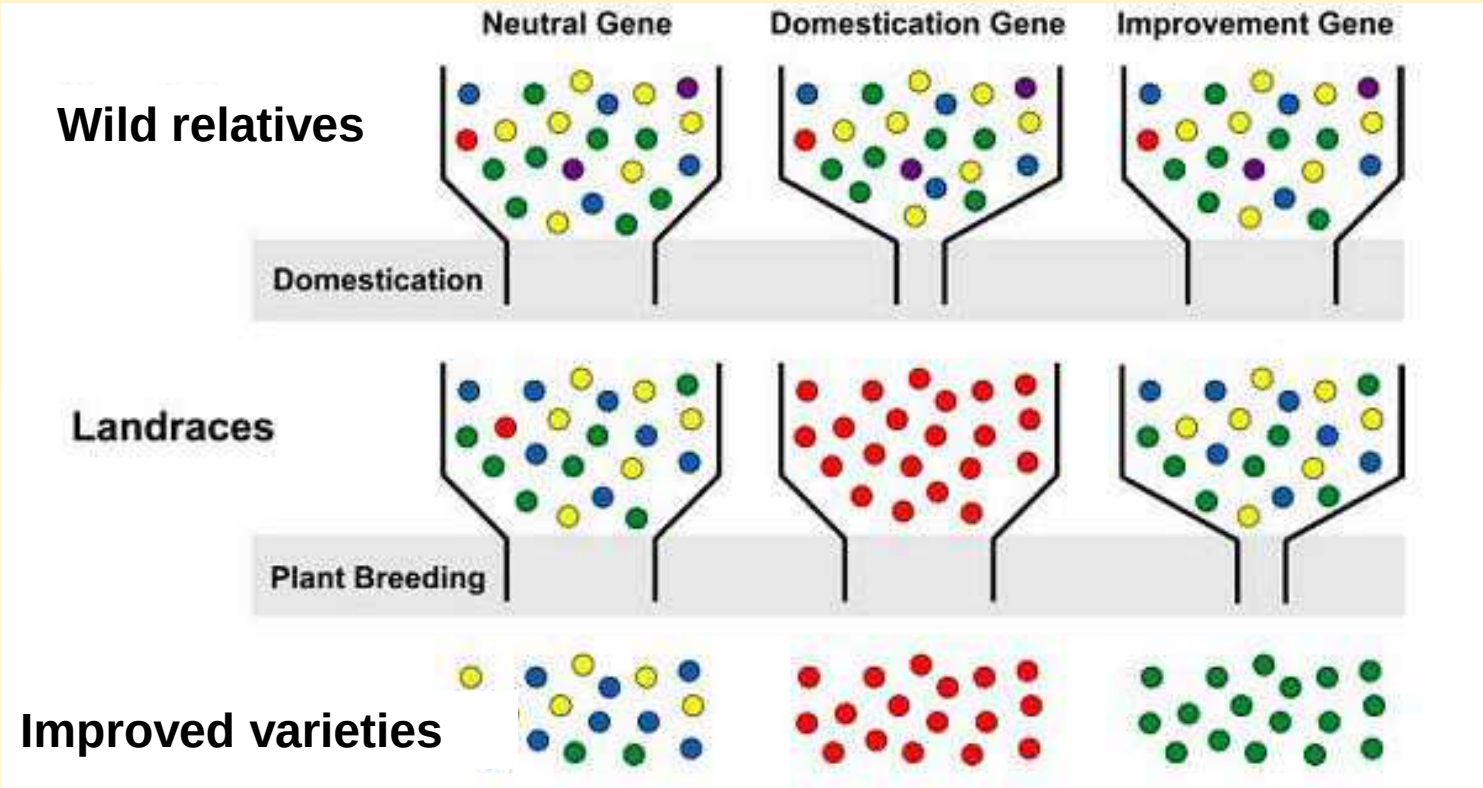
- Morphological/Phenotypic
- Biochemical
- Molecular

Health testing

- Current methods: Grafting on Ipomoea setosa, NCM ELISA & PCR
- Molecular tools (PCR, RT-PCR, Q-PCR)
- ClonDiag Microarray

Clonal identification-Why is it important

Domestication and selection



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Agriculture facing multiple challenges:

- Global warming effects
- Population growth
- Erosion of genetic progress
- Pests and diseases
- Consumer expectations

How do we measure genetic diversity

- Morphological/ phenotypic markers
- Biochemical markers
- Molecular markers

Methods of clonal identification

Morphological/Phenotypic

Descriptor	Phenotypes
Root	
Shape	1= round; 2= round elliptic; 3= elliptic; 4= ovate; 5= obovate; 6= oblong; 7= long oblong; 8= long elliptic; 9= long irregular or curved
Surface defects	0= absent; 1= alligator-like skin; 2= veins; 3= shallow horizontal constrictions; 4= deep horizontal constrictions; 5= shallow longitudinal grooves; 6= deep longitudinal grooves; 7= deep constrictions and deep grooves
Skin color	1= white; 2= cream; 3= yellow; 4= orange; 5= brownish orange; 6= pink; 7= red; 8= purple-red; 9= dark purple
Skin color intensity	1= pale; 2= intermediate; 3= dark
Flesh color	1= white; 2= cream; 3= dark cream; 4= pale yellow; 5= dark yellow; 6= pale orange; 7= intermediate orange; 8= dark orange; 9= strongly pigmented with anthocyanins
Flesh secondary color	0= absent; 1= white; 2= cream; 3= yellow; 4= orange; 5= pink; 6= red; 7= purple red; 8= purple; 9= dark purple
Leaf	
General outline	1= rounded; 2= reniform; 3= cordate; 4= triangular; 5= hastate; 6= lobed; 7= almost divided
Lobe type	1= no lateral lobes; 2= very slight (teeth); 3= slight; 4= moderate; 5= deep; 6= very deep
Lobe number	1= one; 2= two; 3= three
Shape of central lobe	1= toothed; 2= triangular; 3= semi-circular; 4= semi-elliptic; 5= elliptic; 6= lanceolate; 7= oblanceolate; 8= linear
Mature leaf color	1= yellow-green; 2= green; 3= green with purple edge; 4= greyish-green; 5= green with purple veins on upper surface; 6= slightly purple; 7= mostly purple; 8= green upper surface, purple lower surface; 9= purple on both surfaces
Immature leaf color	1= yellow-green; 2= green; 3= green with purple edge; 4= greyish-green; 5= green with purple veins on upper surface; 6= slightly purple; 7= mostly purple; 8= green upper surface, purple lower surface; 9= purple on both surfaces
Leaf size	3= small (<8 cm); 5= medium (8-15 cm); 7= large (16-25 cm); 9= very large (>25 cm)
Petiole pigmentation	1= green; 2= Green with purple near stem; 3= Green with purple near leaf; 4= Green with purple at both ends; 5= Green with purple spots throughout petiole; 6= Green with purple stripes; 7= purple with green near leaf; 8= some petioles purple, some others green; 9= totally or mostly purple

.....Cont.

Molecular

Characteristics	Isozyme	RFLPs	RAPDs	Sequence-tagged SSRs	AFLPs	PCR sequencing
Development costs	Low	Medium	Low	High	Low	High
Level of polymorphism	Medium	Medium	Medium	High	Medium	Medium
Automation possible	No	No	Yes/No	Yes/No	Yes/No	Yes
Cost of automation	Low	Medium	Medium	High	High	High
Repeatability	Low	High	Low	High	Medium	High
Level of training required	Low	Low	Low	Low/ Medium	Medium	High
Cost ($ per assay)	High (2.00)	High (2.00)	Low (1.00)	Low (1.50)	Medium (1.50)	High (2.00)
Radioactivity used	No	Yes/No	No	Yes/No	Yes/No	Yes/No
Samples/ day (without automation)	30-40	20	50	50	50	20

SNP
GBS
DArTSeq

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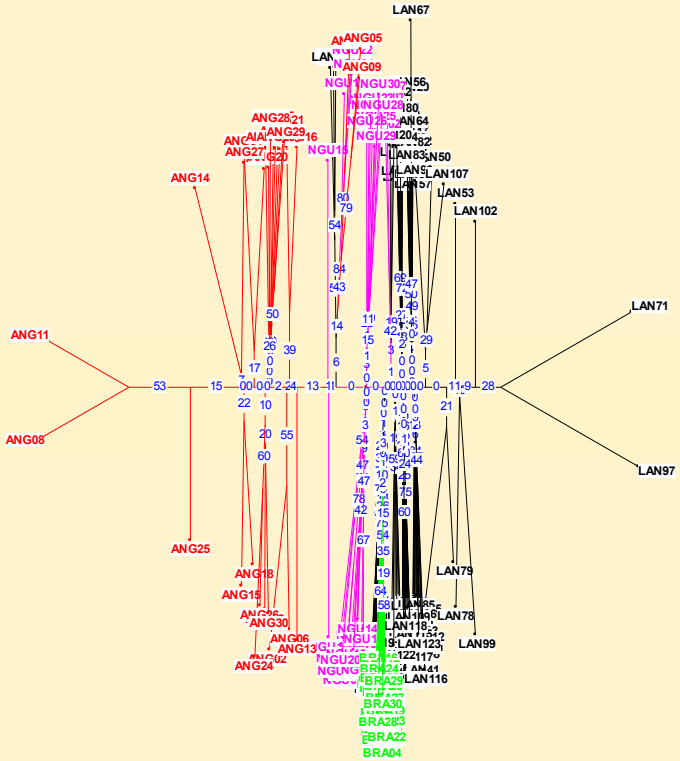
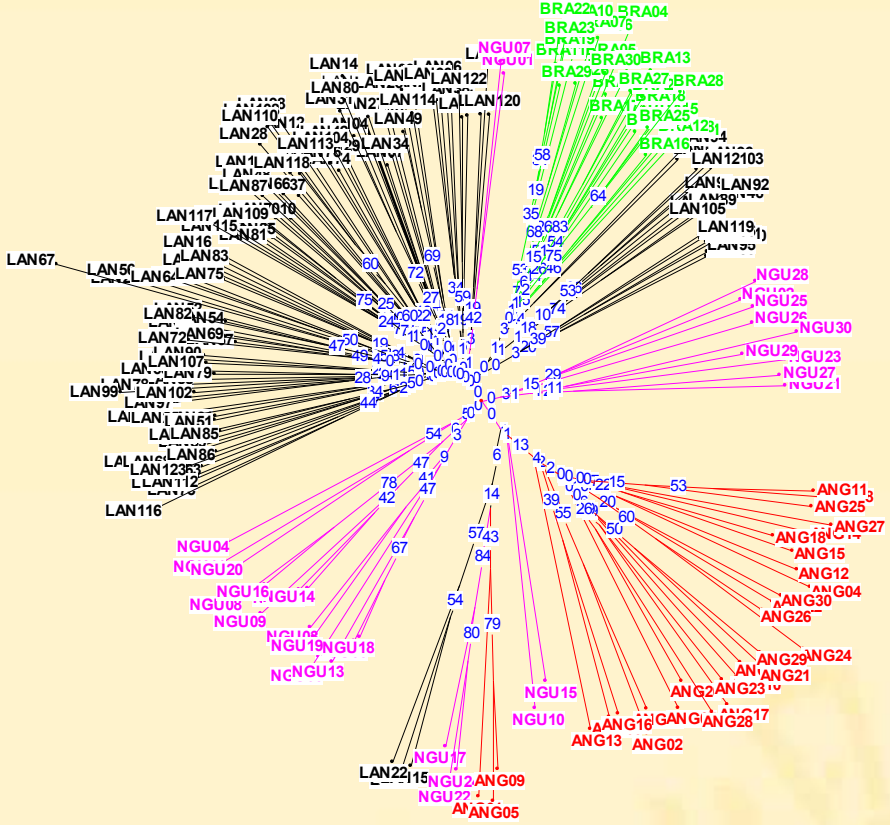


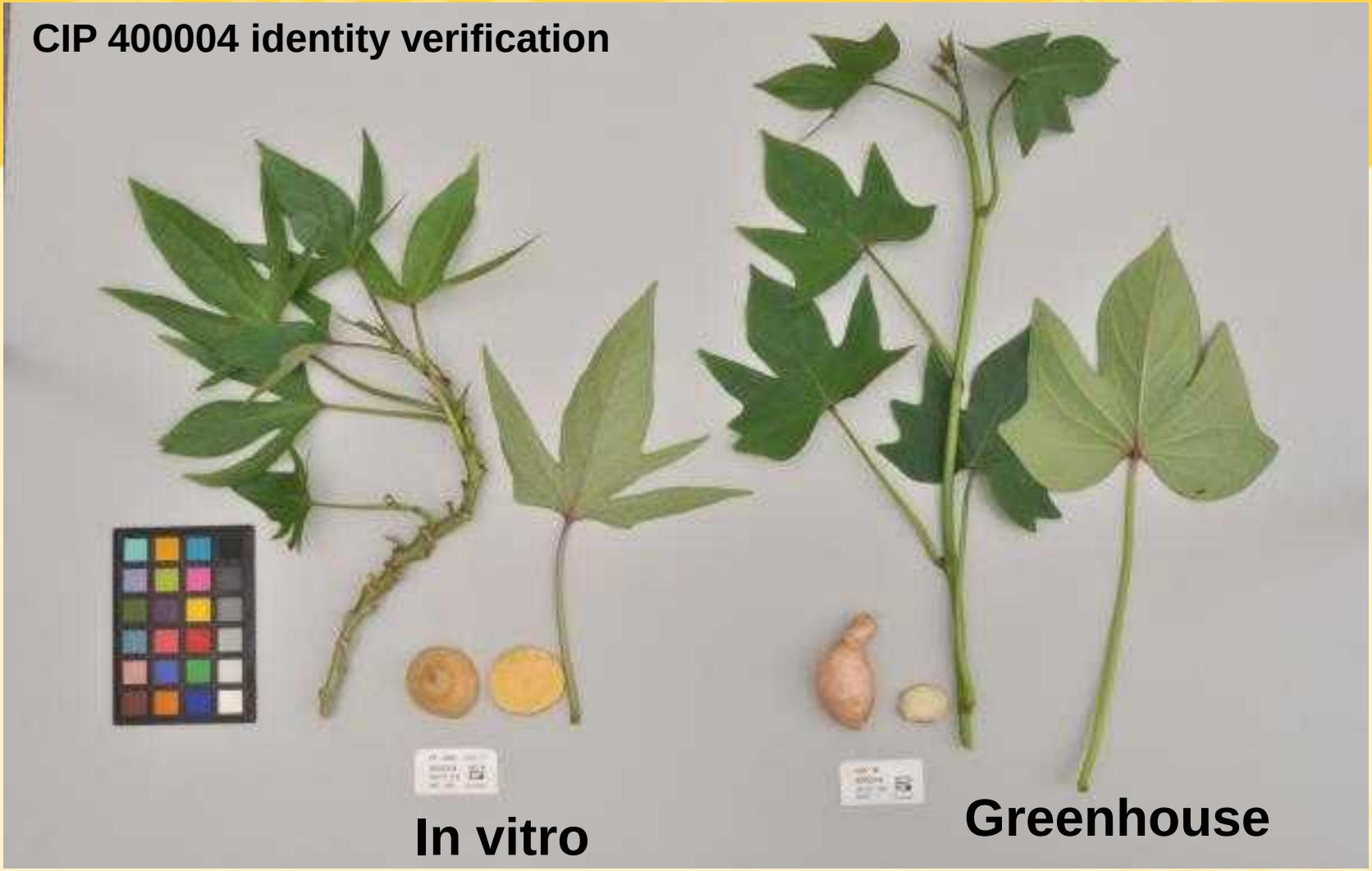


The challenges / The expectations

- RE: Verification of Fake “Orange-Chingova” in Zambia 2/3/2015
- RE: The current problem with CIP 400004 (CEMSA 74-228, INIVIT 104) 9/4/2015
- RE: Urgent issue Naspot 11/ Naspot 1 21/5/2015

Figure: Leaf and root cross section: L “Orange-Chingova” and **R** Orange-Chingova

CIP 400004 identity verification



Greenhouse Lima

Greenhouse CIP Nairobi

???

Overcoming Bottle-Necks

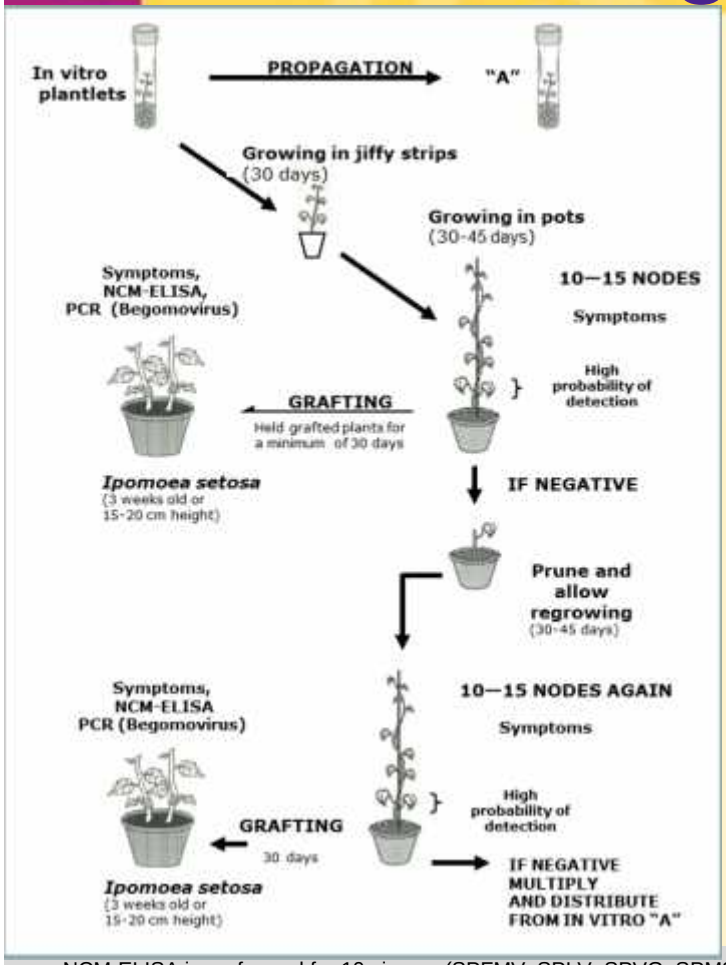
Information management

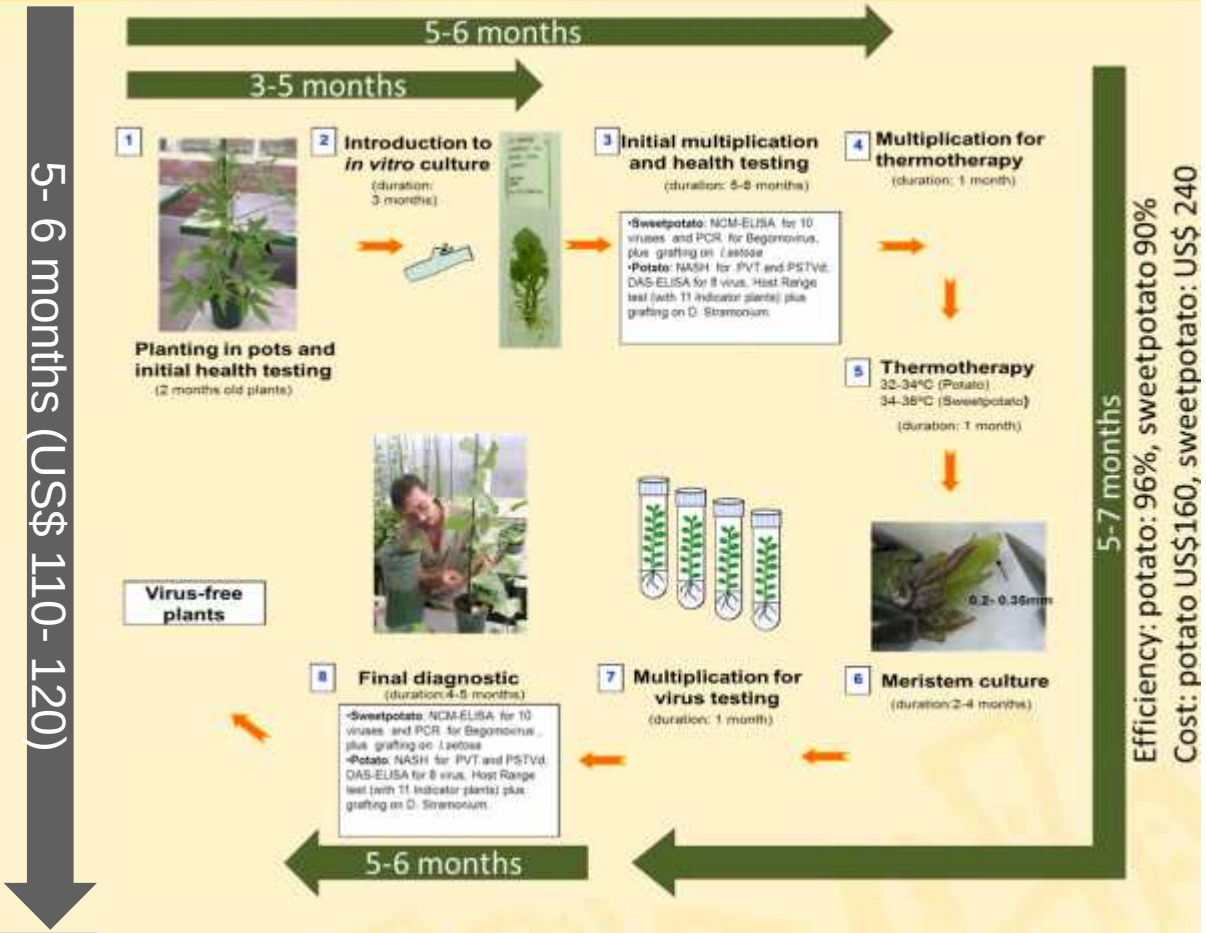
- Standardized data formats
- Tools for accurate, field-based phenotyping
- Data repositories (distributed centralized) - CIPTCL
- Data-analysis & visualization tools
- Support & assistance

Conclusion

- Genetic diversity is basic ingredient of all breeding & conservation of plant genetic resources
- Some specific considerations for *in vitro* conservation
    - Genetic stability and integrity
    - Genetic changes- Somaclonal variation
- Bridging two worldviews
    - Genomes & genes = Germplasm & traits

Virus testing: current methods

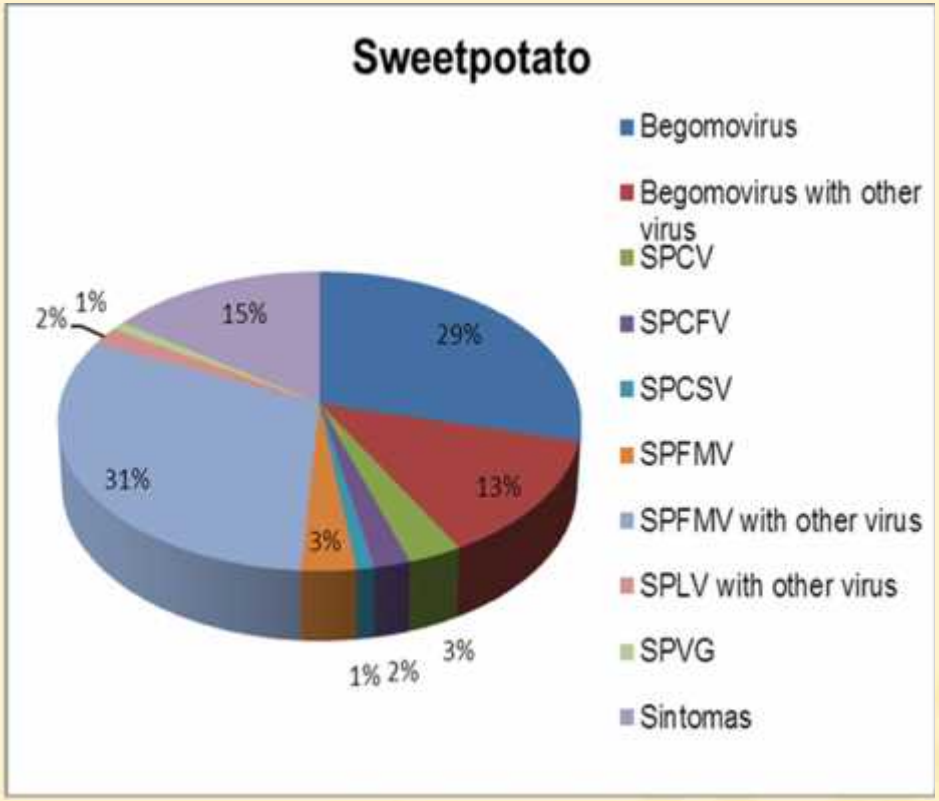




NCM-ELISA is performed for 10 viruses (SPFMV, SPLV, SPVG, SPMSV, SPMMV, SPCSV, SPCFV, SPC6V, SPCV, and CMV).

Virus testing: why such a extensive process

1. Low virus titers in plants = unreliable detection directly from sweetpotato
2. Lack of adequate laboratory tests for some viruses
3. International guidelines for clonally propagated crops



Can we improve the current process?

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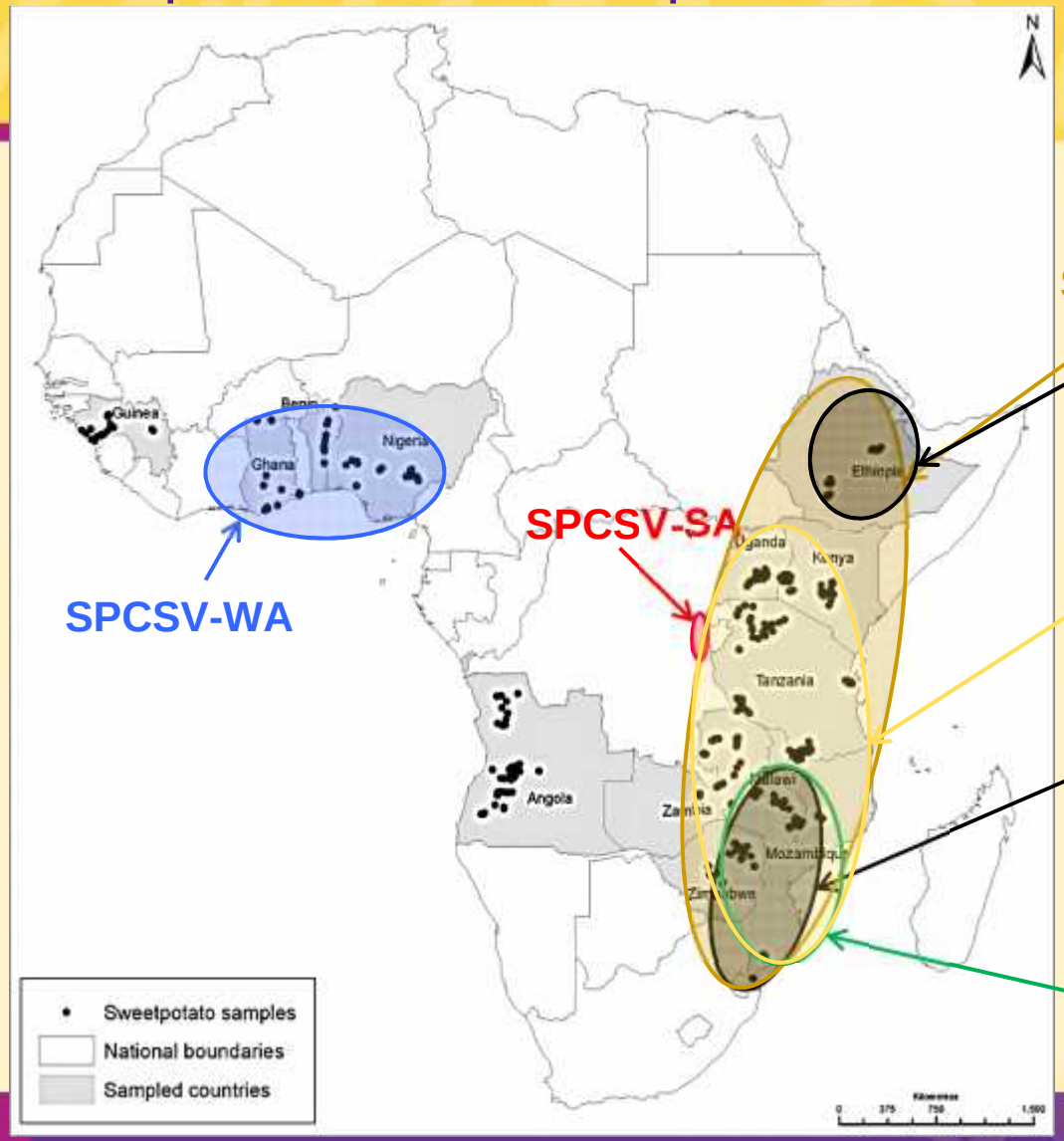
Generic, highly sensitive & fast test directly from in-vitro plants: molecular tests

- PCR/multiplex PCR
- 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



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raw Illumina sRNA reads

SPCSV-EA → SPVG: 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

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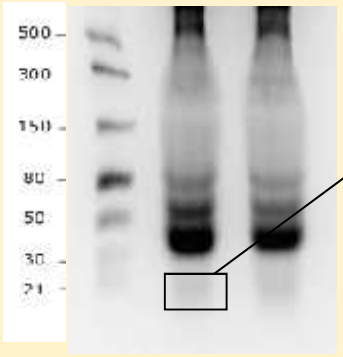
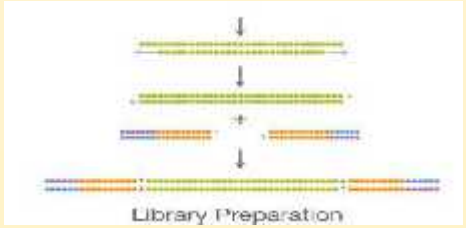
2 weeks, 2x 48 samples

~ 30 US$/sample

Extract RNA & run in 4% agarose gel



Cut and purify 20-30 nt band, prepare library



Send to sequencing provider

3-5 days

<1 US$/sample

Bio-informatics: VirusDetect v1.1

<1 day

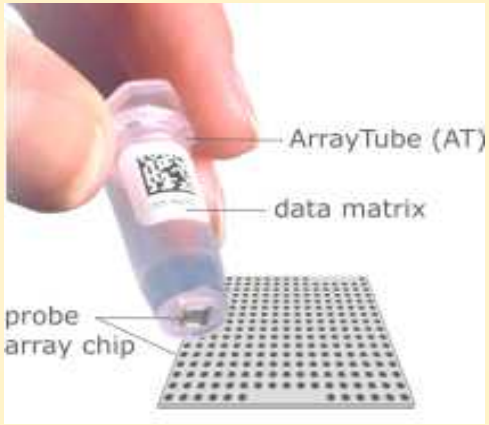
cue + 3 days

21-42 US$/sample

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)



LAMP: addressing the bottlenecks for field use

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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

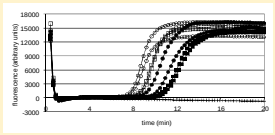
Improving non-instrumented detection methods

colour change/ precipitation

LFD detection

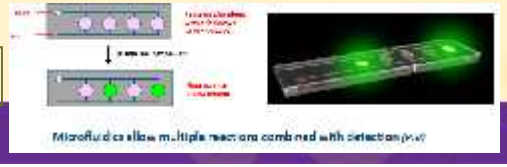
multiplex detection

real-time: Genie



real-time

microfluidics



in the field

Summary

- Current virus testing procedures effective, but time consuming and expensive, slowing down germplasm exchange
- NGS sequencing data contributes to improving primer design for PCR and LAMP, but may by itself be the ultimate generic method
- 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