

# Regional sweetpotato support platforms for virus clean up and testing for the production of clean plants

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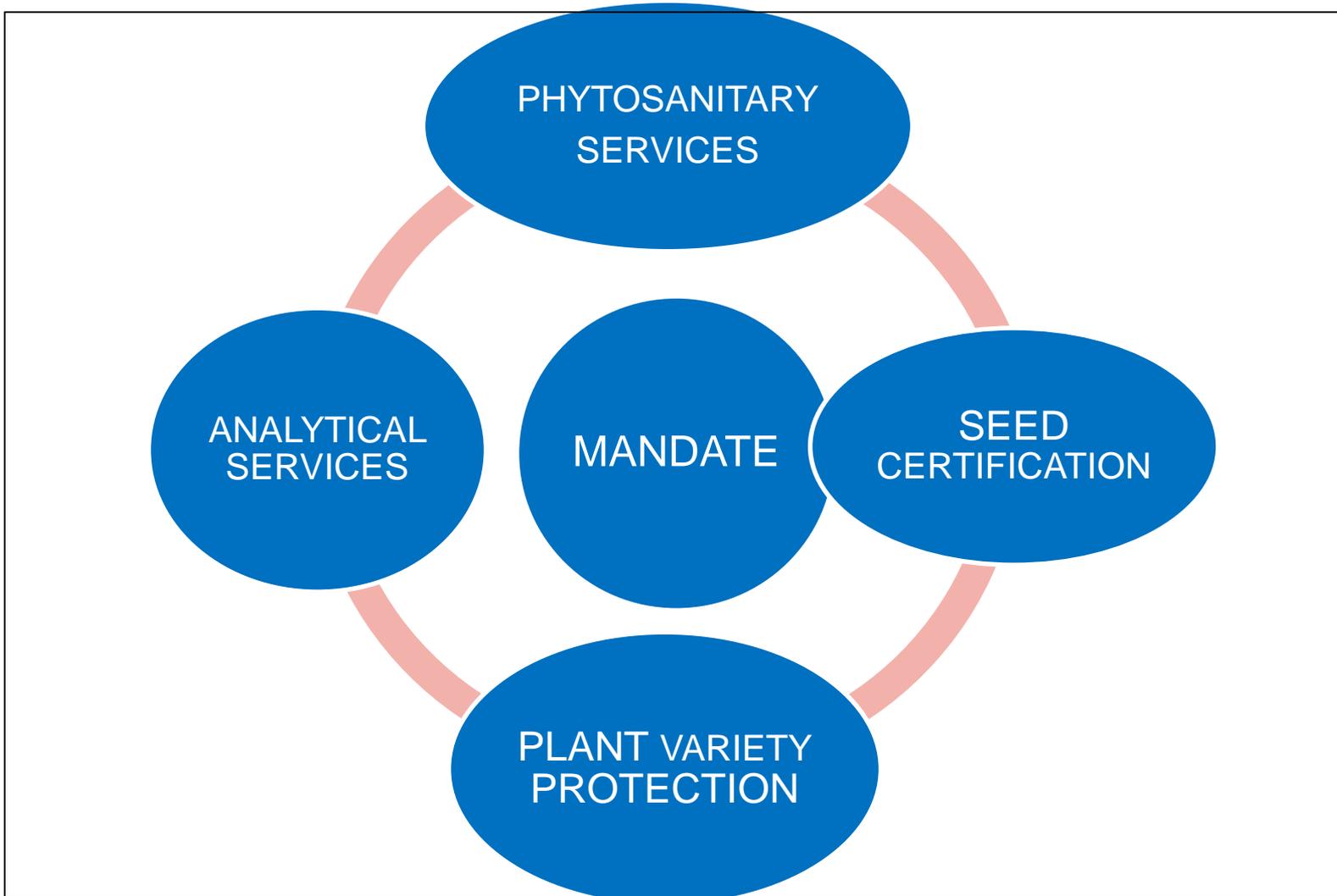


# KEPHIS

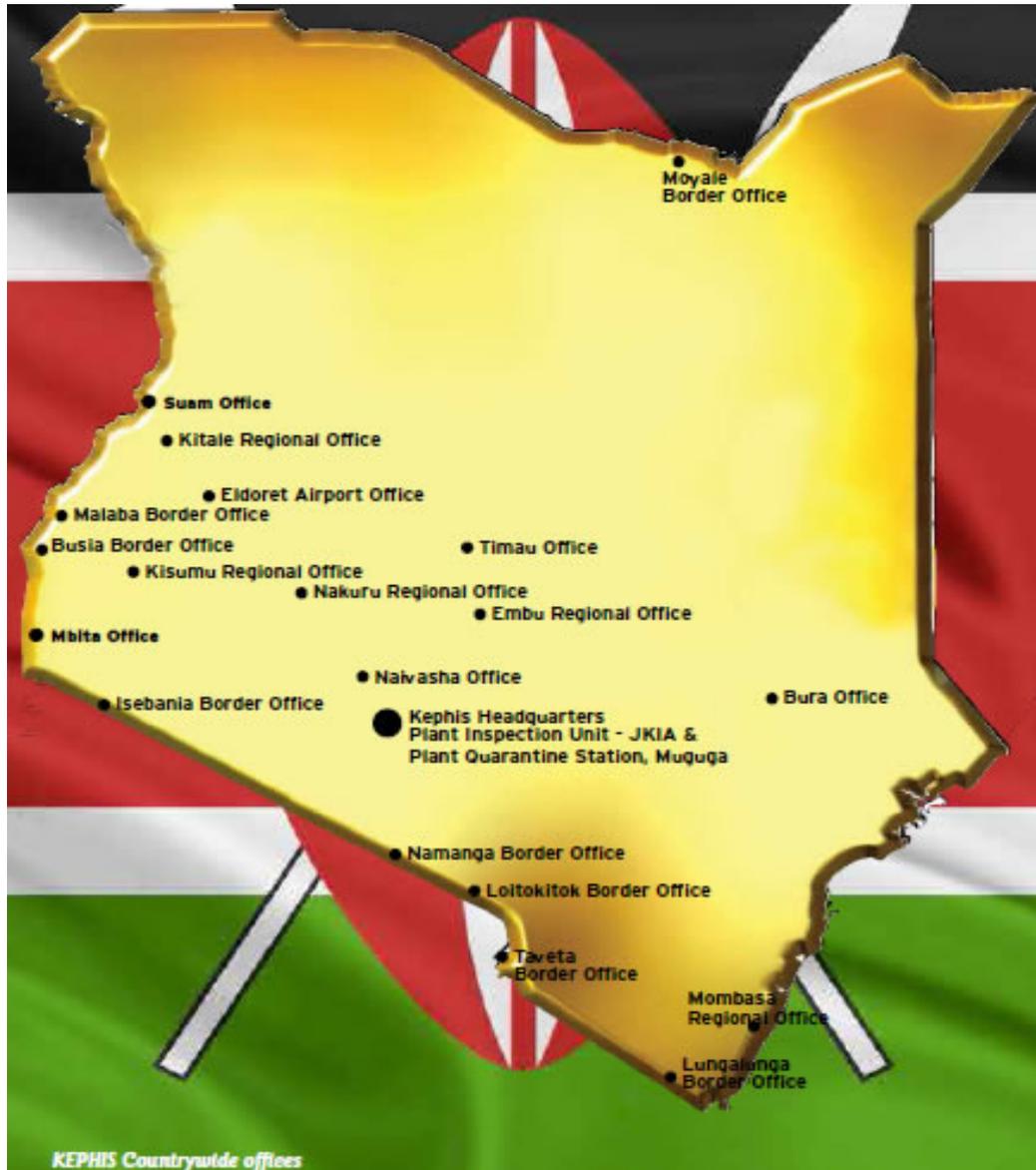


**KEPHIS is a state corporation offers regulatory services in agricultural sector. It is the official NPPO Kenya.**

# SUMMARY OF KEPHIS MANDATE



# KEPHIS offices



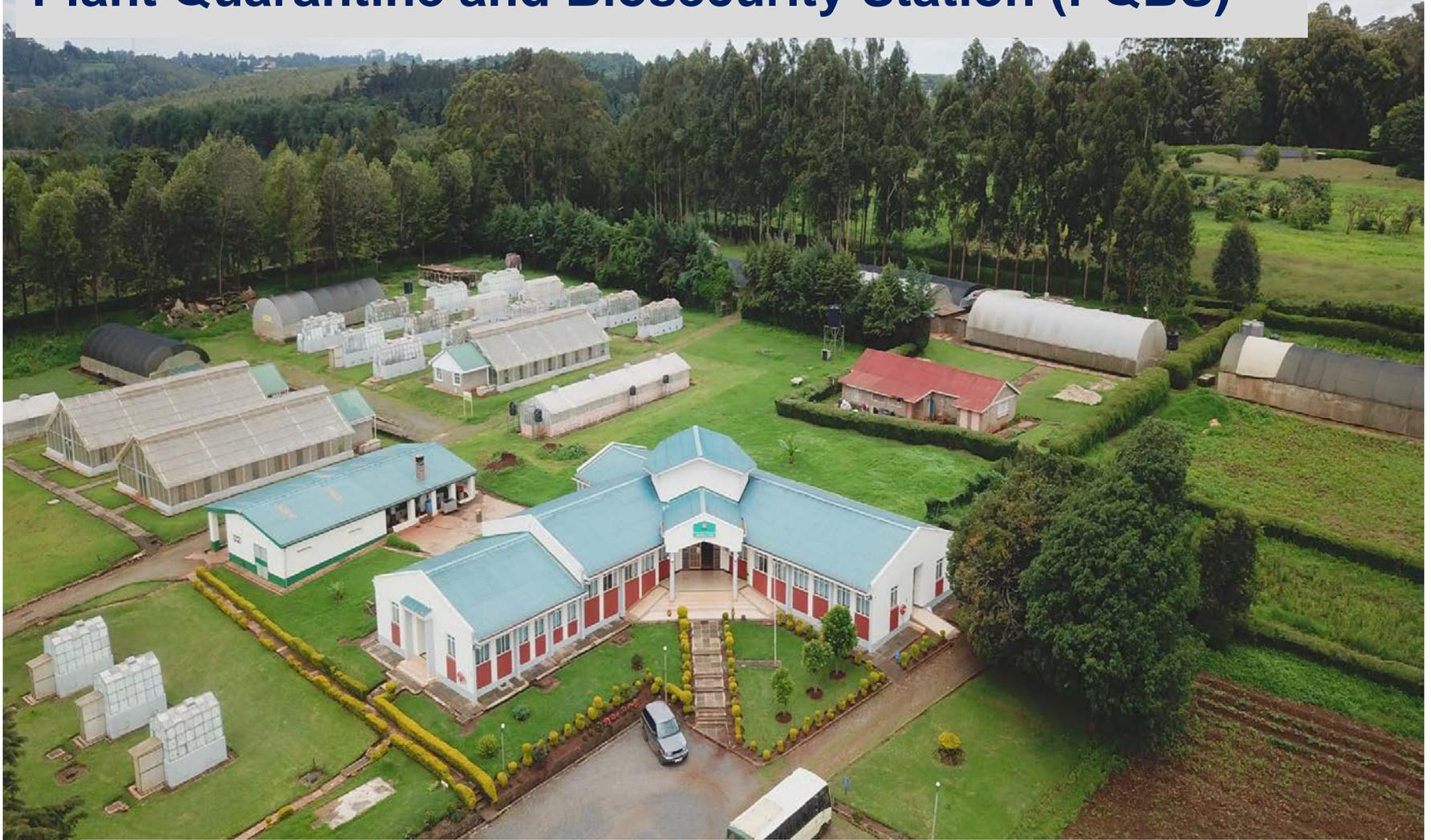
**Headquarter in Nairobi**

**Plant protection**

- **Offices in all major entry points**

- **Quarantine & Bio-security station**

# Plant Quarantine and Biosecurity Station (PQBS)



The PQBS has made efforts to improve infrastructure to facilitate germplasm cleanup and exchange

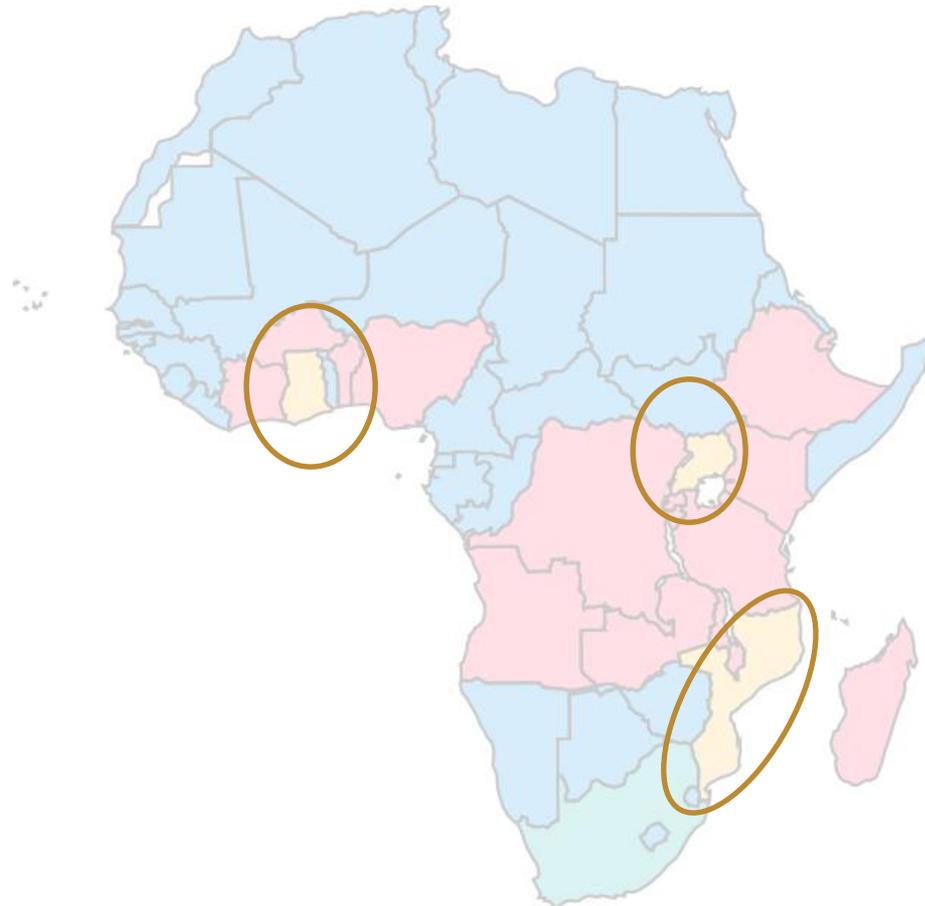
# **Role of KEPHIS-PQBS**

- **Quarantine regulations**
- **Role in pest identification (ISO/IEC 17025:2005 accredited labs)**
- **Regional COMESA reference laboratory for plant health**
- **Offers trainings on virus indexing and clean-up and other programs through COPE (Centre of Phytosanitary excellence)**
- **Role in as centre of excellence in clean germplasm exchange**

# Germplasm management KEPHIS-CIP

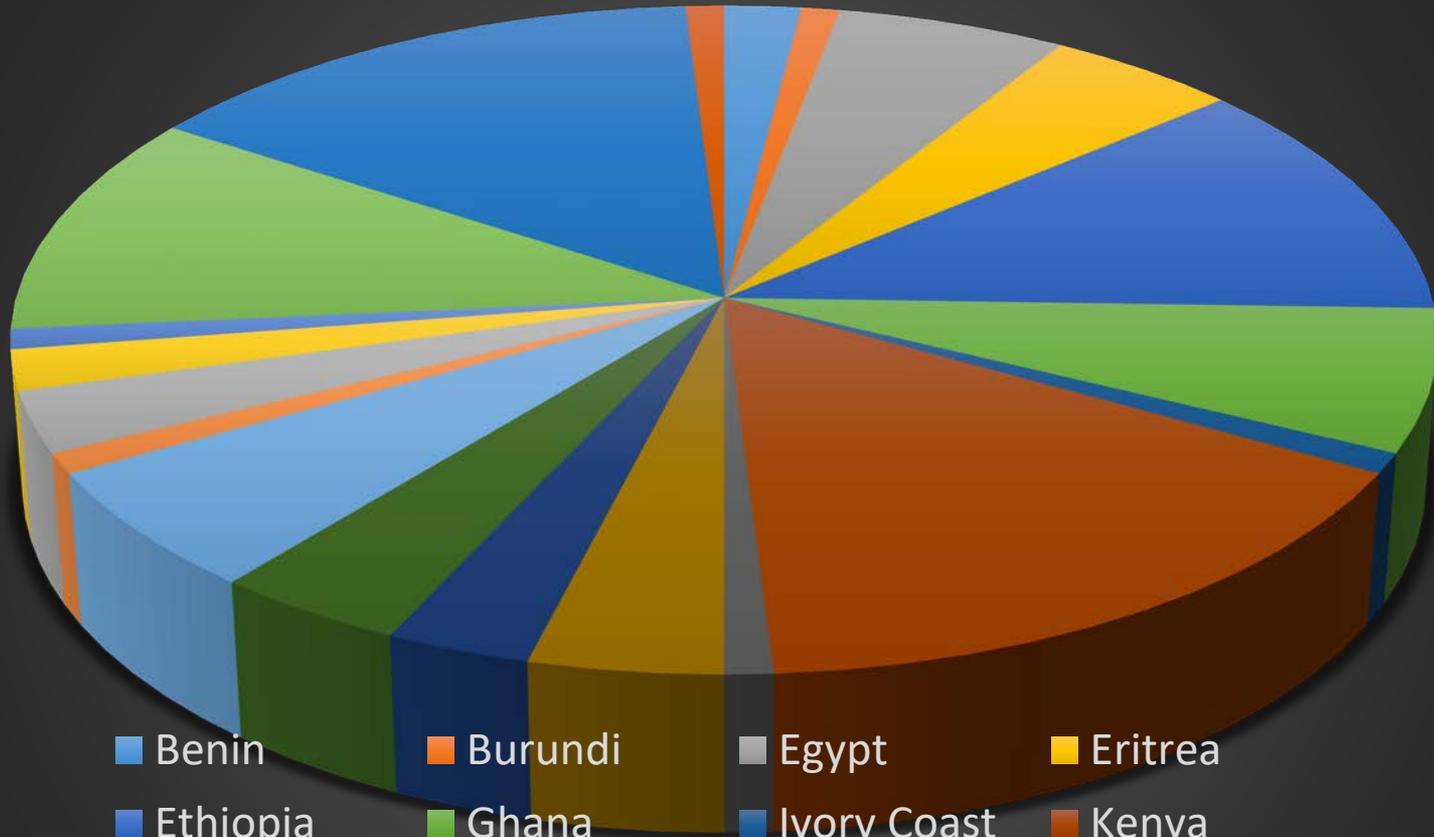
- Collection of 800 Sweetpotato varieties from Africa and around the world
- Conserved as *In vitro* *In vivo*
- Support breeders and researchers by providing superior material for
  - Improvement/evaluation/research
    - Improved nutrition
    - Virus/weevil resistant
    - Drought tolerant/water efficient

# SPHI Participating Countries



● Target Country   ● Others   ● Support Platform   ● Research Training

# CIP\_KEPHIS Sweetpotato distribution in SSA from 2010 to 2016



- |            |            |                |                |
|------------|------------|----------------|----------------|
| ■ Benin    | ■ Burundi  | ■ Egypt        | ■ Eritrea      |
| ■ Ethiopia | ■ Ghana    | ■ Ivory Coast  | ■ Kenya        |
| ■ Liberia  | ■ Malawi   | ■ Mozambique   | ■ Nigeria      |
| ■ Rwanda   | ■ Senegal  | ■ Sierra Leone | ■ South Africa |
| ■ Sudan    | ■ Tanzania | ■ Uganda       | ■ Zimbabwe     |

# What areas are we supporting?

## 1. Challenges of virus testing

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



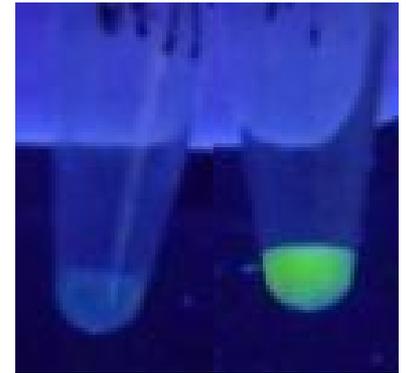


Sweetpotato showing virus various symptoms on var. A-Ejumula

# Improving the current process

Molecular tests are generic, highly sensitive & fast

- PCR/multiplex PCR
- Tube-arrays for sensitive detection of all viruses/pathogens of a crop at once
- Field detection method with high sensitivity and ease of use -> LAMP
- In the future: microfluidic LAMP
- NGS: towards universal viral diagnostics and sequencing



Sequencing by Synthesis

# ClonDiag arrays: sensitive detection of multiple viruses

1

- Multiplex PCR (sensitive) with labeled nucleotides

2

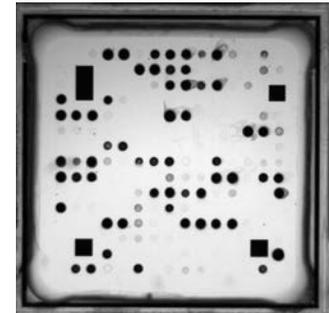
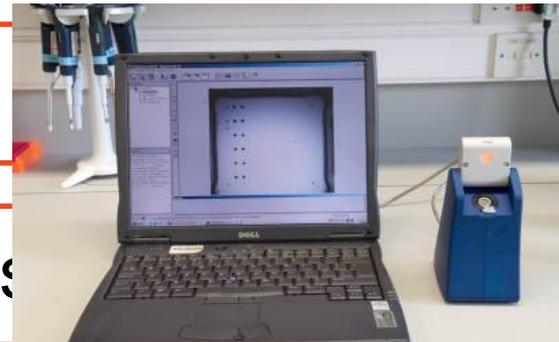
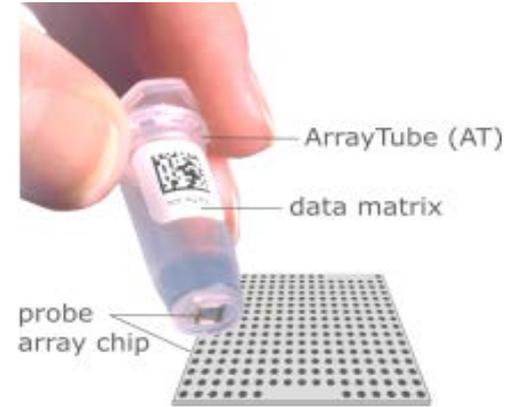
- Hybridization to probes on array

3

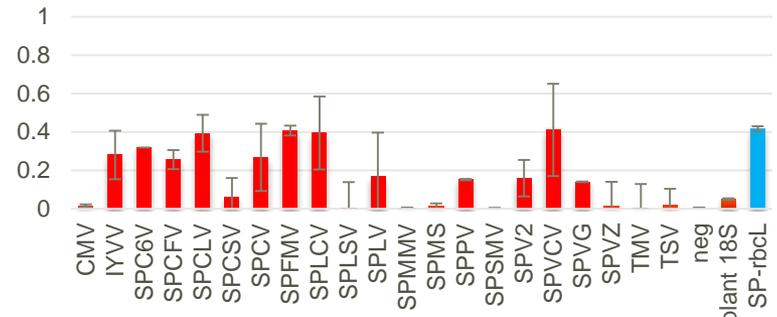
- ELISA

4

- Detection and analysis



Probes and primers designed by FERA



ClonDiag detecting 13 viruses in one sample

# Validation of the 4<sup>th</sup> iteration array

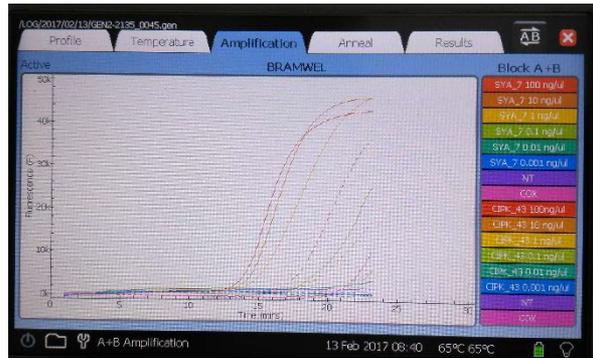
KEPHIS array reader: 100% consistent with biological indexing for infection status

## What have we achieved so far?

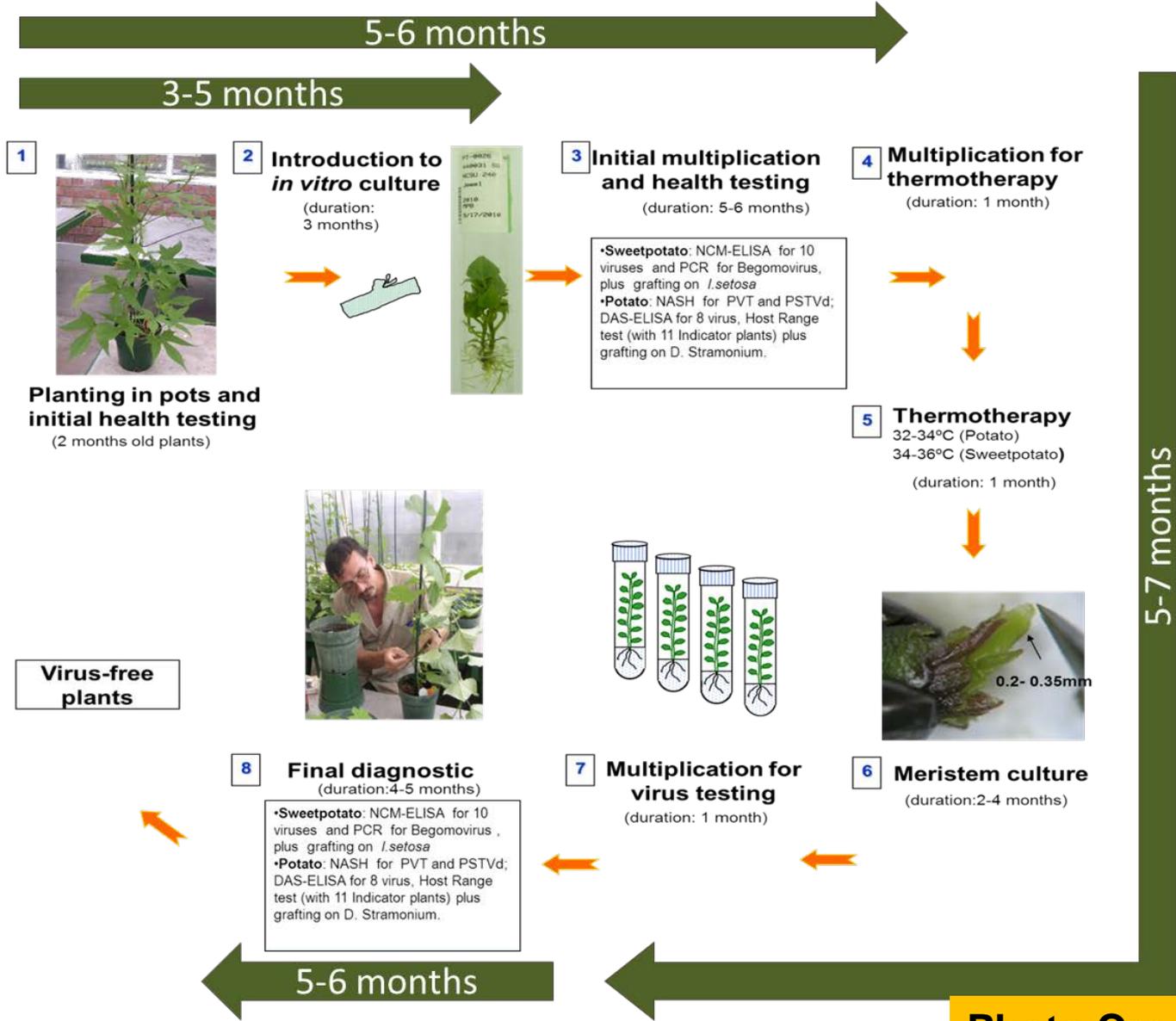
- 1) The present ClonDiag microarray can simultaneously detect all the ten viruses detected by NCM ELISA but also an additional five viruses
- 2) Time to results for grafting/NCM ELISA is 6-12 months while ClonDiag is two days
- 3) ClonDiag costs USD 70 per sample and detects up to 21 viruses while grafting/NCM ELISA costs USD 130 to test 10 viruses per sample
- 4) ClonDiag detected all viruses also detected by indicator host and NCM-ELISA
- 5) The sensitivity of the ClonDiag test is higher than that of NCM
- 6) The ClonDiag test appears to be suitable for routine diagnosis of sweetpotato viruses



# LAMP field testing in Kakamega



# 2. Current Virus clean up process



Efficiency: potato: 96%, sweetpotato 90%  
Cost: potato US\$160, sweetpotato: US\$ 240

# Improving the virus clean up



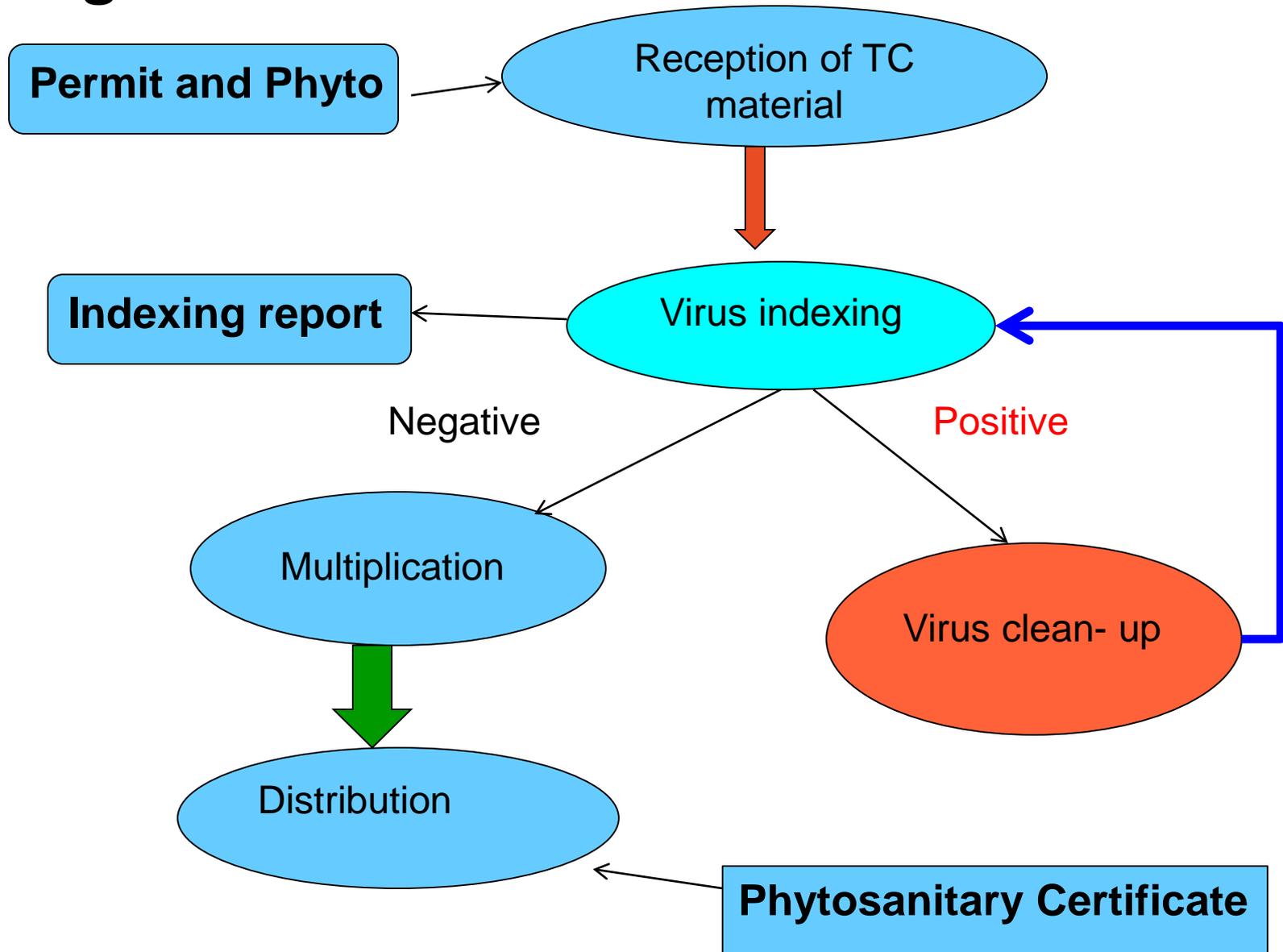
Meristem Tissue culture



Thermotherapy Chamber



# Role in as centre of excellence in clean germplasm exchange



# 3. Capacity building



Tissue culture training

Virus diagnostics training



# 4. Fingerprinting 100 best bet

## Do we keep all the 800 varieties at KEPHIS?

- 1) Coordinate the acquisition of 100 Best Bet Sweetpotato Germplasm in SSA
- 2) Fingerprint
  - 1) Molecular markers-SSRs
  - 2) Phenotypic descriptors
- 3) Virus cleaning, testing and indexing
- 4) Conserve invitro and invivo (double protection)
- 5) Backup copies at Lima Genebank
- 6) Accessible to all researchers/NGOs/farmers

# Countries that have sent in varieties for fingerprinting

Country	Number of varieties	Received	Comments
Burkina Faso	7	7/7	Yes
Burundi	2	2/2	Yes
Cote d'Ivoire	6	2/6	Yes
Ethiopia	3	3/3	Yes
Ghana	13	10/13	Yes
Kenya	6	6/6	Yes
Madagascar	3	0/3	No
Malawi	9	1/9	No
Mozambique	24	24/24	Yes
Nigeria	9	1/9	Yes
Rwanda	4	2/4	No
S. Africa	5	1/5	Yes
Sierra Leone	6	5/6	No
Tanzania	3	3/3	No
Zambia	5	5/5	No
Uganda	11	11/11	Yes



**THANK YOU**