Rapid field based diagnostic tool for the detection of sweetpotato viruses is now a reality

- Extensive testing of LAMP (Loop Mediated Isothermal Amplification) has shown a high sensitivity and specificity in detecting sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), and begomoviruses comparable to using quantitative polymerase chain reaction (RT-QPCR).
- On-site detection of SPFMV, SPCSV, and begomoviruses was simple to perform in different geographical regions.
- LAMP assay has potential as a rapid diagnostic tool for SPFMV, SPCSV, and begomoviruses.
- At a current cost of $6.5 per assay, we anticipate the tool when used with bulked samples, which will be highly cost-competitive compared to biological, serological and molecular techniques.

SASHA Sweetpotato Action for Security and Health in Africa

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Fig 1. Farmers waiting for real time results from the lab under a tree in Lungalunga-Coastal Kenya (Credit: M. Mwandini)

What was the problem?

Current diagnostic tests available for the detection of sweetpotato viruses require expensive laboratory equipment to perform and a high level of experience or are not sensitive enough.

What objectives did we set?

- To develop a thermostabilized field-based LAMP test for SPFMV, SPCSV and begomoviruses that are user-friendly for field diagnosticians.
- To demonstrate that LAMP can be deployed in a non-laboratory situation – providing real-time results, such as in a field setting for use during seed certification or virus incidence survey (Fig. 1).

Where did we work?

The LAMP assay was developed, and the reaction condition optimized at the Kenya Plant Health Inspection Service (KEPHIS) at Muguga and tested in under different field conditions in Kenya.

What did we achieve during SASHA Phase 2?

Parameters optimized included: new primer design for SPCSV/begomoviruses, establishing the effect of

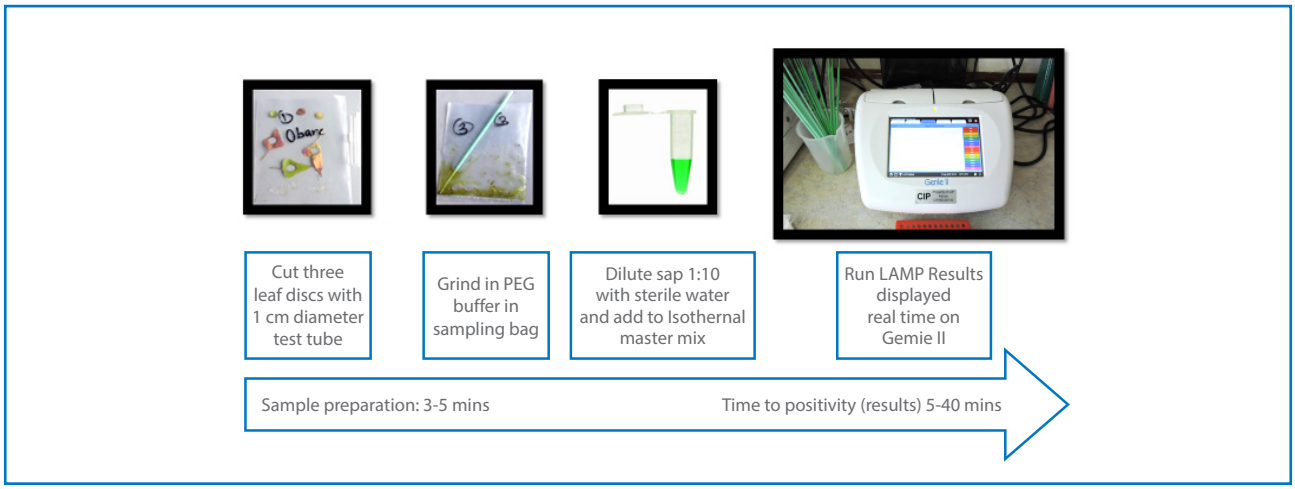

Fig 2. LAMP field sweetpotato virus detection workflow

extraction method on the LAMP assays, sensitivity, specificity, reproducibility and robustness of the assay. Further, we tested the field operability of LAMP assay in four sweetpotato growing regions with different environmental conditions to assess its performance.

We optimized the performance of lyophilized thermostable isothermal master mix with enhanced Reverse transcriptase (RT) activity which is in a ready-to-use form and requires no cold chain.

We adapted simple Polyethylene Glycol (PEG) extraction method that involved macerating leaves in a plastic bag with alkaline buffer and doesn't require sophisticated and expensive Lab equipment (Fig.2). Further, crude extract remains stable relatively long at room temperature (>1 hour) making it possible to test a sample for the three viruses (SPFMV, SPCSV, begomovirus). Furthermore, we designed degenerate primers to be able to detect different begomo species.

We have established that LAMP is equally sensitive and reproducible against more sensitive RT-QPCR in the detection of SPFMV to a concentration of 0.01 ng/ul of sample. Time to positivity for a concentration of 100 ng/ul of the sample was approximately 15 minutes while time to positivity at a concentration of to 0.01 ng/ul of the sample was 30 minutes. This was reproducible between the two extraction methods (PEG and Ambion RNA extraction Kit).

Field testing worked well at the four sites and gave the following times to positivity (TTP): SPFMV 5-20 mins; SPCSV 15-35 mins; and begomoviruses 15-40 mins (Fig.3).

Where there any key challenges or lessons learned?

- SPCSV and begomoviruses have a long TTP and redesigned primers will further improve performance.
- There is a need to lyophilize primers together with LAMP reagents.

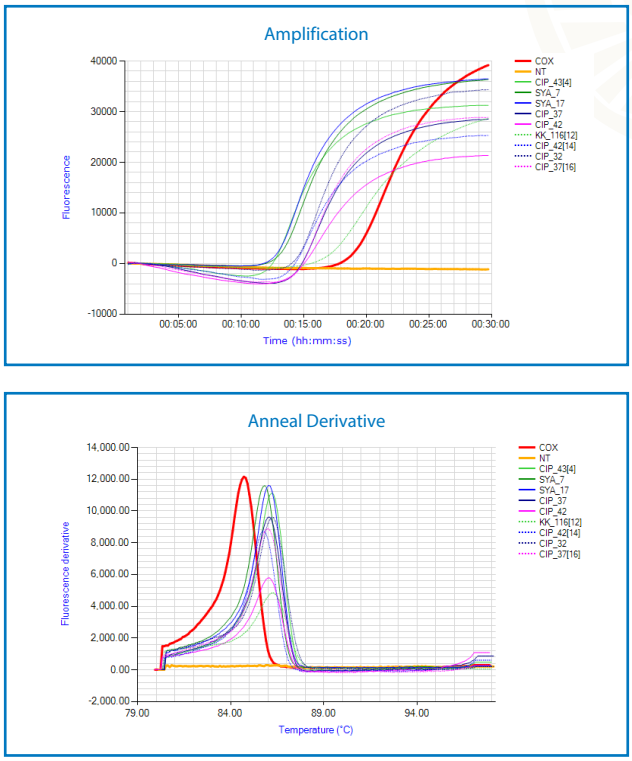

Fig 3. Top - Real-time detection of positive SPFMV samples depicted by the sigmoid curves. Red – COX positive plant control and brown –negative control. TTP of 12-19 minutes. **Bottom** - Anneal derivatives peaks confirming positive and negative samples –. Red – COX positive plant control and brown – negative control.

What's next?

- Evaluate cost and benefits of LAMP assay use by field inspectors.
- Develop a LAMP assay for more sweetpotato viruses.
- In collaboration with regulatory bodies, customize a LAMP kit that is affordable and accessible will ensure a high adoption in the region.

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