SASHA
Sweetpotato Action for Security and Health in Africa

Simple, sensitive loop-mediated isothermal amplification (LAMP) to detect key viruses in the field

AUG 2018

More than 20 viruses have been reported that can pose a major constraint to sweetpotato production. Current diagnostic tests available for sweetpotato viruses are either not sensitive enough to reliably detect viruses directly from sweetpotato or require expensive laboratory equipment to perform and a high level of experience. We demonstrate that LAMP can be deployed in a non-laboratory situation, such as in a field setting for use during inspections for seed certification or incidence surveys.

Fig. 1 Portable LAMP Genie II machine for real time detection of sweetpotato viruses in field

What is the problem?

Crop loss due to sweetpotato viruses can be managed by accurate diagnosis. Several methods have been used for the detection of sweetpotato viruses. Use of indicator plants like *Ipomoea setosa* has been the gold standard for detecting sweetpotato viruses; coupled with nitrocellulose membrane enzyme-linked immunosorbent assays (NCM-ELISA). However, using *I. setosa* takes many months and NCM-ELISA has some limitations in detecting sweetpotato viruses, namely availability of antibody for target virus, limited sensitivity, cost to produce an antibody, requirement of relatively large sample volumes, strain variation and time to complete ELISA to name a few. Currently, many advanced molecular techniques have advantages over NCM-ELISA. However, most of these techniques require an expensive laboratory set up, high technical expertise and expensive reagents (Table 1).

What do we want to achieve?

The aim is to develop a thermostabilized field-based LAMP test for Sweet potato feathery mottle virus (SPFMV), Sweet potato chlorotic stunt virus (SPCSV) and begomoviruses. The test should be user friendly for field diagnosticians. To achieve this, we are currently using room temperature stable lyophilized master mix provided from a commercial company and a simple one step extraction method.

Partners:

- International Potato Center (CIP)
- Food and Environment Research Agency (FERA) in the UK
- Kenya Phytosanitary Inspectorate Service (KEPHIS)

Table 1: Detection methods used for detection of sweetpotato viruses and their attributes

Diagnostic Techniques	Identify virus presence	Identify specific known viruses	Identify new viruses	Repro-ducibility	Time	Skill of Labor	Cost Per Sample
Biological indexing	●	●	●	Poor	3 Months	High	High
NCM-ELISA		●		Good	2 days	High	Moderately high
PCR[1]/RT-PCR		●		Good	4-8 hours	High/ experienced	High
RT-qPCR		●●		Excellent	2-4 hours	High/ experienced	High
LAMP		●●		Good	30-60 mins	High	Moderately high
NGS	●	●●	●●	Good	3 days	High/ very experienced	Very high

[1] Polymerase chain reaction (PCR) is a widely used technique used in molecular biology to exponentially amplify a single copy or a few copies of a specific segment of DNA to generate thousands to millions of copies of a particular DNA sequence. Reverse transcription PCR, or RT-PCR, allows the use of RNA as a template. An additional step allows the detection and amplification of RNA. Quantitative reverse transcription PCR (RT-qPCR) is used when the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA or messenger RNA (mRNA). Next-generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies for DNA and RNA.

Where are we working?

LAMP was tested in lab at FERA, CIP-HQ, KEPHIS and shown to work well with basic nucleic acid extraction methods. Development of simple field extraction method was done at CIP-HQ. Further optimization and new primer design for Sweet potato Chlorotic Stunt Virus (SPCSV)/begomovirus was done at KEPHIS to establish parameters like: sensitivity, specificity, reproducibility and robustness of assay.

How are we going to make it happen?

Currently we are validating the tool in the field. We have successfully detected SPFMV, SPCSV, and Begomoviruses at two sites in Kenya. We are optimizing performance of lyophilized thermostable isothermal master mix with enhanced reverse transcription activity. We propose to target three more regions with different environmental conditions to assess its performance.

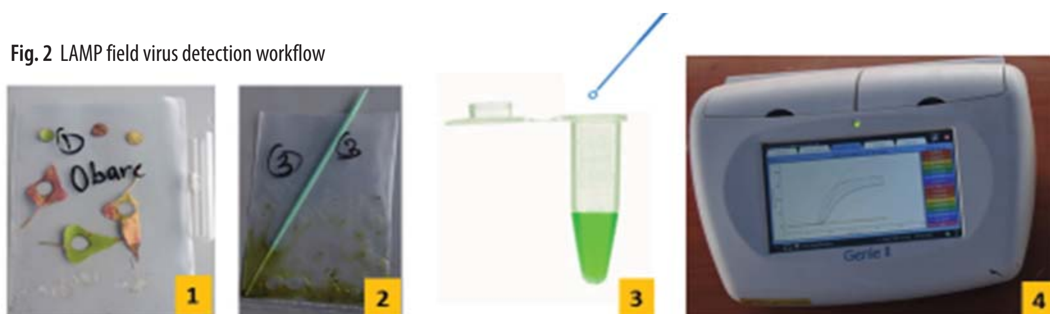
What have we achieved so far?

A simple extraction method for sweetpotato leaves was adopted and involves macerating leaves in a plastic bag with alkaline buffer. Clearly, this approach doesn't require sophisticated and expensive laboratory equipment. The crude extract remains stable relatively long at room temperature (>1 hour) and this is convenient for testing LAMP in the field. We are extensively field testing a thermostabilized LAMP assay for SPFMV, SPCSV and begomoviruses which is in a ready-to-use form and requires no cold chain (Fig. 2).

What's next?

Once validated, the cost of utilizing the LAMP per sample needs to be determined. Ideally, the LAMP would evolve into a commercial kit. The major clientele would be for agents and farmers inspecting "seed" multipliers to determine the quality of the material being produced and/or sold. Over time, more viruses could be detecting using LAMP type systems.

Fig. 2 LAMP field virus detection workflow



LAMP field virus detection workflow

1 Cut three leaf discs	**2** Grind in PEG buffer (sap/buffer)	**3** Dilute sap 1:10 and add to isothermal master mix	**4** Run LAMP - Results displayed real time

CONTACTS

Jan Kreuze (CIP)
j.kreuze@cgiar.org

Bramwel Wanjala (CIP)
b.wanjala@cgiar.org