Improving Virus Diagnostics

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A Loop Mediated isothermal Amplification (LAMP) protocol for Sweet potato feathery mottle virus (SPFMV) was successfully tested in the field in Kakamega, Kenya utilizing a rapid extraction method and partially lyophilized reagents and enabled virus detection on site within 30 minutes. A fourth iteration of another diagnostic tool, ClonDiag arrays, was successfully tested at the Kenya Plant Health Inspectorate Service (KEPHIS) and a validation plan set up.

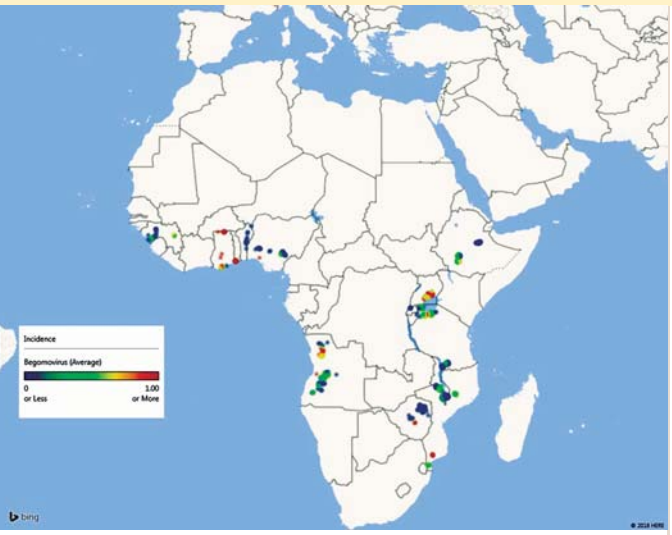

Fig1. Average number (per field) of begomoviruses found infecting each collected sweetpotato sample in different regions of Africa.

What is the problem?

Virus infection, by a number of different types of viruses, is among the most important constraints of sweetpotato production globally and especially in Sub-Saharan Africa (SSA). Among the more than 30 described viruses infecting sweetpotato, *Sweet potato chlorotic stunt virus* (SPCSV) and SPFMV are considered the most wide-spread and devastating, particularly when occurring in combination to cause the sweet potato virus disease (SPVD), which has been reported throughout SSA. A recent project determined the prevalence of different viruses and virus strains infecting sweetpotato and their distribution over the African continent, highlighting the common occurrence of begomoviruses in addition to SPFMV and SPCSV (Fig. 1). This information is essential to enable adequate control of the viruses in each region either through breeding for resistance to the appropriate viruses, production of disease-free or "clean" planting material tested for the appropriate viruses or cultural methods of preventing virus spread in the field.
Diagnostic tests are not available for all viruses and currently available tests are either not sensitive enough to reliably detect viruses directly from sweetpotato, or require expensive laboratory equipment and a high level of experience. Thus, improved diagnostic methods are required.

What do we want to achieve?

We would like to have appropriate diagnostic methods and protocols to detect the most important and prevalent viruses present in sweetpotato. The diagnostic methods developed should be easy to use, not too costly, highly sensitive and able to detect all important viruses, preferably simultaneously. These tools, once developed, can be used to guide breeding and other control strategies to target the appropriate viruses for each country or sub-region, and support phytosanitary processes to prevent the spread of viruses to new areas. We also want to determine the potential impact begomoviruses may have on sweetpotato yields; a study with these viruses has never been done before in the SSA context.

Where are we working?

Surveys for begomovirus detection and next generation sequencing were performed in sweetpotato growing regions of Kenya and will complement those performed previously in other African countries. Begomovirus yield trials will be performed at two locations in Kenya. The diagnostic methods for virus detection are being developed at CIP-Lima, Food and Environment Research Agency (FERA) at York in the UK, and at the KEPHIS – Plant Quarantine and Biosafety Station (PQBS) Muguga. Validation and testing of the diagnostic methods has been done at CIP-Lima, KEPHIS and Mikocheni Agricultural Research Institute (MARI) in Dar es Salaam, Tanzania.

SASHA Sweetpotato Action for Security and Health in Africa

CIP INTERNATIONAL POTATO CENTER A CGIAR RESEARCH CENTER

SPHI Sweetpotato for Profit and Health Initiative
Reaching 10 million African households by 2020

Key Partners

Major partners are the Food and Environment Research Agency (FERA) in the UK, the Kenya Plant Health Inspectorate Service – Plant Quarantine and Biosafety Station (KEPHIS-PQBS) and Mikocheni Agricultural Research Institute (MARI) in Tanzania.

Fig 2. Macerating samples in a plastic bag while performing LAMP assay for SPFMV in the field

How are we making it happen?

We have been using a generic virus detection method developed at CIP called small RNA sequencing and assembly (sRSA) to determine all viruses infecting sweetpotato in SSA: the pan-African sweetpotato virome. We will apply the same method to samples currently collected from Kenya, which was not included previously. In parallel, we have been developing and testing two different diagnostic methods for detecting sweetpotato viruses: micro-arrays in a test tube (ClonDiag arrays) and an isothermal amplification method (LAMP). These two methods have different applications; ClonDiag is able to detect all viruses, but requires laboratory conditions, whereas LAMP detects only single viruses at a time but can be applied directly in the field.

What have we achieved so far?

Virus collections were previously made from 13 countries across SSA and led to the identification of 3,193 viruses from 1,168 samples including the complete or near complete genomes of several new sweetpotato viruses that were previously unknown, or were only known by the symptoms they cause in the indicator plant *I. setosa*. This also revealed the underlying genetic variation of known viruses in distinct geographic regions of Africa, including new strains of Sweet potato mild mottle virus, SPCSV and Sweet potato chlorotic fleck virus.

After analyzing available technologies at the beginning of the project, development of a sweet potato virus micro-array using the ClonDiag system was selected as the best option for a single generic test platform. Four successive iterations of a universal diagnostic sweetpotato virus array were developed and tested, each iteration improving on the previous version, but also adding new viruses (and variants) as they were discovered by sRSA. Currently the array has been tested in CIP-Lima, MARI and KEPHIS. The array will be validated between the laboratories in CIP-Lima and KEPHIS, Muguga using the virus collection set up at KEPHIS. A mobile phone app, programmed in HTML5 for cross platform functionality, was developed for analyzing results from the ClonDiag array.

LAMP assays were also developed for the most common sweetpotato viruses, including SPFMV and SPCSV. LAMP has advantages for virus diagnosis in the field as it works at a single temperature. A simple extraction method for sweetpotato leaves was developed consisting of macerating leaves in an alkaline solution in a plastic bag and using the extract directly for the reaction. A field test was successful in detecting SPFMV within 30 minutes (Fig. 2 and 3). Currently we are focusing our efforts in developing an appropriate format to run and visualize results of LAMP tests under field conditions.

The sweetpotato virologist from CIP-HQ, made six visits to the SSA region to carry out training in sweetpotato virus testing (indexing) and virus elimination (cleaning). Training on thermotherapy, grafting to indicator plants, NCM-ELISA testing, and PCR testing for sweetpotato leaf curl virus (SPLCV) have been conducted in Kenya, Mozambique and Tanzania. FERA provided two courses, one in the UK and one at Biosciences in eastern and central Africa (BecA) hub Nairobi on the use of LAMP and ClonDiag arrays for virus detection. In addition, the CIP virologist provided a course on RNA extraction and bioinformatics sRSA at Makerere University in Uganda and performed RNA extractions for sRSA with scientists at Kumasi in Ghana, and made several personal training visits to MARI and KEPHIS to support ClonDiag array, LAMP and (real-time) PCR implementation.

Fig. 3 Amplification curve on a Genie II instrument showing detection of SPFMV in 5 minutes.

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