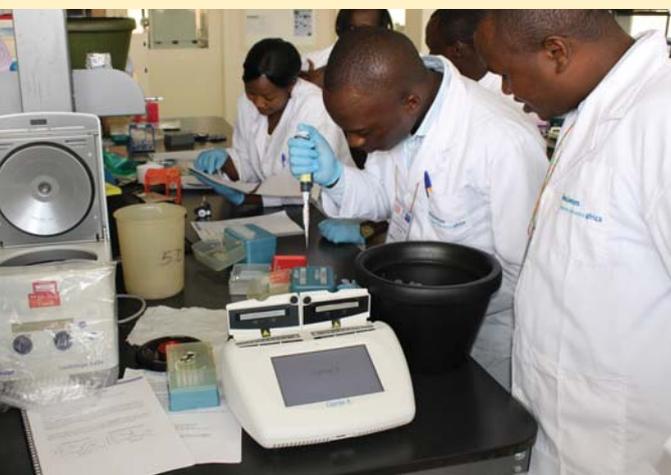


# Improving Sweetpotato Virus Diagnostics

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Genomes of virus species and strains infecting sweetpotato in East, Southern and West Africa have been determined by next generation sequencing. A new diagnostic tool to detect all sweetpotato viruses simultaneously has been developed and will be progressively improved.



■ Training in sweetpotato diagnostics at the BecA hub in Nairobi, using tube arrays and LAMP (credit J. Kreuze)

## ✦ What is the problem?

Virus infection, by a number of different types of viruses, is one of the most important constraints of sweetpotato production globally, especially in Sub-Saharan Africa (SSA). Among the more than 30 described viruses infecting sweetpotato, Sweet potato chlorotic stunt virus (SPCSV) and *Sweet potato feathery mottle virus* (SPFMV) are considered to be the most widespread and are particularly devastating when occurring in combination to cause the sweetpotato virus disease (SPVD). SPVD has been reported occurring throughout SSA. However, beyond a few countries in East Africa, there is no clear information about the prevalence and distribution of different viruses and virus strains infecting sweetpotato. 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 planting material tested for the appropriate viruses and cultural methods 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 to perform and a high level of experience. Thus, improved diagnostic methods are required.

## ✦ What do we want to achieve?

We would like to have an easily accessible distribution map of all relevant viruses and virus strains infecting sweetpotato throughout SSA as well as appropriate diagnostic methods and protocols to detect them. The diagnostic methods developed should be simple to use, not too costly, highly sensitive and able to detect all viruses, preferably simultaneously. These tools, once developed, would 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 relevant viruses to new areas.

## ✦ Where are we working?

Virus surveys for next generation sequencing were performed in Uganda, Mozambique, and Ghana and from a complimentary project in Ethiopia, Kenya, Tanzania, Malawi, Zambia, Zimbabwe, Angola, Nigeria, Benin and Guinea. Virus genome sequencing data is being assembled and analyzed at CIP in Lima, Peru and the diagnostic methods for their detection are being developed at CIP-Lima and FERA at York in the UK. Validation and testing of the diagnostic methods is being done at CIP-Lima and MARI in Dar es Salaam, Tanzania.

## ✦ 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. In parallel we have been developing and testing two different diagnostic methods for detecting sweetpotato



### Key Partners

Major partners are the Food and Environment Research Agency (FERA) in the UK and Mikocheni Agricultural Research Institute (MARI) in Tanzania.

viruses: micro-arrays in a test tube (ClonDiag arrays) and an isothermal amplification method (LAMP). These two methods have different potential application points. The first, ClonDiag arrays, are sensitive and can detect all viruses simultaneously. They require laboratory conditions and are appropriate for use at equipped central hubs for testing and distributing materials. The second, LAMP, can be developed into a diagnostic test for use directly in the field and may thus provide support for obtaining rapid results during field monitoring.

### What have we achieved so far and What's next?

Virus collections have been made from 13 countries across SSA. While many samples are still being processed, results from Mozambique, Ghana, Ethiopia, Malawi and CIPs virus collection have already lead to the characterization of the complete or near complete genomes of several new sweetpotato viruses that were previously unknown, or only were 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 and assessing their merits at the beginning of the project, we concluded that the development of a sweetpotato virus micro-array using the simple Clondiag system would be the best option for a single generic test platform. Three successive iterations of a universal diagnostic sweetpotato virus array have been developed and tested; each iteration improving on the previous version, but also adding new viruses (and variants) as they were discovered in the African sweetpotato virome project. A fourth iteration is planned for the second half of 2014. A mobile phone application, programmed in HTML5 for cross platform functionality, was developed for analyzing results from the ClonDiag array. MARI is currently in the process of validating the array and apps functionality.

LAMP assays were also developed for the two main viruses SPFMV and SPCSV. LAMP is advantageous for field-based diagnosis of virus as it works at a single temperature. A easy extraction method for sweetpotato leaves was developed, consisting of macerating leaves in a simple buffer by grinding them in a plastic bag, or shaking them in a tube containing metal balls. Currently we are focusing our efforts in developing an appropriate format to run and visualize results of LAMP tests under field conditions.



Collecting leaf samples for virus characterization by sRSA in Malawi (credit E. Abidin)

During SASHA Phase 1 (2009-2014), a CIP sweetpotato virologist from headquarters made six visits to the SSA region to conduct trainings 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 both Kenya (KEPHIS PQS) and Mozambique. FERA provided two courses, one in the UK and one at BecA Nairobi on the use of LAMP and ClonDiag arrays for virus detection. In addition, a CIP virologist conducted a course on RNA extraction and bioinformatics sRSA at Makerere University in Uganda and performed RNA extractions for sRSA with a Crops Research Institute scientist in Ghana.



Screenshot of the diagnostic array reader app designed to read results of the sweetpotato virus tube array (credit M. Flores)

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