Improving Sweetpotato Virus Diagnostics

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.

■ Fig. 1 Prototype of Vrius Diagnostic Reader on Smart Phone (credit J. Torres)

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 cholorotic 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 from 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 as part of the SASHA project and in Ethiopia, Kenya, Tanzania, Malawi, Zambia, Zimbabwe, Angola, Nigeria, Benin and Guinea financed by a complimentary project. Virus genome sequencing data are being assembled and analyzed at the International Potato Center (CIP) in Lima, Peru and the diagnostic methods for their detection are being developed at CIP-Lima and Food and Environment Research Agency (FERA) at York in the United Kingdom. Validation and testing of the diagnostic methods is being done at CIP-Lima, Mikocheni Agriculture Research Institute (MARI) in Dar es Salaam, Tanzania and the Kenya Plant Health I nspectorate Service (KEPHIS) in Muguga.

How are we making it happen?

We have been using a generic virus detection method developed at CIP called small RNA

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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 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 Guinea, Ghana, Benin, Nigeria, Angola, Zimbabwe, Malawi, Mozambique, Tanzania and Ethiopia 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, Sweet potato C6 virus, SPCSV and Sweet potato chlorotic fleck virus. After finalizing analysis during the next year, the results will be made available online through geo-referenced maps, including sequence data and pictures of plants.

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 has been developed and tested, each iteration improving on the previous version, but also adding new viruses (and variants) as they were discovered (Fig 2). A fourth iteration is planned for the second half of 2015. A mobile phone application (Fig. 1), programmed in HTML5 for cross platform functionality, was developed for analyzing results from the *ClonDiag* array. MARI has validated the 3rd iteration array under their conditions. A lens system will be developed to ensure adequate quality pictures can be taken with all smartphone models.

▬ **Fig. 2 Red squares indicate where Clondiag virus array still not matching results from RNA sequencing**

LAMP assays were also developed for the two main viruses SPFMV and SPCSV and 6 additional viruses (Fig. 3). LAMP is advantageous for field-based diagnosis of virus as it works at a single temperature. An 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. LAMP assays can be dried down in ready to use reaction tubes and run in a field portable device, in a process of 15-30 min. Validation with field samples from Africa showed SPFMV and SPCSV assays were fit for use in east Africa and we will start testing under field conditions during the next year.

▬ **Fig. 3 Schematic showing LAMP primer design**

▬ **Fig. 4 CIP virologist Jan Kreuze coordinates SASHA virus diagnostics research (credit J. Torres)**