SASHA
Sweetpotato Action for Security and Health in Africa

ClonDiag microarray: An efficient tool for parallel detection of sweetpotato viruses

AUG 2017

The ClonDiag diagnostic tool will facilitate the sustained availability of quality, disease-free planting material, at required quantities and in a timely fashion.

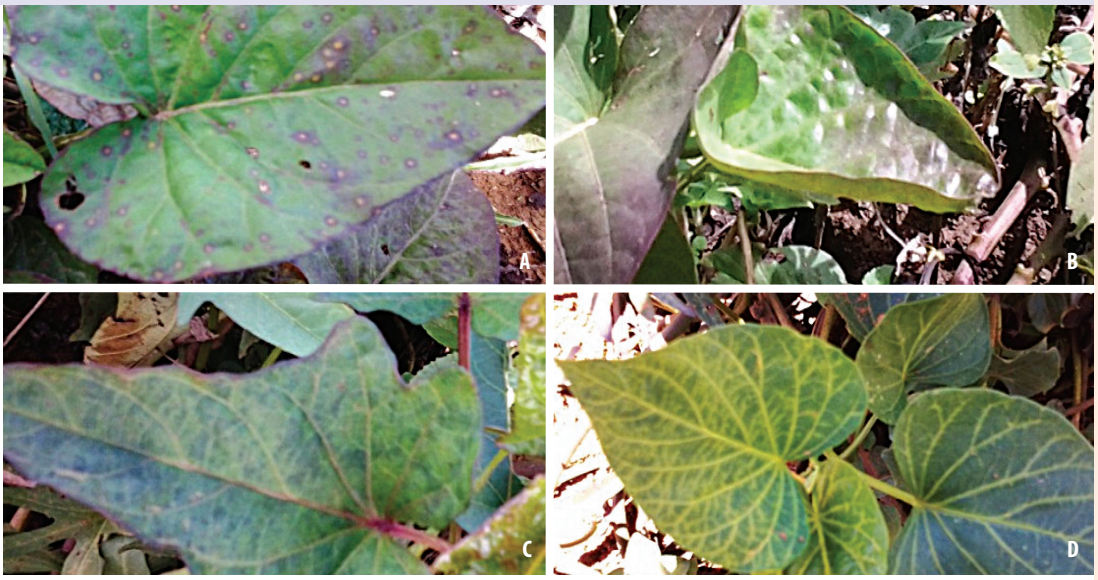

Fig. 1 Different symptom expression in sweetpotato varieties: A – purple rings, B – Roll up, C – mottling and D – chlorosis and vein clearing (credit B. Wanjala)

What is the problem?

Viruses are a major constraint to sweetpotato production (Fig. 1), with more than 20 viruses reported. Viral diseases are economically important and impact on research and movement of germplasm across regions. Low virus titers, uneven virus distribution within the plant, presence of inhibitors, the occurrence of mixed infections, diverse viral strains; make diagnosis of sweetpotato viruses difficult. Current Nitrocellulose membrane ELISA diagnostic tests are not sensitive enough to reliably detect viruses directly from sweetpotato. On the other hand, available molecular tests require expensive laboratory equipment to perform and a high level of experience.

What do we want to achieve?

The current phytosanitary screening/cleaning process requires the introduction of plant material into tissue culture followed by screening for more than ten viruses. The plants undergo two testing regimes; with each round of virus testing taking six months to a year or even more to verify if it is virus-free (Fig. 2). Greenhouse grafting to host plants to confirm the health status make the current process lengthy. We aim at having a test that can significantly reduce this time. In addition, the test should detect the more than ten sweetpotato viruses at one go.

Where are we working?

This work is a collaboration between the Kenya Plant Health Inspectorate Service (KEPHIS)-Muguga, Kenya, the Food Environment Research Agency (FERA), and CIP. Once validation is completed the ClonDiag will be available for use throughout sub-Saharan Africa.

How are we going to make it happen?

An improved iteration of the ClonDiag array was used for inter-laboratory testing conducted between CIP Lima and CIP Nairobi. Twenty-five samples previously tested with NCM ELISA were identified and sub-samples exchanged with Lima. Parallel tests were conducted in Lima and Nairobi, to ensure that

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Sweetpotato for Profit and Health Initiative
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Partners

- Food Environment Research Agency (FERA) - UK
- Kenya Plant Health Inspectorate Service (KEPHIS), Muguga-Kenya

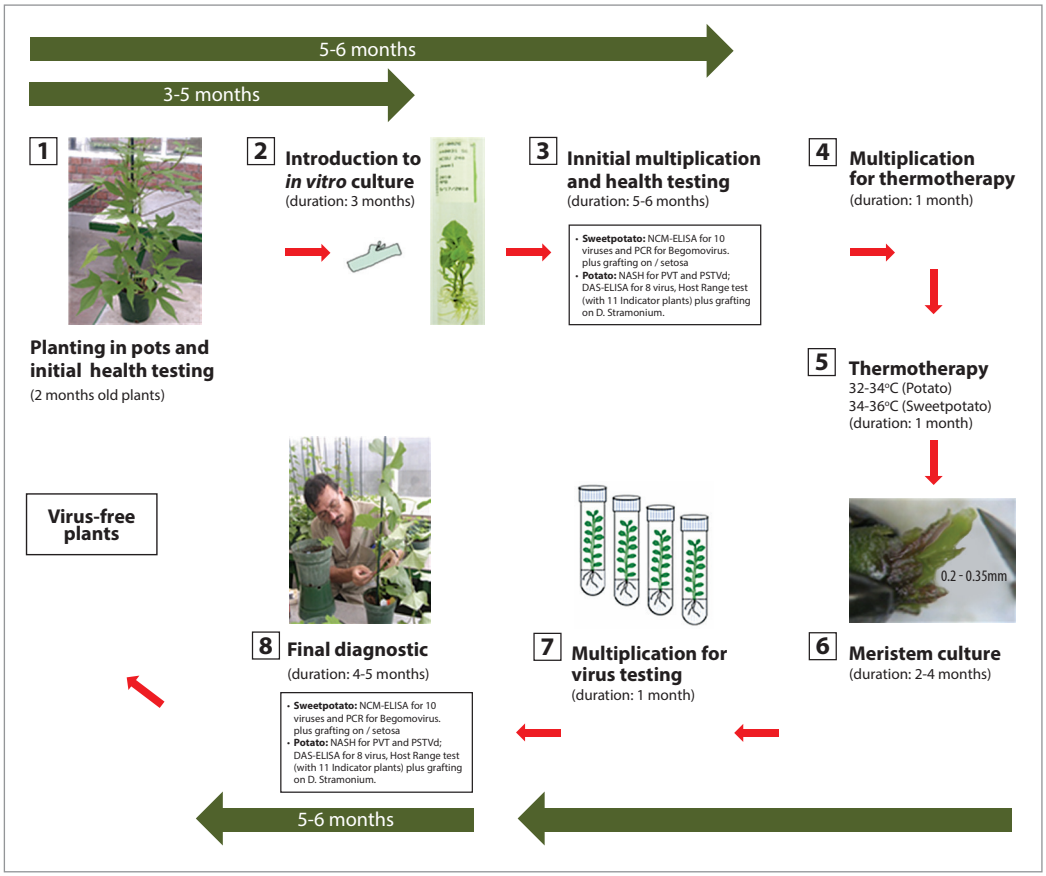

Fig. 2 Steps and timelines for virus clean up and testing by conventional grafting onto *Ipomoea setosa* and NCM ELISA method. Virus clean-up process and current testing by grafting onto *Ipomoea setosa* and NCM ELISA method takes up to 12 months (credit J. Kreuze)

the test meets the criteria of sensitivity, specificity, repeatability and accuracy.

What have we achieved so far?

1) The present ClonDiag microarray can simultaneously detect all the ten viruses detected by NCM ELISA but also an additional five viruses (Fig. 3).
2) Time to results for grafting/NCM ELISA is 6-12 months while ClonDiag is two days.
3) ClonDiag costs USD 70 per sample and detects up to 21 viruses while grafting/NCM ELISA costs USD 130 to test 10 viruses per sample.
4) ClonDiag detected all viruses also detected by indicator host and NCM-ELISA.
5) The sensitivity of the ClonDiag test is higher than that of NCM (Fig. 4).
6) The ClonDiag test appears to be suitable for routine diagnosis of sweetpotato viruses.

What's next?

We will confirm all viruses detected in samples by Next Generation Sequencing. Based on these results, any discrepancies will be assessed and final improvements to the array will be made.

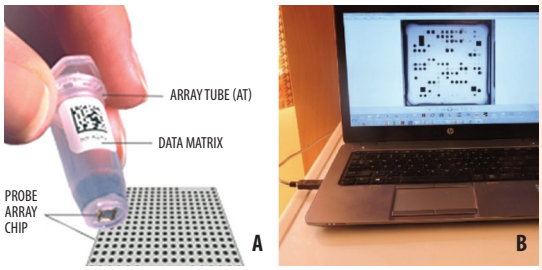

Fig. 3 Microarray tube with 21 probes on array chip (A) and Array tube reader connected onto a computer analyzing virus spots (B)

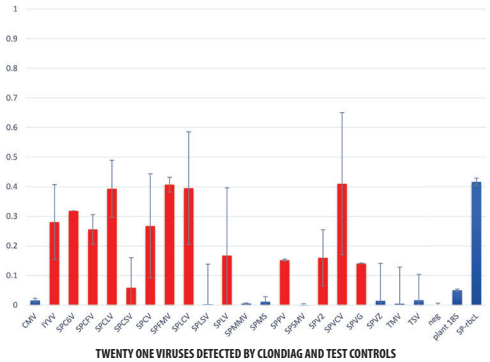

Fig. 4 Parallel detection capability of ClonDiag. Thirteen of the twenty-one viruses detected in single run.

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