Participants taken through the process of setting up LAMP assay in the screenhouse for the detection of sweet potato viruses at KEPHIS Plant Quarantine Biosecurity Station (PQBS) Muguga. Photo Credit: Bramwel Wanjala

Rapid diagnostics tool targets sweet potato viruses

By Murimi Gitari

SWEET potato, is an important food crop in sub-Saharan Africa, not only for food security but also for nutrition and income security. It is grown globally in approximately nine million hectares in 110 countries globally.

However, the production of this food crop has faced a number of challenges, with viruses posing the greatest challenge. There are more than 20 identified viruses. These diseases impact both research and germplasm movement. The insensitivity of existing diagnostic techniques, as well as the necessity for expensive equipment and substantial knowledge, make it difficult to detect, measure, and isolate viruses directly from sweet potato, according to Bramwel Wanjala, a plant biochemist and molecular biologist at the Kenya Agricultural and Livestock Research Organization (KALRO).

The good news is that scientists have developed a new disease tool for sweet potato seed quality management. Known as Loop-Mediated Isothermal Amplification Assay (LAMP), it is a powerful molecular diagnostic technique used for rapid and specific detection of nucleic acids (NAs).

The tool can be used to detect sweet potato feathery mottle virus (SPFMV), sweet potato virus (SPVSV) and begomoviruses related to sweet potato leaf curl virus (SPLCV) with laboratory validation recording 100 percent diagnostic sensitivity for all the three viruses.

"In LAMP, the target sequence is amplified at a constant temperature of 60-65 ˚C with two or three sets of primers and a polymerase with strong strand displacement activity as well as replication activity. Typically, four separate primers are used to amplify six unique sections of the target gene, increasing specificity. An additional pair of "loop primers" might hasten the process," Mr Wanjala says.

He adds that the current diagnostic procedures are not sensitive enough to detect viruses directly from sweet potato and need the use of expensive laboratory equipment as well as extensive knowledge. LAMP is very sensitive and specific for both

DNA and RNA amplification; it is inexpensive, and its properties make it potentially appropriate for on-site tests.

LAMP tests have been developed for many sweet potato viruses, including SPFMV, SPVG, sweet potato chlorotic stunt virus (SPCSV) and SPLCV (and related sweepoviruses). Recent research has constantly shown that the potyviruses SPFMV and SPCSV are the most common and harmful viruses globally.

"The LAMP assay has the potential to be more cost-effective, quicker, sensitive, and need less sample processing than traditional laboratory assays. This makes it suited for detecting plant viruses on-site or in the field. LAMP assay can be conducted with persons with limited molecular biology experience as it easy to perform. This has been supported by inspectors from National Plant Protection Organizations (NPPOs) in Kenya, Uganda, and Tanzania who have undergone training on the onsite use of the LAMP assay. Plans are underway to conduct inter-lab validation and packaging it into affordable kits to ensure high adoption in sub-Saharan Africa," Mr Wanjala says.

He notes that the LAMP assay can be used for field surveys and monitoring of the phytosanitary status of pre-basic seed production under quarantine or in a certification program. This will ensure that pathogen-free plant material enters the seed system.

There are however a few challenges in using LAMP assays in field settings that include: sample preparation (DNA/RNA extraction), LAMP reagents as they require utilization of cold storage to protect the integrity and stability of reagents, using a stable power supply, and detecting LAMP products.

"The study adopted alkaline polyethylene glycol (APEG) quick extraction method that negates the need of RNA/DNA purification that is difficult to perform under field conditions. We used thermostable lyophilized reagents in Genie strip tubes, which obviated the need cold chain storage. All LAMP assays results were detected real-time on a rechargeable, portable Genie® II (OptiGene Ltd., UK), that has an inbuilt battery that can last for up to 6 hours," he highlights.

Mr Bramwel Wanjala conducting real time detection of sweetpotato viruses in the field at KALRO Kiboko. Photo Credit: Bramwel Wanjala