ABSTRACT

Cassava is an important food crop in tropical and semi-tropical regions. Its production in East and Central African regions has been devastated by Cassava brown streak disease (CBSD), caused by two distinct virus species, *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV), both being referred to as CBSVs. Different control methods have been adopted, with the use of clean planting materials being the most common since there are no resistant varieties available in the market. Use of clean planting materials requires availability of effective virus detection methods. Several molecular detection techniques with different costs and efficacy are used to detect CBSD-causative viruses including reverse transcription-polymerase chain reaction (RT-PCR), real time-polymerase chain reaction (qRT-PCR), loop-mediated isothermal amplification (LAMP). However, these methods are expensive; require specialized laboratories and highly skilled manpower. A highly efficient and cost effective detection method is needed for daily screening of CBSVs in cassava planting materials.

In this study, TAS-ELISA technique using monoclonal antibodies (MAbs) and mixed CBSVs-antibodies was validated for detection and discrimination of CBSVs by determining their specificity and sensitivity in comparison to RT-PCR, which was used as a gold standard technique. Validation also involved determination of the cost and cost effectiveness ratios of both techniques for routine detection of CBSV and UCBSV in cassava. Screenhouse samples were used in determining the sensitivity of MAbs in ten-fold dilutions, best leaf position for reliable detection of CBSVs and specificity. Cost effectiveness of the two diagnostic techniques and the specificity of monoclonal and mixed antibodies were also determined using field samples.

RT-PCR was more sensitive in less diluted samples detecting the CBSV viruses than TAS-ELISA technique which detected 100% positive samples from 1:20 to 1:1:10-4 w/v. The specificity of the antibodies showed that both monoclonal antibodies (CBSV and UCBSV MAbs) had the highest virus mean absorbance of 1.634 nM and 1.1173 nM respectively, in co-infected samples. The combined antibodies had a specificity of 100% in both virus species. The sensitivity of antibodies in different plant positions was higher in low plant leaves in both MAbs but most in UCBSV-MAbs with 64.7%. Cost-effectiveness of both CBSD diagnostic techniques had 452.06 and 558.98 US$ for TAS-ELISA and RT-PCR respectively, while analyzing 100 cassava leaf samples. This means that TAS-ELISA was cheaper and can analyze 24 samples more than RT-PCR. However, RT-PCR assay was 100% effective compared to TAS-ELISA with 60.8% and 59.09% true positive detection for CBSV and UCBSV-MAb, respectively. TAS-ELISA took more time than RT-PCR to analyze the same number by a difference of 10 hours and 30 minutes. Moreover, TAS-ELISA had least cost-effectiveness ratio of 7.53 US$/% effectiveness than 5.58 US$/% effectiveness for RT-PCR.

This study found that CBSVs MAbs in TAS-ELISA was more sensitive below the manufacturer recommended ratio. The monoclonal antibodies were less specific compared to CBSVs mixed monoclonal antibodies and RT-PCR technique. The later technique was more specific in discriminating the two virus species than TAS-ELISA using MAbs. The lower cassava plant leaves
are the best positions sampling for CBSVs detection when using CBSVs-MAbs in TAS-ELISA. RT-PCR technique is most cost-effective than CBSVs-MAbs for reliable detection of the viruses on routine basis, CBSVs-MAbs using TAS-ELISA can be used where there is no access to RT-PCR.