ABSTRACT

Background: Cassava leaf samples can degrade quickly during storage and transportation from distant areas. Proper sampling and the use of efficient cheap storage methods are critical to obtain sufficient quality of DNA and RNA for plant virus epidemiology and to improve understanding on disease control. This is practical when samples are collected from isolated zones distant from a laboratory, or in developing countries that lack a supply of money and materials for virus diagnostics.

Results: The effect of sample storage duration on nucleic acid (NA) quality on virus detection was investigated in this study. A simple, rapid, and cost-effective CTAB-based approach (M3) for single NA extraction was optimized and evaluated together with two existing CTAB-based methods (M1 and M2) for extraction of NA from fresh and herbarium cassava leaves stored at; 1, 8, 26, and 56 months. The quantity of DNA and quality of both DNA and RNA were evaluated using Nanodrop 2000c UV–vis Spectrophotometer and agarose gel electrophoreses, and the rate of sample degradation was estimated using a simple mathematical model in Matlab computational software.

The results show that there is no significant difference between M1 and M2 in the mean concentration of DNA but there was a significant difference between M3 and the other two methods at $p < 0.005$. The mean concentration of DNA extracted using M3 was higher at 1 and 8 months of age. M3 and M2 produced high concentrations at the age of 26 and 56 months. Using a developed scale for quality score, M3 and M2 produced high-quality DNA from fresh samples. All methods produced poor-quality DNA and RNA at the age of 8 and 26 months and no visual bands at the age of 56 months. Statistically, there was a significant difference in the mean quality of DNA between M1 and M2 but there was no significant difference between M3 and the other two methods at $p < 0.005$. However, Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) were readily detected by RT-PCR from RNA isolated using M3. The quality of DNA declined per storage time at the rate of 0.0493 and 0.0521/month while RNA was 0.0678 and 0.0744/month. Modified CTAB extracted a sufficient amount of NA of high quality for a third of the time (28/95min) compared to the existing two methods.

Conclusion: Our method will provide the cost-effective, quick, and simple processing of fresh and dry samples which will quicken and guide the decision process on when and what type of sample to process for plant disease management and surveillance actions.