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Research Application Summary

Evaluation of cassava genotype response to hot water therapy for efficient cassava virus elimination

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Abstract

Cassava Brown Streak (CBSV) and Cassava Mosaic Viruses (CMV) are serious constraints to cassava production. In this work, the rate of shoot regeneration and disease incidence on shoots following hot water therapy as a technique for virus elimination was evaluated. RT-PCR was used to detect the presence of CBSV and CMV using the CTAB extraction protocol. Stem cutting of cultivars whose leaves were confirmed positive with RT PCR were subjected to hot water treatment at different temperature ranges and time intervals. Shoot regeneration and symptom appearance after thermotherapy treatment was studied and analyzed. All the shoots tested positive for both CMV and CBSV, with 40% exhibiting co-infected with both CBSV and CBSV-Ug.

Key words: Cassava brown streak, Cassava Mosaic, hot water therapy, shoot regeneration, virus elimination

Résumé

La striure brune de manioc (Cassava Brown Streak (CBSV) et le virus de mosaïque de manioc (Cassava Mosaic Virus (CMV) sont de sérieuses contraintes à la production de manioc. Dans ce travail, le taux de régénération des pousses et l'incidence de la maladie sur les pousses après un traitement à l'eau chaude comme une technique pour l'élimination du virus a été évaluée. La RT-PCR a été utilisée pour détecter la présence de CBSV et de CMV en utilisant le protocole d'extraction CTAB. La tige coupée des cultivars dont les feuilles ont été confirmées positifs à la RT PCR ont été soumis à un traitement de l'eau chaude à différentes catégories de température et des intervalles de temps. La régénération de pousses et le symptôme d'apparence après le traitement de thermothérapie ont été étudiés et analysés. Toutes les pousses ont été testés positifs à la fois pour le CMV et le CBSV, avec 40% présentant une co-infection avec les deux CBSV et CBSV-Ug.

Mots clés: Manioc striure brune, Mosaïque de manioc, thérapie de l'eau chaude, pousse de la régénération, l'élimination du virus

Background

Cassava production and productivity is affected by viral diseases notably cassava Mosaic and Cassava brown streak perpetuated through propagation of infected stem cuttings and whitefly vector (*Bemisia tabaci Gennadius*) (Legg and Fauquet, 2004; Alicai *et al*., 2007; Mware *et al.*, 2009; Ogwok *et al.*, 2015). Conservation of disease free cassava germplasm for the foreseeable future serves the most effective and reliable way of reserving propagation material with desired traits before they become extinct. Development of a healthy phytosanitary measure and conservation strategy to free and conserve the cassava germplasm is therefore important (Anegbeh *et al.*, 2004). The use of Heat therapy on vegetatively propagated plants is a techniques that has reported success in virus elimination. (Faccioli and Marani, 1998). The high temperatures in thermotherapy hinder virus replication by making surrounding conditions unfavorable for viral survival (Walkey, 1976). The present study evaluates the rate of shoot regeneration and symptom development following hot water therapy in order to eliminate CBSV and CMV for production of clean cassava materials and their conservation for future use in Uganda.

Methods

Five best performing varieties including two landraces and three elite varieties of field grown cassava at the National Crops Resources, Research Institute (NaCRRI) Namulonge, i.e., TME 204, Alado Alado, Magana, Ariwala, and Bao were selected for the experiment. Infected leaves and stem sections showing clear CBSD and CMD symptoms were picked from the field. Leaves for CBSD detection were wrapped in clean aluminium foil, labeled and immediately frozen on ice in an icebox. Two young leaves for CMD indexing were put in a 1.5 eppendorf tube and 70% ethanol added. The collected leaf samples were kept frozen at -80^{0}C until needed. Stakes were also obtained from cassava plants from which leaves were collected and were labeled according to variety.

The leaf samples were ground in sterile mortars and total DNA from CMV and RNA for CBSV was extracted from the resulting powder following the CTAB protocol (Lodhi *et al*., 1994). Extracted DNA and RNA were treated with DNase I and RNase H according to manufacturer recommendations (Life Technologies, Thermo Fisher Scientific). Nucleic acid purity, quantity and integrity of each sample was assessed using a NanoDrop (Model 2000C; Thermo Scientific). Two micrograms (2µg) of total RNA for CBSV was converted to cDNA using RevertAid First Strand cDNA Synthesis Kit following manufacturer recommendations. Presence of UCBSV and CBSV in each sample was detected by subjecting 1 of cDNA to conventional RT-PCR assays as described previously (Ogwok *et al*, 2012) using the primers CBSDF2 and CBSVR (Mbanzibwa *et al*., 2011), which amplify c.437 and 343 nucleotides of the 3'-terminal sequence of the UCBSV and CBSV genomes, respectively. Presence of EACM-UG and ACMV in each sample was detected by subjecting 1µL of DNA to conventional PCR assays using the primers; ACMV-AL1/F/AR0/R used for the detection of open reading frames (ORFs) AC1 and AV2 of ACMV; UV-AL1/F/ACMVCP/R3 for ORFs AC1 and AV1 specific for Ugandan EACMV strain EACMV-Ug. (Ogbe *et al.*, 2005).

Stakes of confirmed positive leaf samples were subjected to hot water therapy by incubation at 40°C, 45°C, 50°C for 30 minutes and 55°C and 60°C for 20 minutes respectively before potting in a screen house. Shoot regeneration was monitored weekly by recording the number of regenerated shoots, shoot length and leaf numbers for eight weeks. Symptom appearance on the shoots was also recorded.

Results and discussions

Preliminary results indicated that 100% of the shoots tested positive with CMV and CBSV with 40% co- infected with both CBSV and CBSV-Ug. Cassava stem cuttings from virus-infected plants subjected to hot water treatment gave promising regeneration response results. Shoot regeneration ranged from 0% at 60°C to 87% at 40°C. Highest symptom incidence (100%) was observed on plants grown from untreated diseased cuttings (controls), treated plants at 40°C recorded 55.5% CBSD and 34% CMD incidence while plants treated at 45°C recorded 18% CBSD and 63% CMV incidence. Generally a low disease incidence was recorded with increasing temperatures that is 50°C recorded 25% CBSD and 28.57% CMV incidence and 55°C recorded 0% CBSD and 25% CMV incidence. This could be because temperature is known to dramatically affect plant-virus interactions with an attenuation of virus-induced symptoms at high temperature therefore, the increase in temperature might have led to an increase in virus-derived siRNA accumulation, resulting in the development of fewer symptoms (Szittya *et al*, 2003).

Although information on severity has not been reported in this work, according to visual observations, samples with mixed infection showed severe symptoms even at higher temperature (25%) at 55°C. This is could be attributed to higher virus titres in the samples (Zinga *et al.*, 2008). In all cultivars, TME 204 produced the highest number of shoots, leaves and shoot height than the rest, followed by Magana, Bao, Ariwala and Alado Alado (Figs. 1, 2 and 3). However different varieties produced maximum number of shoots at different

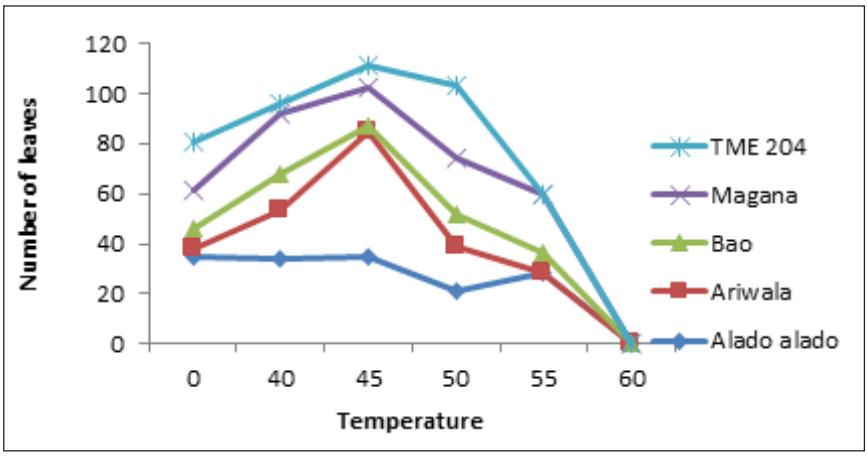

Figure 1. Standard deviation of total number of leaves produced by the different varieties at increasing temperatures

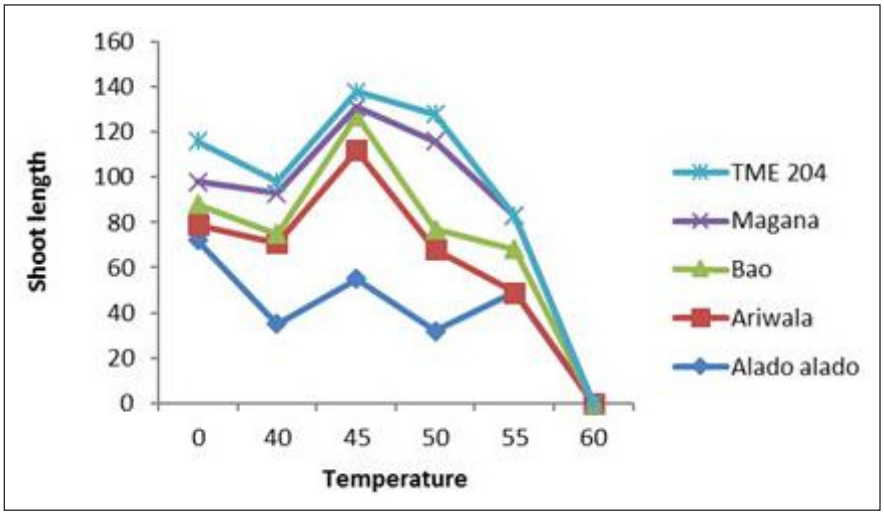

Figure.2. Standard deviation of total shoot length produced by the different varieties at increasing temperatures

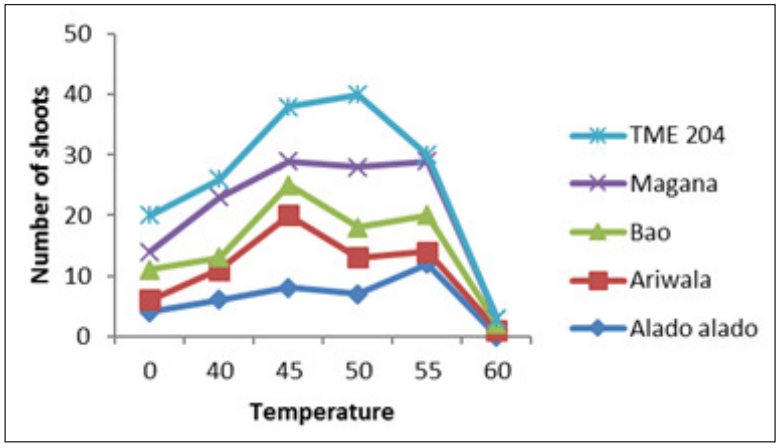

Figure 3. Standard deviation of total number of shoots produced by the different varieties at increasing temperatures

temperatures, TME 204 at 50^{0}C, Magana at 55^{0}C, Bao at 45^{0}C, Ariwala at 45^{0}C and Alado alado at 55^{0}C (Fig. 3). This could imply that different varieties can tolerate different temperature ranges to obtain maximum shoot regeneration following hot water therapy.

Conclusions

Preliminary results indicate that hot-water therapy can reduce or eliminate CBSV and CMVs from cassava cultivars as previously reported. This technique could be used to reduce loss of farmer preferred cassava varieties and distribution of infected cassava propagation material. The technique could further support the *in-situ* and *ex-situ* conservation strategy.

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