OPTIMISATION OF *IN VITRO* TECHNIQUES FOR *CASSAVA BROWN STREAK VIRUS* ELIMINATION FROM INFECTED CASSAVA CLONES IN UGANDA

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ABSTRACT

Cassava brown streak disease (CBSD) caused by *Cassava brown streak virus* (CBSV) is an economically important disease of cassava (*Manihot esculenta* Crantz). CBSD is a destructive disease damaging leaves, stems and roots. Several strategies have been proposed for the management of viral diseases. *In vitro* meristem tip culture and thermotherapy techniques can be employed to effect elimination of CBSV from infected cassava clones. However, these techniques had hitherto not been used for elimination of CBSV from local cassava cultivars in Uganda. Thus, the aim of the study was to optimise *in vitro* techniques for CBSV elimination from infected cassava cultivars in Uganda.

Farmers' fields in 15 districts of Uganda representative of four major agroecological zones of the country were surveyed to record preferred cassava cultivars and CBSD prevalence. From each district, 10 farmers' cassava fields were selected and 30 plants in each cassava field assessed. Stem cuttings of plants apparently affected by CBSD in farmers' fields were collected and established in a screenhouse at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). Sprouted cassava cuttings were diagnosed to confirm CBSV infection using reverse transcription-polymerase chain reaction (RT-PCR) technique with virus specific primers. Using semi-solid half strength Murashige and Skoog (MS) basal medium, hormone concentration and heat treatment regimes were varied to determine optimum conditions for micropropagation of farmers' preferred cassava cultivars and CBSV elimination. Single node cuttings from young cassava stems of 10 commonly grown cassava cultivars were used. Nodal cuttings were thoroughly sterilised and cultured for four weeks on ½ MS medium supplemented with 6-benzyl amino purine (BAP) and 2,4-dichlorophenoxy acetic acid (2,4-D) concentration (mg/l)

combinations of 0.0 and 0.0, 0.5 and 0.1, 1.0 and 0.2, 1.5 and 0.3, and 2.0 and 0.4, respectively. *In vitro* plantlets were used for thermotherapy by exposing them to four temperature regimes of 30-34, 34-38, 36-40 and 38-42^{o}C for 8 hours darkness and 16 hours light, respectively, for four weeks. Meristem tips (~0.3 mm) were excised and cultured on the optimum medium from above for 8 weeks. Plantlets were weaned for 14 weeks and then indexed for CBSV using RT-PCR to establish CBSV elimination efficiency.

The field survey results showed variation in cultivar preferences between regions and some cultivars were found in more than one region. CBSD symptoms were observed on 22 cassava cultivars in seven districts out of 144 cassava cultivars recorded in the 15 districts surveyed. Incidence and severity significantly ($P \leq 0.001$) varied between and within districts and cultivars. *In vitro* micropropagation of cassava showed highest overall plantlet height of 2.5 cm on MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l 2,4-D. Overall number of nodes (leaves) of 4.8 per plantlet was highest on medium without BAP and 2,4-D. *In vitro* thermotherapy enhanced recovery of CBSV free plants. CBSV elimination efficiency significantly ($P \leq 0.001$) varied between temperature regimes and increased with increase in temperature. Conversely, cassava plantlet mortality rate increased with increase in temperature regime. Highest CBSV elimination efficiency of 40% with 49% plantlet survival was observed at 36^{o}C for 8 hours darkness and 40^{o}C for 16 hours light.

These results confirm the occurrence of CBSD on many farmers' preferred cassava cultivars hence, the risk of increased spread of the disease. Results also indicate that *in vitro* techniques can enhance CBSV elimination thus providing an option for management of CBSD. This can be applied to clean, disseminate and conserve popular but CBSD susceptible cultivars.