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Efficacy of *Ipomoea setosa* as an alternative detection technique for propagation of virus-free sweetpotato

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Abstract

Virus diseases pose major limitations to sweetpotato production in sub-Saharan Africa and use of virus-clean planting material is a management option commonly embraced among growers. However, virus detection and *in vitro* processes of producing clean material are expensive, thus making such materials unaffordable to most smallholder farmers. The objective of this study was to examine the efficacy of *Ipomoea setosa* as a cheaper virus-detection method and macro-propagation of virus-tested sweetpotato vines. The efficiency of virus detection using *Ipomoea setosa* was compared to polymerase chain reaction (PCR)/reverse transcriptase (RT)-PCR. The rate of virus-tested plantlet multiplication was also compared for screen house macro-propagation *vis-à-vis* laboratory micro-propagation; and the yield of planting material from these different sources compared in screen house and field trials. The rate of degeneration in the field of sweetpotato derived from macro-propagation *versus* micro-propagation material, was determined for four generations. Sweetpotato viruses were significantly ($P<0.05$) better detected using *I. setosa* than PCR/RT-PCR; and detection results varied with viruses and cultivars. The rate of plantlet multiplication was significantly ($P<0.05$) higher for macro-propagation than micro-propagation. Tissue culture-derived and screen house-derived materials did not differ in yield for both screen house and field experiments. Sweetpotato yield was highest in the first generation and declined significantly ($P<0.05$) from generation two through four. Virus-clean macro-propagated material has potential to give good yields, but farmers should use such materials for one season, after which they should get new clean materials from screen house multipliers.

Key words: Micro-propagation, reverse transcriptase, virus detection

Introduction

Viruses cause the most damaging diseases of sweetpotato in sub-Saharan Africa. The most important viruses are *Sweetpotato chlorotic stunt virus* (SPCSV) and *Sweetpotato feathery mottle virus* (SPFMV), especially in dual infections when they cause severe sweetpotato virus disease (SPVD), and a yield loss of 60- 98% (Gibson *et al*., 1998; Adikini *et al*., 2015). Co-infections involving SPCSV and *Sweetpotato mild mottle virus* (SPMMV) have also been assayed (Mukasa *et al*., 2006; Tugume *et al*., 2016) and may cause up to 80% yield reduction (Mukasa *et al*., 2006). In most single viral infections, yield losses of up to 50% are reported (Gutierrez *et al*., 2003; Mukasa *et al*., 2006; Adikini *et al*., 2015). SPVD causes severe conspicuous symptoms and farmers can select against SPVD-affected plants (Karyeija *et al*., 2000; Ngailo *et al*., 2013). However, most single virus-infected sweetpotato plants do not show symptoms (Mukasa *et al*., 2006). *Sweetpotato chlorotic stunt virus*, *Sweetpotato feathery mottle virus*, *Sweetpotato mild mottle virus*, *Sweetpotato chlorotic fleck virus* (SPCFV), and sweepoviruses; including *Sweetpotato leaf curl Uganda virus* (SPLCUV); have been found in Uganda (Wasswa *et al*., 2011; Tugume *et al*., 2016; Wanjala *et al*., 2021).

Use of virus-infected symptomless vines as planting material is known for rapid spread of the virus (Adikini *et al*., 2015); thus use of virus-clean planting material can potentially reduce the spread. To achieve this, tissue culture-derived sweetpotato is usually screened for viruses using expensive nucleic-acid based detection methods, before being hardened and multiplied in screen houses for distribution to farmers (Mutandwa, 2008). Such procedures are most feasible in developed countries where sweetpotato farmers operate on large scale. In developing countries, especially in sub-Saharan Africa, such laboratories for sweetpotato micro-propagation are few (Masiga *et al*., 2013) resulting into failure to supply enough clean materials to meet the demand. This culminates into high cost of tissue culture-derived plantlets (Ssamula and Mukasa, 2016).

Previous studies on sweetpotato viruses in farmers' fields in East Africa have shown that for some sweet potato cultivars, there is always a proportion of plants that is virus free (Gibson and Kreuze 2015; Ssamula *et al*., 2019). These virus free plant vines can be cheaply tested for virus infections by grafting onto the indicator plant *Ipomoea setosa*, a near-universal indicator plant for sweetpotato viruses (Clark and Moyer, 1988). *Ipomoea setosa* can simultaneously screen against a broad range of sweetpotato viruses and vines found to be virus-free could then be directly macro-propagated in a simple screen house. All this could, thus easily be done by nursery operators near farmers localities. The objective of this study was to examine the

efficacy of *Ipomoea setosa* as a cheaper virus-detection method and macro-propagation of virus-tested sweetpotato vines.

Methodology

Study site

All studies were conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Uganda. Virus detection and propagation experiments were done in 2017, screen house yield experiment was done during first season of 2018; while field yield experiments were done for four consecutive seasons of 2018 and 2019. MUARIK is located at a latitude of 0°28'N of the equator, longitude of 32°37'E and at a mean altitude of 1,200 m above sea level. MUARIK receives annual rain fall of about 13,000 mm (Huxley, 1960), and is a high disease pressure zone for sweetpotato viruses (Mukasa *et al*., 2003).

Detection of sweetpotato viruses

Source of virus isolates

The three most prevalent viruses in East Africa were used in this study. These included two RNA viruses (i) SPCSV (East Africa strain; GenBank accession no. DQ864362) (Aritua *et al*., 2008); (ii) SPFMV (East Africa strain; GenBank accession no. FJ795762) (Tugume *et al*., 2010); and (iii) one DNA virus SPLCUV (GenBank accession no. FR751068) (Wasswa *et al*., 2011). These viruses were previously partially sequenced to confirm their identity (Ssamula *et al*., 2019) using primers sourced from Hylabs - Israel (Table 1). The isolates were maintained in *I. setosa* as the source of inoculum, in an insect proof screen house at MUARIK.

Plant material and virus detection

The plant materials used were of sweetpotato cultivars Resisto, Beauregard, New Kawogo and NASPOT 11. These were obtained from MUARIK sweetpotato collections that were maintained virus free. Studies show that these cultivars respond differently to virus attack; cultivars Resisto and Beauregard are susceptible to SPFMV and SPCSV; and relatively tolerant to SPLCUV (Gibson *et al*., 2014; Ssamula *et al*., 2019). New Kawogo is susceptible to SPLCUV but moderately tolerant to the other viruses (Wasswa *et al*., 2011). NASPOT 11 is highly tolerant to all these viruses (Mwanga *et al*., 2011; Ssamula *et al*., 2019).

Detection experiment set up

Eighty vines, each with 4 nodes, of the four sweetpotato cultivars were grown in an insect proof screen house at MUARIK. Each vine was grown in a bucket filled with 2.5 kg of sterilised soil mixture (loam soil, lake sand and cow manure in a 3:1:1

Table 1. Primer sequences for the viruses used in the study

Virus or gene	Forward and reverse primer names	Primer sequence (5' - 3')	Fragment length (bp)	Reference
SPCSV	SPCSV-UGF SPCSV-UGR	GACGTTCCGATACGATTGAC TCATCATCAGTGTTGCTGCT	550	Ssamula *et al*. (2019)
SPFMV	SPFMV-ILF SPFMV-ILR	CTCCACCACCCACAATAACTG CAGTTGTCGTGTGCCTCTCCG	810	Ssamula *et al*. (2019)
SPLCUV	SPG3 SPG4	ACTTCGAGACAGCTATCGTGCC AGCATGGATTCACGCACAGG	1148	Li *et al.* (2004)
Cox	Cox F Cox R	ACTGGAACAGCCAGAGGAGA ATGCAATCTTCCATGGGTTC	159	Park *et al.* (2012)

Sweet potato feathery mottle virus (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato leaf curl Uganda virus* (SPLCUV), Cytochrome C oxidase (Cox)

ratio). The experiment was laid in a completely randomised design. Plants were watered daily and sprayed weekly with imidacloprid, at a rate of 30 ml per 20 litres of water, to eliminate virus vectors. One week after planting, plants were singly inoculated with SPFMV, SPCSV and SPLCUV by side-grafting scions of infected *I. setosa* (each scion was ~25 mm). Ten mock-inoculated plants per cultivar per virus were included as a control. Treatments were completely randomised. Plants were tested for virus infection at 1 week post inoculation, using PCR/RT-PCR. Fifty plants infected by each virus for each cultivar were then used for the comparison with PCR/RT-PCR assay *vis-à-vis I. setosa* infectivity assay. This procedure was repeated twice to ensure validity.

Ipomoea setosa infectivity assay

Three shoot portions/tips (scions of ~50 mm) were cut from each of the 50 sweetpotato plants per cultivar (as well as the mock inoculated plants), two weeks after graft inoculation. The lowest leaf of each scion was removed and preserved at -20 °C for detection using PCR/RT-PCR; thus providing a composite sample of three leaves per plant. The three scions were then singly side grafted to one week-old virus-clean *I. setosa* plants. Symptom development on *I. setosa* typical of virus infections (Fig. 1) was monitored for 4 weeks. If any of the three scions tested positive, the original sweetpotato plant from which the scions were obtained was considered positive.

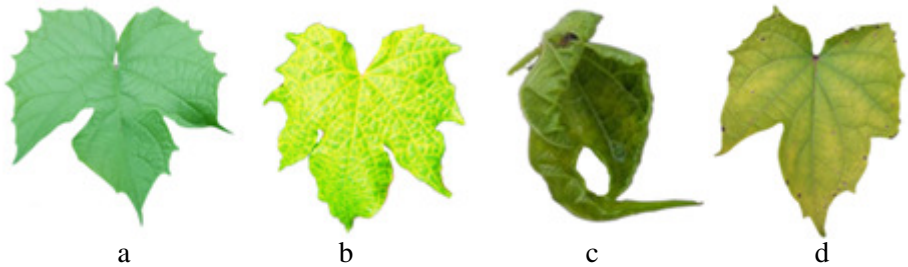

Figure 1. *Ipomoea setosa* leaves showing symptoms induced by sweetpotato viruses. (a) Healthy leaf of a control plant; (b) feathery mottling induced by *Sweet potato feathery mottle virus*; (c) leaf-curling induced by *Sweet potato leaf curl Uganda virus*; (d) leaf chlorosis induced by *Sweet potato chlorotic stunt virus.*

Nucleic acid extraction and virus identification by PCR/RT-PCR

For SPLCUV, total plant nucleic acid extraction was performed from leaves using a cetyl trimethyl ammonium bromide method (Maruthi *et al*., 2002). For RNA viruses, RNA was extracted from leaves using the TRI Reagent protocol, following the supplier's manual (Bio Labs, Jerusalem, Israel). Nucleic acids were quantified using a NanoDrop-ND-1000 spectrophotometer (Thermo Scientific; Bargal Analytical Instruments, Airport City, Israel). The samples were standardised to 500 ng μl^{-1} and evaluated on a 1.5% agarose gel. For RNA virus, complementary DNA (cDNA)

was synthesised using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Tamar, Israel), following the manufacturer's manual.

The 25-µl PCR master mix consisted of 8.5 µl of water, 12.5 µl of PCR mix (HyLabs Ready Mix (×2), HyLabs, Rehovot, Israel), 1 µl of each primer [(10 pmol (Table 1)] and 2 µl of DNA/cDNA (500 ngµl^{-1}). For SPCSV cDNA was denatured at 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 55 °C (annealing) for 30 s and 72 °C (extension) for 45 s; and final extension step of 72 °C for 10 min (Ssamula *et al*., 2019). The PCR conditions for SPFMV included an initial heating step at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 40 s and 72 °C for 40 s; and the reaction was ended with a final extension step of 72 °C for 8 min (Ssamula *et al*., 2019). For SPLCUV, PCR conditions included an initial step of 94 °C for 2 min; 11 cycles of 94 °C for 40 s, 50 °C for 40 s, 72 °C for 90 s; 24 cycles of 94 °C for 40 s, 52 °C for 40 s, 72 °C for 90 s; and 72 °C for 10 min (Li *et al.*, 2004). The PCR condition for housekeeping gene Cytochrome C Oxidase, (Cox) included an initial heating step at 94 °C for 4 min; 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s; and final extension step of 72 °C for 5 min (Park *et al.*, 2012). The house keeping gene, Cytochrome C Oxidase, was used to confirm reactions.

Amplicons were separated on a 1.5% agarose gel in 0.5% Tris ethylene diamine tetra acetic acid buffer, stained with ethidium bromide. The gel was run at voltage of 80 for 40 minutes and products viewed under ultraviolet light and documented by an OmniDoc gel documentation system (Clever Scientific, Image Care – Uganda) (Fig. 2).

Data analysis

For both the *I. setosa* and PCR/RT-PCR assays, the number of virus-infected plants out of the fifty graft-inoculated plants for each virus per cultivar for the two repeats was recorded and averaged, and percentage detection efficiency calculated for each method. The data were subsequently analysed using XLSTAT Chi-square goodness of fit (Addinsoft, 2017) and Student's T test ($P<0.05$).

Rate of sweetpotato regeneration

Planting material production

Twenty plants of each of the sweetpotato cultivars Resisto, Beauregard, New Kawogo and NASPOT 11 from accessions, found virus free, were individually planted in pots filled with 1 kg of sterilised soil mixture (loam soil, lake sand and cow manure in a 3:1:1 ratio) in an insect proof screen house at MUARIK. One week after establishment, a shoot tip (scion of ~50 mm) from each plant was picked and side-

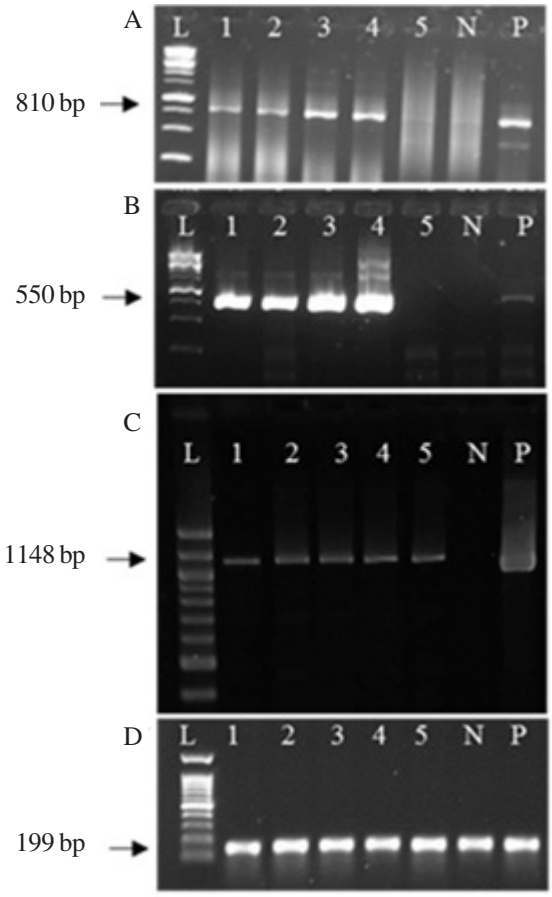

Figure 2. Sample gels of PCR showing virus amplified products in sweetpotato cultivar New Kawogo. Plate A depicts SPFMV gel picture, plate B depicts SPCSV gel picture and plate C depicts SPLCUV gel picture. Lanes L = 1kb ladder, 1-5 = virus positive plants, N = negative control sweetpotato plant of cultivar New Kawogo and P= positive control *I. setosa* plant. Plate D depicts the gel picture of the host *Cytochrome C oxidase* reference gene.

grafted to one week-old *I. setosa* in pots with 1 kg of soil mixture substrate as above. Plants were watered daily and sprayed weekly with imidacloprid (30 ml per 20 litres of water) to eliminate virus vectors.

Sixteen scions of each sweetpotato cultivar continued growing on *I. setosa* root stock up to when each scion was at least 12 internodes. Shoots of *I. setosa* were trimmed off at the 5th week to enable sweetpotato scions to grow faster. Six of the scions were evaluated for regeneration rate in the screenhouse (macro-propagation) and six other scions were evaluated for micro-propagation efficiency/rate. The four remaining scions of the original twenty grafted plants, were maintained as extras to replace any cultivars contaminated during micro-propagation.

Macro-propagation efficiency

Six virus-negative scions were removed from *I. setosa* root stocks and sliced into cuttings of two nodes to provide six two-node cuttings. The cuttings were planted in 1 m x 1 m x 0.15 m (L x W x H) raised flat beds in a screen house. The floor of the screen house was made of loose soil. The bed soil mixture consisted of 3:1:1 ratio of black soil: lake sand: cow manure. Then two-node cuttings were planted in the beds with one node buried in soil at spacing of 15 cm x 15 cm, each bed accommodating 36 cuttings of one cultivar. Further multiplication was done monthly for 4 months by cutting two-node vines from previous beds (leaving two nodes at the base for regrowth) for planting at similar spacing in the subsequent same size beds. Treatments were laid in a completely randomised design and at the end of the experiment (4 months), vines were left to grow to provide planting material for the subsequent yield experiment.

Micro-propagation efficiency

Six virus-negative scions (plus four extra scions to replace contaminated cultures) for each cultivar were cut off the *I. setosa* graft and prepared for *in vitro* micro-propagation. The two-node cuttings were surface sterilised by washing with double distilled water and liquid soap. The cuttings were then rinsed thrice with double distilled water, sterilised in 15% jik (0.00525% w/v sodium hypochlorite) for 15 minutes, followed by sterilisation in 70% ethanol for 5 minutes; and finally rinsed four times in double distilled water (Rukarwa *et al*., 2010; Tadda *et al*., 2022). The explants were cultured individually in baby jars on semi-solid half strength Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962), supplemented with 0.5 mg l^{-1} BAP and 0.1 mg l^{-1} 2,4-D (Abubakar *et al*., 2018).

The cultures were incubated under white fluorescent light with a 14 hour photoperiod at 25±1 ^{o}C. Sub-culturing/multiplication was done monthly for up to four months. The experiment was laid in a completely randomised design. During weaning, plantlets were carefully washed using tap water to remove the media. The water used for washing contained bio-cure fungicide (*Trichoderma veraliti*) at a rate of 120 ml per 150 litres of water to kill fungal contaminants. Plants were then hardened for a month in high humidity chambers in a shaded screen house in pots with potting mixture of 3:1:1 black soil: saw dust: lake sand. Vines were left to grow and these provided planting material for the subsequent yield experiment.

Yield and degeneration rate

Screen house and field experimental setups for yield assessment were conducted at MUARIK, using cultivars Resisto, Beauregard, New Kawogo and NASPOT 11. For the screen house experiment, three types/categories of planting material; namely tissue-culture derived, screen house-derived, as mentioned above; and a new

component of farmer-derived was added. Visually clean farmer-derived planting material was included as a control, to mimic farmers' practice of picking apparently health material from previous crop when planting new fields. Sweetpotato plants were planted in five flat-bottomed basins (0.0005 ha) per planting material category for each cultivar. Three cuttings were planted per basin at three points (this is the normal practice of planting sweetpotato in mounds at MUARIK). Each basin contained 40 kg of field soil, as this was the estimated amount contained in a mound used for growing sweetpotato in the field at MUARIK. Plants were watered daily and sprayed weekly with imidacloprid, at a rate of 30 ml per 20 litres of water to eliminate virus vectors. Treatments were laid in a completely randomised design and plants were harvested 16 weeks after planting.

For the field experiments, tests compared two categories of planting material; namely, tissue-culture derived and screen house-derived planting material. The trials were conducted for four consecutive seasons during 2018 and 2019, with the previous season providing visually-clean planting material for the subsequent season (acting as a control and mimicking farmers' practice). Mean while clean material from screen house (generation 1 - G1) of each cultivar were included for each season. Therefore, the second field trial consisted of cuttings from each original field (G2) and fresh virus-tested cuttings from the screen house (G1). The third trial consisted of cuttings from G2 and G1 of the second trial forming G3 and G2, respectively, and healthy cuttings from the screen house (G1). Fourth trial consisted of G1, G2, G3 and G4 (design adopted from Adikini *et al*., 2015).

Four plots of each of the planting material category per cultivar were arranged in a replicated randomised Latin square. Each plot had four mounds of a given cultivar (two mounds for tissue culture-derived material and two mounds for screen house-derived material) giving a total of eight mounds (0.0008 ha) for each planting material category per cultivar. Each mound was approximately 1 m in diameter and 0.4 m high and spaced 1.5 m apart, with nothing planted in between. Three cuttings were planted per mound at three points. For each season, weeding was done three times using a hand hoe. To ensure uniform seasonal influence, only season four trials that consisted all the four generations (G1, G2, G3 and G4) were considered for data collection and analysis, and plants were harvested 16 weeks after planting. For each of the generations, each mound per planting material category per cultivar was harvested and data recorded separately.

For both screen house and field, experimental data were collected on total root weight, marketable root weight (between 150 – 1000 g), total root number and marketable root number.

Data analysis

Data were collected and compared for number of plantlets produced from screen house macro-propagation and tissue culture micro-propagation and subjected to analysis of variance (ANOVA), using GenStat 14.0 for Windows. Mean root number and weight values; and yield and degeneration rate from macro-propagated and micro-propagated planting material were also subjected to ANOVA. Means were separated using Fischer's protected LSD test at 5%.

Results

Sweetpotato virus detection using I. setosa vis-à-vis PCR/RT-PCR assay

Virus detection by either method varied with viruses and sweetpotato cultivars (Table 2). Generally, sweetpotato viruses were better detected using *I. setosa* compared to PCR/RT-PCR; although in some plants, viruses were not detected (P>0.05) by either

Table 2. Number (and percentage detection efficiency) of virus positive plants of different sweetpotato cultivars tested using *I. setosa* indicator plant *vis-à-vis* PCR/RT-PCR

Cultivar	Virus tested	Virus detection assay	
		Using *I. setosa*	Using PCR/RT-PCR
Resisto	SPCSV	50a (100%)	50a (100%)
	SPFMV	50a (100%)	50a (100%)
	SPLCUV	41a (82%)	41a (82%)
Beauregard	SPCSV	50a (100%)	50a (100%)
	SPFMV	50a (100%)	50a (100%)
	SPLCUV	50a (100%)	39b (78%)
New Kawogo	SPCSV	50a (100%)	40b (80%)
	SPFMV	33a (66%)	33a (66%)
	SPLCUV	50a (100%)	50a (100%)
NASPOT 11	SPCSV	50a (100%)	50a (100%)
	SPFMV	20a (40%)	20a (40%)
	SPLCUV	27a (54%)	16b (32%)
Average percentage detection		87%	82%

Values in a row followed by the same letter are not significantly different at P<0.05. Different categories of detection assays (for each cultivar for different virus infections) above were statistically significant at 95% with a chi-squared value equal to 7.3 (df = 1)

technique (Table 2). Across cultivars, SPCSV was the most frequently detected by either *I. setosa* or PCR/RT-PCR, followed by SPLCUV; while SPFMV was the least frequently detected by either method (Table 2). All viruses were detected similarly by both methods in all plants of cultivar Resisto (Table 2). However, variations in virus detection methods were observed in other cultivars, more SPCSV infections being detected in New Kawogo using *I. setosa* than PCR/RT-PCR (Table 2; $P<0.05$) and also more SPLCUV infections detected in Beauregard and NASPOT 11 using *I. setosa* compared to PCR/RT-PCR (Table 2; $P<0.05$). No viruses were detected using either method for mock –inoculated (control) plants (results not presented).

Sweetpotato regeneration

Sweetpotato cultivars varied widely in number of plantlets produced with some cultivars responding better than others. In general, macro-propagation in the screen house produced significantly more plantlets overall compared to *in vitro* micro-propagation (Table 3; $P<0.05$). Cultivars, Resisto and NASPOT 11, produced significantly more plantlets through macro-propagation than micro-propagation. There was no significant difference ($P>0.05$) in number of plantlets between macro-propagation and micro-propagation for cultivars Beauregard and New Kawogo (Table 3). Considering macro-propagation for the four months, cultivar NASPOT 11 produced the highest number of plantlets (1302 per month); followed by Resisto, then New Kawogo and finally Beauregard (656 per month). With micro-propagation, the number ranged between 384 plantlets per month (New Kawogo) and 313 plantlets per month (Beauregard) (Table 3).

Table 3. Mean monthly number of sweetpotato plantlets produced per cultivar using macro-propagation and micro-propagation pooled over a period of four months

Sweetpotato cultivars	Number of plantlets	
	Macro-propagation	Micro-propagation
Resisto	1247a	378b
Beauregard	656a	313a
New Kawogo	838a	384a
NASPOT 11	1302a	374b
Mean	1011a	362b
LSD	497.5	
CV (%)	144.6	

Values in a row followed by the same letter are not significantly different at $P<0.05$, LSD is the least significant difference at $P = 0.05$, CV is the coefficient of variation

Yield and degeneration rate

In the screen house, yield was not significantly affected ($P>0.05$) by cultivar differences. However, planting material category significantly affected total storage root yield ($P<0.001$), marketable storage root yield ($P<0.001$), total number of storage roots ($P<0.01$) and marketable number of storage roots ($P<0.001$; Table 4). The effect of cultivar by planting material type interaction also significantly affected total storage root yield ($P<0.05$) and marketable storage root yield ($P<0.05$) and marketable number of storage roots ($P<0.05$); but not total number of storage roots.

For the field trial, significant differences ($P<0.01$) were detected among cultivars for total storage root yield, marketable storage root yield, total number of storage roots and marketable number of storage roots (Table 4). Additionally, total storage root yield, marketable storage root yield, total number of storage roots and marketable number of storage roots were all significantly affected by generation/cycle in field production ($P<0.001$; Table 4). Planting material source individually or in interaction with other factors did not significantly affect total storage root yield, marketable storage root yield, total number of storage roots and marketable number of storage roots. The interaction between cultivar and generation was significant for total storage root yield ($P<0.001$), marketable storage root yield ($P<0.001$), total number of storage roots ($P<0.01$) and marketable number of storage roots ($P<0.001$; Table 4).

In the screen house experiment, the mean storage root yields across cultivars of tissue culture-derived material and screen house-derived material were not significantly different (Tables 4 and 5; $P>0.05$). However, the farmer-derived material generally yielded less, though not for NASPOT 11. This trend held for number of storage roots ($P<0.01$), number of marketable storage roots ($P<0.001$) and marketable storage root yield ($P<0.05$) (Tables 4 and 5). The root yield from the cultivars was in the order Resisto>Beauregard>NASPOT 11>New Kawogo (Table 5).

For the field trial, screen house-derived material and tissue culture-derived material again yielded similarly in terms of total storage root yield, marketable storage root yield, total number of storage roots and marketable number of storage roots (Tables 4 and 5). Of the macro-propagated materials, NASPOT 11 had the highest yields and New Kawogo the lowest for all yield parameters; whereas of the micro-propagated materials, Resisto had the highest yield and New Kawogo the lowest (Table 5).

The yield of sweetpotato cultivars for the different generations generally decreased with increasing generations for both tissue culture-derived and screen house-derived material (Table 6). Yield degeneration was greatest in cultivars Resisto and Beauregard,

Table 4. ANOVA to test differences in yield of different propagation material types of sweetpotato cultivars in screen house and field across generations

Source of variation	d.f.	Mean squares			
		Total storage root yield (t ha^{-1})	Marketable storage rootyield (t ha^{-1})	Total number of storage roots	Marketable number of storage roots
Screen house trial					
Replication (mounds)	4	1.341	1.145	2.2250	0.1000
Cultivar (Cv)	3	4.947ns	2.980ns	1.7500ns	0.3722ns
Planting material type	2	37.368***	36.732***	5.7167**	6.3500 ***
Cv X Planting material type	6	7.490*	7.070*	1.4500ns	1.1722*
Error	44	2.652	2.419	0.8795	0.3727
Total	59				
Field trial					
Replication	3	132.36	117.50	5.066	4.160
Cultivar (Cv)	3	70.34**	70.29**	10.743**	8.702**
Generation	3	646.87***	692.20***	60.254***	72.921***
Planting material type	1	39.85ns	48.13ns	0.098ns	0.035ns
Cv X Generation	9	95.25***	82.43***	8.469***	7.362***
Cv X Planting material type	3	21.88ns	24.48ns	0.202ns	0.150ns
Generation X Planting material type	3	6.35ns	6.11ns	0.254ns	0.410ns
Cv X Generation X Planting material type	9	13.23ns	11.82ns	0.636ns	0.594ns
Error	221	17.08	15.66	2.684	1.945
Total	255				

ns indicates non significance; * indicates significance at P<0.05, ** indicates significance at P<0.01, *** indicates significance at P<0.001

Table 5. Mean yield of sweetpotato cultivars in the screen house and field using different planting material types

Screen house trial	Total storage root yield (t ha^{-1})			Marketable storage root yield (t ha^{-1})			Total number of storage roots			Marketable number of storage roots		
	TC	SH	FD	TC	SH	FD	TC	SH	FD	TC	SH	FD
Resisto	5.64	5.00	1.30	5.06	4.22	0.64	2.80	2.40	1.20	2.00	1.60	0.20
Beauregard	4.54	4.84	0.46	3.96	4.28	0.00	2.40	2.60	0.60	1.60	1.80	0.00
New Kawogo	2.88	3.30	1.94	2.70	2.88	1.74	1.40	1.60	1.00	1.00	1.20	0.60
NASPOT 11	3.90	3.76	3.76	3.50	3.36	3.22	2.00	1.80	2.00	1.40	1.20	1.20
Mean	4.24a	4.22a	1.87b	3.80a	3.68a	1.40b	2.15a	2.10a	1.20b	1.50a	1.45a	0.50b
Field trial												
Resisto	18.06	18.00		17.19	17.12		4.75	4.66		4.031	3.969	
Beauregard	17.84	19.38		16.78	18.47		5.25	5.38		4.469	4.625	
New Kawogo	16.62	16.28		15.53	15.22		5.06	4.94		4.094	4.062	
NASPOT 11	17.72	19.75		16.44	18.59		5.69	5.62		4.750	4.781	
Mean	17.56a	18.35a		16.48a	17.35a		5.19a	5.15a		4.336a	4.359a	

Means in a row with same letters are not significantly different for each yield·parameter ($P<0.05$). TC is tissue culture-derived planting material, SH is screen house-derived planting material, FD is farmer-derived planting material

Table 6. Mean yield of sweet potato cultivars for the different generations using different planting material types at MUARIK

Cultivar	Generation	Total storage root yield (t ha^{-1})		Marketable storage rootyield (t ha^{-1})		Total number of storage roots		Marketable number of storage roots	
		TC	SH	TC	SH	TC	SH	TC	SH
Resisto	G1	23.75a	23.88a	22.88a	23.38a	6.62a	6.62a	6.00a	6.13a
	G2	19.75b	20.00b	19.00b	19.00b	5.38b	5.62b	4.75b	4.88b
	G3	15.62c	14.50c	14.25c	13.25c	3.75c	3.38c	2.63c	2.50c
	G4	13.12d	13.62c	12.62d	12.88c	3.25c	3.00c	2.75c	2.38c
Beauregard	G1	26.88a	24.50a	25.38a	23.38a	7.38a	7.12a	6.50a	6.38a
	G2	19.88b	21.38b	19.00b	20.62b	5.50b	6.00b	4.88b	5.50b
	G3	12.38c	15.75c	11.38c	14.88c	4.00c	3.88d	3.25c	3.13c
	G4	12.25c	15.88c	11.38c	15.00d	4.12c	4.50c	3.25c	3.50c
New Kawogo	G1	17.38a	17.38a	16.75a	16.50a	5.50a	5.25a	4.75a	4.63a
	G2	16.50a	15.75b	15.50a	14.88b	5.12a	4.88a	4.13b	4.00b
	G3	16.12a	16.50b	14.88ab	15.12b	4.75ab	5.00a	3.75b	3.88b
	G4	16.50a	15.50bc	15.00b	14.38b	4.88ab	4.62ab	3.75b	3.75b
NASPOT 11	G1	19.88a	22.25a	19.00a	21.50a	5.75a	6.38a	5.00a	5.88a
	G2	18.00b	20.50b	16.75b	19.00b	6.25a	6.12a	5.13a	5.00b
	G3	16.25c	19.88b	14.75c	18.75b	5.25ab	5.12b	4.38b	4.25c
	G4	16.75c	16.38c	15.25c	15.12c	5.50ab	4.88b	4.50b	4.00c

G1 to G5 represents number of cycles in field production. Means in a column with the same letters among different generations for each cultivar are not significantly different for each yield parameter ($P<0.05$). TC is tissue culture-derived planting material, SH is screen house-derived planting material.

moderate in NASPOT 11 and least pronounced in cultivar New Kawogo, even though the initial yield for generation one was lowest for cultivar New Kawogo than in other cultivars (Table 6). The initial decrease in yield from generation one to two and then to three was significant and greatest ($P<0.05$; Table 7). There was a relatively less decrease in yield from the third to fourth generation. Across generations, screen house-derived material performed better than tissue culture-derived material for total storage root yield, marketable storage root yield and marketable number of storage roots. For total number of storage roots, tissue culture-derived material performed better than screen house-derived material (Table 7).

Discussion

This study is the first of its kind to show that virus-free shoots of many sweetpotato plants can be easily identified using *I. setosa* and thereafter directly macro-propagated in the screen house, thus eliminating expensive tests and tissue culture and making practical locally-available virus-clean material in Africa. In fact, in some cases, detection using *I. setosa* picked virus particles where the PCR/RT-PCR did not. The lesser efficacy of PCR/RT-PCR shown here is in agreement with earlier researchers, for instance, Lotrakul *et al*. (1998). Similarly, Kokkinos and Clark (2006) while working on SPLCV, found 45% detection by conventional PCR vs. 90% detection by quantitative (q)PCR. However, qPCR is even more expensive and rarely used in Africa than PCR/RT-PCR.

The observed variation in detection efficiency is probably because *I. setosa* is extremely susceptible to virus infections (Clark and Moyer, 1988; Ssamula *et al*., 2019) and thus is capable of replicating even very dismal concentrations of viruses. In contrast, PCR/RT-PCR is usually less sensitive when the starting virus concentration is extremely low (Peters *et al*., 2004). Also, plant sap may have inhibitors affecting PCR amplification and, thus affect its efficiency (Schrader *et al*., 2012). The relatively low sensitivity of PCR/RT-PCR could as well be due to uneven distribution of viruses in the plant (Ssamula *et al* 2019). In fact, some plants expected to be positive after successfully grafting infected material, tested negative by *I. setosa* and PCR/RT-PCR. Therefore, a leaf sample taken from a virus-free portion of the plant for PCR/RT-PCR will test negative. However, when *I. setosa* is used as a detection technique, a relatively larger sample (a scion) is taken thus reducing the chances of missing the virus.

Our failure to detect viruses in some plants by both *I. setosa* and PCR/RT-PCR (Table 2) could as well be due to the possibility of reversion from virus infections as was also observed by others (Wasswa *et al*., 2011; Gibson *et al*., 2014; Ssamula *et al*., 2019). Failure to detect expected viruses was most common in cultivar NASPOT

Table 7. Mean yield of sweetpotato for the different field generations using different planting material types

	Total storage root yield (t ha^{-1})		Marketable storage rootyield (t ha^{-1})		Total number of storage roots		Marketable number of storage roots	
Generations	TC	SH	TC	SH	TC	SH	TC	SH
Generation 1	21.97a	22.00a	21.00a	21.19a	6.31a	6.34a	5.562a	5.750a
Generation 2	18.53b	19.41b	17.56b	18.38b	5.56b	5.66b	4.719b	4.844b
Generation 3	15.09c	16.66c	13.81c	15.50c	4.44c	4.34c	3.500c	3.438c
Generation 4	14.66c	15.34c	13.56c	14.34c	4.44c	4.25c	3.562c	3.406c
Mean	17.56	18.35	16.48	17.35	5.19	5.15	4.34	4.36
CV (%)	8.0		8.0		5.4		5.9	
LSD	1.440		1.369		0.568		0.4845	

Means in a column with same letters are not significantly different for each yield parameter ($P<0.05$). TC is tissue culture-derived planting material, SH is screen house-derived planting material

11, followed New Kawogo and then Beauregard and Resisto. NASPOT 11 and New Kawogo are East African varieties and are documented as tolerant to virus infections (Mwanga *et al*., 2011). In a study by Ssamula *et al*. (2019), NASPOT 11 was found to be very tolerant to virus infections and with high reversion potential. NASPOT 11 was bred from New Kawogo (Mwanga *et al*., 2011) and inoculated New Kawogo also often tested negative. On the other hand, Beauregard and Resisto are American varieties and are very susceptible to virus infections, and in studies by Gibson *et al*. (2014) and Ssamula *et al*. (2019) these cultivars were found to be the least reverting.

For either detection method, SPCSV was the most frequently detected in inoculated plants followed by SPLCUV and then SPFMV (Table 2). This observation is consistent with findings by Ssamula *et al*. (2019). Once plants get infected with East African strain of SPCSV, the RNAse3 and p22 silencing suppressor genes break down resistance (Cuellar *et al*., 2008), making it difficult for plants to revert from it and other co-infecting viruses (Kreuze *et al*., 2005), thus causing SPVD. On the other hand, plants can easily revert from SPLCUV and SPFMV infections. Geminiviruses (SPLCUV) and potyviruses (SPFMV) also have silencing suppressor genes (AC2 for SPLCUV; Voinnet, 2001 and HC-Pro for SPFMV; Kasschau and Carrington, 1995); but these probably do not extensively compromise plant defences. Our findings suggest that, virus free shoots of especially the east African varieties could easily be identified with *I. setosa* and multiplied for the production of virus free plants.

More plantlets were regenerated from macro-propagation than micro-propagation (Table 3) probably because for macro-propagation, plants are more or less in a natural environment. Therefore, the physiological processes such as photosynthesis that are essential for plant growth are resumed almost immediately in scions following planting. In fact, CO_2, light, water, nutrients, temperature and humidity are better under screen house conditions than in *in vitro* environment (Ho, 2002). *In vitro* plants survive in a manipulated environment; the light is artificially provided and this cannot be sufficient for photosynthesis; so plants are provided with an external carbohydrate source for energy requirements (Mehwish *et al*., 2012). Besides, while *in vivo* plants use endogenous hormones that are naturally well balanced for proper cell growth for each cultivar, *in vitro* plants need to be supplemented with external sources of these growth hormones (Abubakar *et al*., 2018), and these may not be optimally balanced across cultivars. Micro-propagation also requires that the explants be sterilised to kill bacterial and fungal contaminants that may out-compete the cultured plantlets (Hammond *et al*., 2014). Through sterilisation, however, the explants are affected and when established on media, they need considerable time to recover from such stress. All these factors affect the *in vitro* multiplication rate. The great

performance of macro-propagation bodes well for our farming communities as it is feasible and cost-effective.

The number of plantlets varied significantly with different cultivars during macro-propagation (Table 3). The difference in growth response could be attributed to genotypic variation that exist in sweetpotato varieties. However, during micro-propagation, there was no significant difference between cultivars for number of plantlets. This observation is in contrary with findings by Rukarwa *et al*. (2010) and Abubakar *et al*. (2018). This variability shows the challenge in optimizing- *in vitro* conditions.

When both propagation types were used to provide planting material, no significant difference in yield was observed within cultivars for screen house. Our observations suggest that macro-propagation can be used to provide as good quality planting material as micro-propagation. However, there were differences among yields of cultivars in the field (Table 4). This suggests the difference between screen house and field environments. Indeed, Villagran and Jaramillo (2020) while studying thermal behavior and airflow patterns in a screen house during daytime hours (6:00 to18:00 h), they observed the air flow patterns inside the screen house to have speed reductions of up to 68% with respect to the speed of the external wind. They also found that the thermal behavior inside the screen house was quite homogeneous; the average temperature values in the structures ranged between 23.9 and 39 °C and the difference with external environment temperature of up to 1.8 °C. It is, therefore, possible that plants in the screen house are stressed and thus cultivars fail to express full potential, even though the environment is more controlled therein (Villagran and Bojacá, 2019).This was also evidenced by the overall higher yields in the field than in the screen house.

Greater sweetpotato yield was observed in generations G1 than in G2, G3 and G4 for both tissue culture and screen house-derived planting material (Tables 6 and 7). The decline in yield with generations was probably due to exposure of sweetpotato plants to virus infections in the field (Adikin *et al*., 2015; Gibson and Kreuze, 2015). Yield degeneration was greatest in cultivars Resisto and Beauregard, moderate in NASPOT 11 and least in New Kawogo. Cultivars Resisto and Beauregard are very susceptible to viruses (Gibson *et al*., 2014; Ssamula *et al*., 2019); while New Kawogo is tolerant to viruses and has been used in breeding for resistance (Mwanga *et al*., 2011). The initial yield for generation one was lowest for cultivar New Kawogo. Despite being tolerant to viruses, cultivar New Kawogo is not a good yielder (Mwanga *et al*., 2011). The low yield degeneration observed in cultivar New Kawogo supports farmers' being able to maintain this cultivar for decades in the field (Mwanga *et al*., 2011). For the high yielding cultivars (Resisto and Beauregard), there was a sharp

decline in yield after the first and second generations for both tissue culture and screen house-derived material. This suggests that at most, in a high disease pressure area, farmers should use sweetpotato material for only one season before they go back for fresh clean planting material.

Conclusion and recommendations

Ipomea setosa performs better than PCR/RT-PCR in detecting different viruses infecting sweetpotato. Virus-clean planting materials multiplied faster *via* macro-propagation in the screen house compared to micro-propagation through tissue culture. Using *I. setosa* as a screening tool has potential in rural areas of sub-Saharan Africa where sweetpotato production is based, but are devoid of laboratory facilities. This study has clearly demonstrated that clean planting material can ensure sustainable sweetpotato production with increased yield compared to using vines of unknown health status normally used by farmers. However, such clean material especially of extremely susceptible cultivars like Resisto and Beauregard, despite having high yield potential, will quickly collapse within the first season of field exposure. Therefore, macro-propagation in the screen house of virus-tested sweetpotato material using *I. setosa* is recommended for outreach/technology transfer to seed multipliers in farmers' localities for rapid multiplication of large quantities of affordable high-quality planting material for efficient distribution to small scale farmers. Farmers should be strongly advised when given clean planting material not to use it for more than one cycle in field production, to obtain the maximum benefit of using clean vine cuttings.

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