Degeneration of cleaned-up, virus-tested sweetpotato seed vines in Tanzania

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ABSTRACT

Viruses pose a major challenge to sweetpotato production in Tanzania. Use of cleaned-up, virus-tested seed vines distributed through a formal seed system is among the proposed strategies to address this challenge. However, virus-tested seed vines can get infected once in the field and it is not known how they will perform following several seasons of on farm propagation. We assessed the performance of virus-tested seed vines and farmer-sourced seed vines of a susceptible variety, Ejumula, and a relatively tolerant variety, Kabode, over five seasons to understand the trend in root yields, vine yields and virus incidences. The experiments were done in high and low virus pressure areas. The most prevalent viruses were sweet potato chlorotic stunt virus (SPCSV) followed by sweet potato feathery mottle virus (SPFMV) and sweet potato leaf curl virus (SPLCV), respectively. Both farmer-sourced and cleaned-up, virus-tested seed of cv. Ejumula were rapidly infected with SPCSV. The incidence of this virus on Ejumula's farmer-sourced material at the high-virus-pressure area reached 100% by the second season. The incidences for all three viruses remained stable for cv. Kabode across the five seasons. Plants generated from cleaned-up, virus-tested seed had lower incidences for all viruses compared to those from farmer-sourced planting material. Virus-tested seed produced significantly higher root yields for cv. Ejumula in the high-virus-pressure site, with a gradual drop across the seasons. The findings show that regular replenishment of clean, virus-tested seed is more economical in high-virus-pressure areas and for more susceptible varieties like cv. Ejumula. They also indicate that farmers may be reluctant to invest in cleaned-up, virus-tested seed in cases where they have virus-tolerant varieties such as cv. Kabode due to lack of obvious virus effect on yields.

1. Introduction

Sweetpotato (*Ipomoea batatas*) productivity in Tanzania is currently very low, averaging 3–6 t/ha, against a potential of over 16 t/ha depending on the cultivar (Sebastiani et al., 2007). Factors contributing to this low yield include limited use of quality seed, poor agronomic practices, low soil fertility, drought and high virus incidences (Ngailo et al., 2016). However, greatest losses in yield have been associated with virus infections (Adam et al., 2015; Ndunguru et al., 2009). Viruses are systemic, hence multiply easily from season to season through vegetative propagation of infected planting material which leads to virus build up over time (across seasons), progressively decreasing yields (Clark et al., 2002). The quality loss caused by this so-called recycling of infected planting material is known as degeneration (Gibson and Kreuze, 2015) or cultivar decline (Bryan et al., 2003; Clark et al., 2002). The viruses contributing to this to a large extent belong to the families of *Closteroviridae, Geminiviridae* and *Potyviridae* (Nakazawa, 2001).

In Tanzania, and East Africa as a whole, sweet potato chlorotic stunt virus (SPCSV; genus *Crinivirus,* family *Closteroviridae*) and sweet potato feathery mottle virus (SPFMV; genus *Potyvirus,* family *Potyviridae*) are the main viruses causing degeneration (Adikini et al., 2016; Mukasa et al., 2003; Tairo et al., 2004). In single infections, SPFMV has been

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reported to cause up to 40% yield losses while SPCSV has been reported to cause up to 52% yield losses (Adikini et al., 2016). However, co-infection leads to a devastating disease complex known as sweet potato virus disease (SPVD) that can cause more than 90% yield losses (Adikini et al., 2016; Clark et al., 2012; Karyeija et al., 2000). SPCSV makes sweetpotato more susceptible to SPFMV through suppression of its natural virus-defense mechanism. Often plants initiate defense mechanisms when attacked by various pathogens including viruses. One mechanism through which sweetpotato responds to virus infection is based on RNA silencing (Baulcombe, 2004; Guo et al., 2016). Here, host proteins identify the viral RNA and cut it into small molecules of 21–24 nt which then trigger a sequence-specific host response that degrades the RNA of the invader virus (Ding and Voinnet, 2007). Two RNA silencing suppression (RSS) proteins (p22 and p26) have been discovered in SPCSV (Kreuze et al., 2005). The RSS p22 has only been found in SPCSV isolates from Uganda while synergistic effects have been shown even in those isolates not encoding it (Cuellar et al., 2008). This implicates p26 as the protein responsible for breaking antiviral resistance (Cuellar et al., 2009). The p26 protein expresses an RNase III-type of protein (RNase3), which has been found in all SPCSV isolates characterized (Kreuze et al., 2021). This RSS protein suppresses the RNA silencing mechanism in sweetpotato therefore making it more vulnerable to SPFMV and other viruses (Kreuze et al., 2005). SPFMV titers have been shown to increase several 100-folds when SPCSV is present compared to when infecting alone (Cuellar et al., 2008; Mukasa et al., 2006). Co-infection with SPFMV does not change SPCSV titers. Sweepoviruses belonging to the family *Geminiviridae* have also been reported to cause up to 80% yield losses even without obvious foliar symptoms (Clark and Hoy, 2006). The effect of sweepoviruses on yield loss is now being appreciated and a recent study by Wanjala et al. (2020) reported an average of 47% yield loss from sweet potato leaf curl virus (SPLCV; genus *Begomovirus,* family *Geminiviridae*) on a moderately resistant Kenyan variety, cv. Kakamega.

The extent of virus-related yield losses varies with cultivar and agroecology. Degeneration is likely to be rapid in high-virus-pressure agroecologies. These include locations where sweetpotato is grown year-round, for instance areas with a bimodal rainfall pattern. This means that there is always a crop in the field and therefore there is always a source of virus inoculum. The year-round cultivation also means that vectors are always present. On the other hand, low-virus-pressure areas experience several months of no rain leading to drying up of most sweetpotato crops and alternate virus hosts e.g., other *Ipomoea* spp. This reduces the amount of virus inoculum and vector populations. There is no variety that is one hundred per cent resistant, but there are some varieties that can produce economically acceptable yields even when infected (Mwanga et al., 2013). Some East African landraces have been shown to perform well in high-virus-pressure areas owing to decades of selection by farmers (Bua et al., 2006; Gibson et al., 2000). This means that with time farmers retained more tolerant varieties through selection of healthy-looking planting material. In addition, some varieties have been reported to show signs of reversion whereby the host recovers from disease symptoms. Reversion from virus infection is the ability of plants that are virus-infected to become mostly virus-free (Gibson & Otim-nape, 1997). On the other hand, high-yielding but susceptible improved varieties can perform well if grown in low-virus-pressure areas with good on-farm management practices.

Degeneration in sweetpotato can be managed through use of clean virus-tested seed vines, breeding for resistance and on-farm management of infections (Thomas-Sharma et al., 2016). Use of clean virus-tested seed vines is now widely promoted in sub-Saharan Africa and quality is a value-proposition for formal sweetpotato seed systems (McEwan, 2016). Virus cleaning is achieved through a combination of thermotherapy and meristem tip culture (Mashilo et al., 2013). This process can eliminate most of the known viruses depending on how it is done. After virus elimination, the plants are tested via techniques such as polymerase chain reaction to confirm that they are virus-free, at least of the known viruses. Planting clean virus-tested seed vines has been shown to mitigate losses caused by viruses (Clark et al., 2010; Dennien, 2015; Gibson et al., 2004).

However, clean virus-tested seed is prone to infection once grown in the open where the seed crop is exposed to virus vectors, i.e., whiteflies and aphids. The rate of infection depends on the genotype and environment. Virus-susceptible varieties can get infected rapidly, especially when grown in high-virus-pressure areas. The risk of infection makes it challenging to promote the use of clean virus-tested seed in sub-Saharan Africa, especially if it leads to a decline in yield. The first-generation seed, which is always virus-free, is expensive due to the cost of clean-up, virus indexing and maintenance and is therefore not recommended for direct planting by farmers to produce storage roots. Therefore, this material must undergo further multiplication on-farm to make it more affordable. Seed systems interventions have taken this into consideration by training seed producers how to minimize infection at farm-level (Adam et al., 2018). Farmers are also advised to avoid too much recycling and buy quality seed from trained seed producers who are multiplying planting material sourced from cleaned-up seed stocks. However, this is challenging because it takes time to change people's mindsets from practices they are accustomed to. Recycling of clean virus-tested seed will eventually lead to yield losses in subsequent plantings (Lewthwaite et al., 2011). Cognizant of the effect of seed recycling, it is important to understand the performance of cleaned-up, virus-tested seed recycled over several seasons in various agroecologies. This will lead to recommendations on its optimal use in addressing degeneration in sweetpotato. This research sought to assess performance of clean virus-tested seed and farmer-sourced seed, recycling it over five seasons, in terms of storage root yield, vine yields and accumulation of SPFMV, SPCSV and SPLCV.

2. Materials and methods

2.1. Location

The experiments were conducted in Nyamang'uta village, Bunda district and Nungwe village, Geita district at the Lake Zone Tanzania. Bunda district is in Mara region whereas Geita district is in Geita region. The site at Nyamang'uta village was located at 1° 58′ 32″ S 33° 59′ 03″ E and at an altitude of 1254 m asl while the site in Nungwe village was located at 2° 46′ 46″ S 32° 00′ 50″ E and at an altitude of 1139 m asl. The two sites were about 310 km apart.

Both Bunda and Geita have two rainfall peaks in a year, November–December, and March–April. However, the amount of rainfall received varies with Geita receiving more rainfall in both seasons. Bunda receives moderate rains in November–December reducing in January and peaking in March–April. This difference gives farmers in Geita two distinct sweetpotato cropping seasons whereas those in Bunda mostly rely on the March–April rains and therefore on one season of cultivation. This leads to year-round cultivation of sweetpotato in Geita while Bunda has a break during which there is no sweetpotato crop in the field. The continuous cultivation in Geita means high virus inoculum in the environment compared to Bunda where plants desiccate, and the infection cycle is broken during the dry period.

2.2. Soil analysis

Six soil samples were collected from each site and analyzed for texture and nutrient contents based on standard procedures as stipulated in the Tanzania National Soil Service manual (National Soil Service (NSS), 1990). Eight soil parameters were tested: particle size, pH, total nitrogen, organic carbon, electric conductivity, available phosphorus, exchangeable bases and cation exchange capacity (CEC). The distribution of soil particle sizes was determined using the Bouyoucos hydrometer method as described by Gee and Bauder (1986). Soil pH was measured potentiometrically in water at a ratio of 1: 2.5 (soil: water) (Thomas, 1996). Organic carbon was determined by the Walkley and

Black wet digestion method (Nelson and Sommers, 1996), total N by the micro-Kjeldahl procedure (Bremner, 1996) and available P by the Bray and Kurtz method (Kuo, 1996). Cation exchange capacity (CEC) was determined by the 1 M neutral ammonium acetate saturation method (Sumner and Miller, 1996). Exchangeable Mg and Ca in the ammonium acetate filtrates were determined by atomic absorption spectrophotometry and K and Na contents by flame photometry (Sumner and Miller, 1996).

2.3. Sourcing of planting material

Cleaned-up, virus-tested planting material sourced from a pre-basic seed vine production screen house at the Tanzania Agricultural Research Institute (TARI) - Ukiriguru and healthy-looking material obtained from farmers' fields were used in this experiment. The pre-basic seed vines multiplied at TARI had been obtained four months earlier from the Kenya Plant Health Inspectorate Service (KEPHIS) Plant Quarantine Station at Muguga, Kenya following virus cleaning via thermotherapy and meristem tip culture, and virus testing via polymerase chain reaction. The farmer-sourced material of both varieties had been under field propagation for four seasons (information from the farmer who provided the material). Two varieties were selected based on their level of resistance to sweet potato virus disease. Cv. Ejumula is highly susceptible to SPVD and does well in low-virus-pressure zones whereas cv. Kabode is moderately resistant and adaptable to most areas.

2.4. Experimental set up and field management

A split-plot design with source of planting material in main plots and the two varieties in subplots was used to minimize the rapid spread of viruses from farmer-sourced to clean virus-tested plants. There were four replications leading to eight main plots and 16 subplots per site. Each subplot measured 3 m × 3 m and comprised of three ridges each measuring 3 m × 1 m. These were planted with thirty cuttings measuring 30 cm long at a spacing of 1 m (between ridges) × 0.3 m (between plants). The subplots were separated by 2 m. Harvesting was done at 120 days after planting. The experiment was repeated for five seasons using planting material recycled from the previous crop.

The experiments followed the local sweetpotato growing seasons meaning that they coincided with the rain seasons therefore not requiring irrigation. However, provisions for irrigation were made to mitigate against erratic rains. No manure or mineral fertilizers were used during the entire experiment. Weeding was done manually by scouting and removing any emerging weeds.

2.5. Field data collection and leaf sampling

Root harvesting was conducted 120 days after planting and the yield per subplot determined by weighing all the roots using a digital scale. The total vine yield per subplot was also determined by weighing all the vines. Ten leaf samples per subplot were randomly collected in each season for virus testing via reverse transcriptase polymerase chain reaction (RT-PCR) for potyviruses, RT-qPCR for SPCSV and PCR for begomoviruses. Each sample comprised of three leaves cut from the top, middle and bottom of a plant. These were collected into coffee-filters and put in zip-lock bags containing silica gel to dehydrate them and prevent rotting.

2.6. Molecular diagnostics for common sweetpotato viruses

The following viruses were targeted in testing: the genus *Potyvirus* - sweet potato feathery mottle virus (SPFMV), sweet potato virus G - SPVG, sweet potato virus C - SPVC, sweet potato virus 2 – (SPV2); genus *Geminivirus* - begomoviruses (generic primers) and genus *Crinivirus* - sweet potato chlorotic stunt virus (SPCSV). Each diagnostic run included three levels of control: positive – for the respective virus; healthy

sweetpotato plant RNA/DNA control and non-template control. The primers used for the various viruses are listed in Table 1.

2.6.1. Total nucleic acid extraction

Isolation of total DNA and RNA was done as described by Doyle and Doyle (1990) with minor modifications. The CTAB extraction buffer comprised of 2% CTAB, 20 mM EDTA, 100 mM Tris-HCl, pH8.0, 1.0% Na sulphite and 2.0% PVP-40. Two steel balls were placed in a well labelled 2.0-ml microcentrifuge tube and 0.02 g of the sample placed inside. One ml of the CTAB buffer was added and the mixture centrifuged at 12,000 rpm for 5 min at room temperature. Seven hundred (700) µl of the supernatant was transferred into a new set of microfuge tubes. An equal volume of chloroform/isoamyl alcohol (24:1) was added and mixed thoroughly by shaking. This was then centrifuged at 12,000 rpm for 5 min and the upper aqueous layer carefully transferred to a new set of microfuge tubes without interfering with the interphase. Ice-cold isopropanol (350 µl) was added, mixed well, and left at room temperature for 15 min. The mixture was then centrifuged at 12,000 rpm for 10 min and the supernatant removed without disturbing the pellet. Five hundred (500) µl of 70% ethanol were added and the mixture centrifuged at 12,000 rpm for 5 min. The supernatant was discarded and the pellet air dried for 10–15 min. The pellet was then resuspended in 100 µl of nuclease-free water (NFW). The purity and concentration of the extracted RNA/DNA were checked using a NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific) followed by a quality check on 2% agarose gel. RNA/DNA concentrations were standardized to 100 ng/µl before use in detection assays.

2.6.2. PCR assays

2.6.2.1. Simultaneous detection of potyviruses (SPFMV, SPVC, SPVG, SPV2).
A multiplex One-Step Reverse Transcriptase (RT) PCR was used to detect the potyviruses. The Invitrogen SuperScript™ III One-Step RT-PCR with Platinum Taq kit was used. The quadruplex RT-PCR primers for the potyviruses and their expected sizes are listed in Table 1. The optimal concentrations for the primer sets were: 2.5 µl (1.25 µM) for SPGF, 0.4 µl (0.2 µM) for SPCF, 2.0 µl SPFF (1.0 µM) and 0.2 µl (0.1 µM) for SP2F in a reaction volume of 20 µl. RT-PCR master mix as recommended by the manufacturer was used. Reaction conditions were: cDNA synthesis for 45 min at 52 °C; initial denaturation at 95 °C for 5 min and 40 cycles consisting of 30 s at 95 °C, 1 min at 55 °C, 1 min at 72 °C, and final extension 5 min at 72 °C. Runs were performed using a GeneAmp 9700 PCR (Applied Biosystems, Foster City, CA, USA). PCR products were analyzed by electrophoresis on a 1.5% agarose gel, 0.5 X TAE buffer; run at 100 V for 1.5 h, stained with GelRed and visualized using a UV transilluminator. Test samples with a distinct product size corresponding to the respective potyvirus listed in Table 1 were presumed positive.

2.6.2.2. Geminivirus (SPLCV) testing.
Sweet potato leaf curl virus was tested by PCR as described by Li et al. (2004) using sweepovirus-specific primers SPG1 and SPG2, designed to amplify a 912-bp fragment. A 25 µl reaction mix with 1X Dream Taq buffer, 2 µl DNA, 0.3 µM forward and reverse primers, 2.0 mM MgCl₂ 0.3 mM dNTPs and 1.0 U Taq polymerase was prepared. The reaction was incubated in a thermocycler machine (GeneAmp 9700 PCR (Applied Biosystems, Foster City, CA, USA) using the following conditions: initial denaturation at 95 °C for 5 min and 40 cycles consisting of 30 s at 95 °C, 1 min at 55 °C, 1 min at 72 °C, and final extension 5 min at 72 °C. PCR products were analyzed by electrophoresis on a 1.5% agarose gel, 0.5 X TAE; run at 100 V for 1.5 h, stained with GelRed and visualized using a UV transilluminator. Samples were scored as positive or negative for begomoviruses if the 912-bp PCR amplicon expected fragment size was produced.

2.6.2.3. SPCSV testing.
One-step quantitative reverse transcription PCR

Table 1
Primers used for the detection of common sweetpotato viruses.

Name	Direction	Sequence (5' – 3')	Assay	Target	Fragment size bp
SPG-F	Forward	GTATGAAGACTCTCTGACAAATTTTG	RT-PCR	SPVG	1191
SPC-F	Sense	GTGAGAAAYCTATGCGCTCTGTT	RT-PCR	SPVC	836
SPF-F	Sense	GGATTAYGGTGTTGACGACACA	RT-PCR	SPFMV	589
SP2–F	Sense	CGTACATTGAAAAGAGAAACAGGATA	RT-PCR	SPV2	369
SPFCG2-R	Reverse	TCGGGACTGAARGAYACGAATTTAA	RT-PCR		
CL43 U	Sense	ATCGGCGTATGTTGGTGGTA	RT-PCR	SPCSV	486
CL43 L	Antisense	GCAGCAGAAGGCTCGTTTAT	RT-PCR	SPCSV	
SPG1[a]	Sense	CCCCKGTGCGWRAATCCAT	PCR	SPLCV	912
SPG2[a]	Antisense	ATCCVAAYWTYCAGGGAGCTAA	PCR	SPLCV	
SPCSV-Uni-E	Sense	CGGAGTTTATTCCCACYTGTYT	RT-qPCR	SPCSV	
SPCSV-Uni-E	Antisense	GGGCAGCCYCACCAA	RT-qPCR	SPCSV	
SPCSV-Uni-E-P	Probe	[FAM]-TCTGTCACGGCTACAGGCGACGTG-[TAMRA]	RT-qPCR	SPCSV	

[a] Primers set SPG1 and SPG2 (Li et al., 2004) can also detect other SPLCV-related sweetpotato begomoviruses (known as 'sweepoviruses') and the sizes of amplified products may be different from 912 bp. All primer sets apart from SPG1 and SPG2 were designed internally within the International Potato Center (CIP).

(RT-qPCR) was used for the detection of sweet potato chlorotic stunt virus (SPCSV) – East African strain (EA). TaqMan One Step PCR Master Mix kit (Applied Biosystems) was used. A 25 μl reaction mix containing 2 μl of template RNA, 0.4 μM each of forward and reverse primer, 0.2 μM TaqMan probe, 12.5 μl of the 2 × Master Mix (Applied Biosystems), MMLV (2U/ul) and 10.45 μl nuclease free water (NFW) was prepared. The following real-time PCR thermal cycling conditions were used: 42 °C for 42 min (cDNA synthesis) and 95 °C for 10 min (hot start activation), followed by 40 cycles of denaturation at 95 °C for 30 s and annealing/extension at 55 °C for 1 min. All samples were run in triplicate. Results for SPCSV-EA reactions were verified after confirming that all the controls were valid. Samples producing a FAM Ct in the range of ≤36 were considered positive for SPCSV-EA.

2.6.2.4. Determination of virus incidence. The number of samples testing positive for various viruses was established through counting and the virus incidence at 95% confidence interval was calculated as follows:

$$\frac{\text{infected samples}}{\text{sample size}} \pm \Phi^{-1}\left(0.0975\sqrt{\frac{\text{infected samples/sample size} * (1 - \text{infected samples/sample size}) * (\text{population size} - \text{sample size})}{(\text{sample size} * (\text{population size} - 1))}}\right)$$

2.7. Data analysis

All data was analyzed in the R program (*version 4.0.5*). The following variables were included in this analysis: root and vine yields, the percentage incidences of SPCSV, SPFMV, SPLCV, and co-infection with SPFMV and SPCSV, i.e., SPVD, simultaneously. We estimated descriptive statistics for each of these variables and disaggregated these estimates by sweet potato variety and sources of vines over a period of 5 planting seasons. The *psych* package was used to estimate descriptive statistics. Figures were created with the package *ggplot2*. We used an analysis of variance to identify the influence of the varieties, sources of vines, and seasons on root and vine yields, and virus incidences under high and low virus conditions. Assumptions of the analysis of variance were evaluated using QQpplots, and residual plots. We used generalized linear models to estimate the influence of varieties, sources of vines, and seasons on the incidence of viruses and yields (Equation (1)). Statistical significance was evaluated at the 0.05 level.

Root yield, vine yield, Virus incidence = Variety + Sources of vines + Season + E

(1)

Generic equation to determine the incidence of the variety type, sources of vines and Seasons on the root and vine yield. Variety represents the two variety types used in the experiments. Sources of vines represent cleaned-up, virus-tested seed and farmer-sourced seed. Season is a continuous variable and represent the number of on-farm propagation cycles. E describes the error of the model.

3. Results

3.1. Soil parameters

The Geita site was dominated by sand particles and grouped as sandy loam (SL) to sand clay loam (SCL) textural class whereas clay (C) soil dominated in Bunda. This indicates that the two sites differed in their physical and mineralogical properties. The soil pH range in Geita site was 5.92–6.98 indicating moderately acidic to almost neutral pH. These extreme ends indicate less possibility of aluminium (Al) or manganese (Mn) toxicity. At the Bunda site the soil pH was between 5.73 and 7.08 which also ranges from moderately acidic to neutral. Organic carbon (OC), which is a measure of organic matter in the soil was low to high in Geita, ranging between 0.77% and 2.11%. The organic carbon in Bunda was between 1.09% and 1.93% which is moderate to high. Total nitrogen (N) was at low to medium levels in both sites ranging from 0.08% to 0.18% in Geita and 0.08%–0.16% in Bunda. The levels of available phosphorus (P) were moderate to very high in both sites ranging from 12 mg/kg to 29 mg/kg in Geita and from 14 mg/kg to 86 mg/kg in Bunda. Exchangeable potassium (K) in both sites was below 0.2 cmol (+)/kg which is very low. Exchangeable calcium (Ca) was between very low (0.89 cmol(+)/kg) to high (19.36 cmol(+)/kg) in Geita and between very low (0.37 cmol(+)/kg) and low (4.68 cmol(+)/kg) in Bunda. In both sites, exchangeable magnesium (Mg) ranged between very low (below 0.3 cmol(+))/kg and low (0.3–1.0 cmol(+)/kg. The cation exchange capacity (CEC) in Geita ranged between 11.2 cmol(+)/kg and 24.4 cmol(+)/kg which is low to moderate. Bunda also had a low to moderate CEC ranging from 11.1 cmol(+)/kg to 16 cmol(+)/kg. The carbon/nitrogen ratio was normal at both sites i.e., Geita (11.61) and Bunda (11.71). A carbon/nitrogen ratio of 10–12 is normal for arable soils.
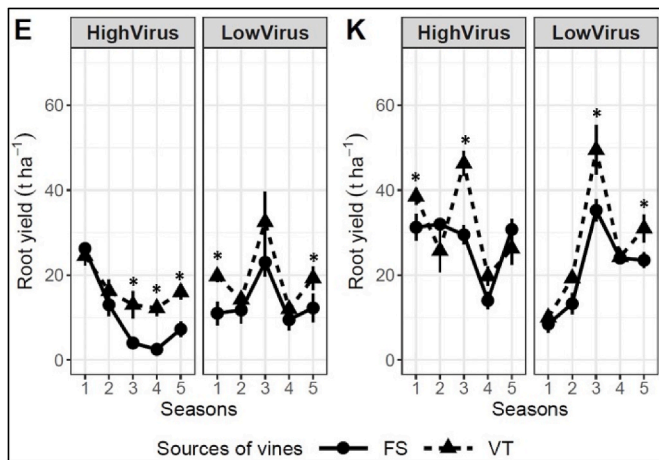
Fig. 1. The trend in root yields for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula (E) and the more resistant cv. Kabode (K), recycled over five seasons in high- and low-virus-pressure environments. (* *means statistically different at P < 0.05*).

3.2. Root yields

Cleaned-up, virus-tested seed vines of the susceptible cv. Ejumula planted in the high-virus-pressure site performed better than farmer-sourced material with significant differences in Seasons 3 to 5 (Fig. 1). Across the seasons, there was very minimal reduction in root yield, irrespective of variety, source of vines and agroecology. Season 3 was an outlier with highest root yield for the tolerant cv. Kabode in both sites and the susceptible cv. Ejumula in the low-virus-pressure site. Kabode variety did much better than Ejumula at both sites regardless of the source of vines. In terms of univariate analysis between root yield and source of vines, the clean virus-tested (VT) vines outshone those sourced from farmers (FS). Root yields at the high-virus-pressure site were significantly influenced by variety, the sources of vines, and the on-farm propagation cycles (p < 0.05). No interactions were identified. Under the low virus conditions, root yield was affected by the interaction between variety and seasons (p < 0.01), and by the sources of vines (p < 0.01).

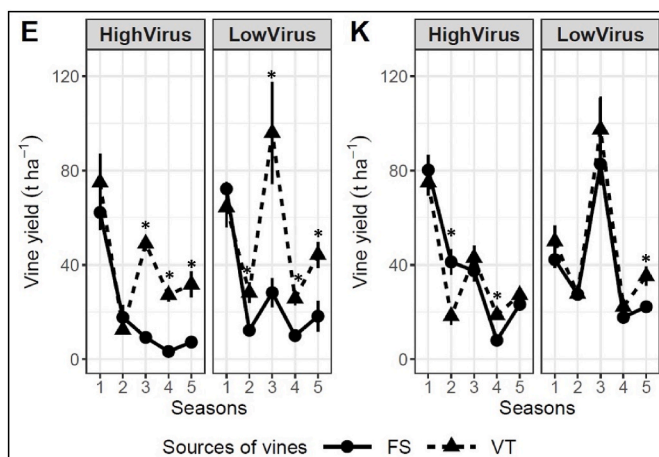### 3.3. Vine yields

In general, cleaned-up, virus-tested planting material of the susceptible cv. Ejumula had higher vine yields than farmer-sourced planting material in both the low- and high-virus-pressure environments (Fig. 2). This was significant for Seasons 3 to 5 at the high-virus-pressure site and Seasons 2 to 5 at the low-virus-pressure site. There was a season-to-season decline in the weight of vines produced by farmer-sourced planting material of this variety at both sites. Cv. Kabode recorded no differences in vine yields for both cleaned-up, virus-tested planting material and farmer-sourced planting material at both sites. A season-to-season decline in vine yields was recorded for both farmer-sourced and virus-tested planting material of cv. Kabode at the high-virus-pressure site, with Season 3 being an outlier. An interaction between the varieties and the sources of vines was observed under high virus conditions (p = 0.01). Under low virus conditions, vine yield was affected by the effect of the seasons (p < 0.01).

3.4. Virus incidences

3.4.1. Incidences of sweet potato chlorotic stunt virus

There was rapid infection of both farmer-sourced and clean virus-tested seed of the susceptible cv. Ejumula with sweet potato chlorotic stunt virus (SPCSV) at both high- and low-virus-pressure sites (Fig. 3). This cultivar was infected from the first season, with farmer-sourced vines recording significantly higher incidences compared to clean virus-tested seed. By the third season infection had reached 100% at the high-virus-pressure site for FS vines. The SPCSV incidences increased gradually with seasons for virus-tested seed of cv. Ejumula at both sites and cv. Kabode at the high-virus-pressure site. The more resistant cv. Kabode had lower SPCSV incidences at both sites compared with cv. Ejumula. The incidence of SPCSV was statistically significant between varieties (p < 0.001) and sources of vine (p < 0.05) for plants grown under high-virus conditions. This was also observed under the low-virus conditions, with significant differences between varieties (p < 0.001) and the sources of vines (p < 0.01).

3.4.2. Incidences of sweet potato feathery mottle virus

Under high-virus conditions, the incidence of SPFMV was influenced by the variety with cv. Ejumula recording higher incidences than cv. Kabode (p < 0.05) (Fig. 4). Under low-virus conditions, the percentage of plants infected with this virus was influenced by the seasons (p < 0.05). Incidences of sweet potato feathery mottle virus (SPFMV) for cv.
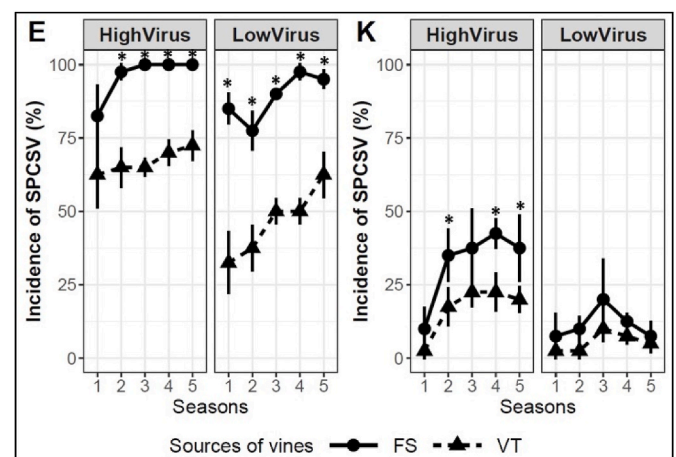

Fig. 2. The trend in vine yields for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula (E) and the more resistant cv. Kabode (K), recycled over five seasons in low- and high-virus-pressure environments. (* *means statistically different at P < 0.05*).

Fig. 3. Trend in sweet potato chlorotic stunt virus (SPCSV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula (E) and the more resistant cv. Kabode (K) recycled over five seasons in low- and high-virus-pressure environments. (* *means statistically different at P < 0.05*).
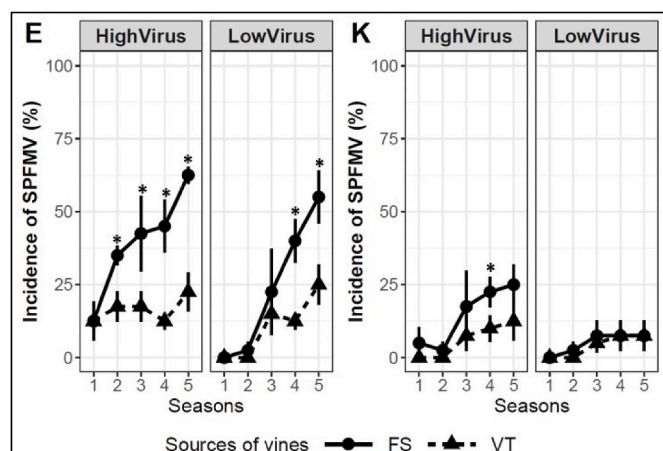
Fig. 4. Trend in sweet potato feathery mottle virus (SPFMV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula (E) and the more resistant cv. Kabode (K) recycled over five seasons in low- and high-virus-pressure environments. (* *means statistically different at P < 0.05*).

Fig. 6. Trend in sweet potato virus disease (SPVD) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula and the more resistant cv. Kabode recycled over five seasons in low and high-virus-pressure environments. (* *means statistically different at P < 0.05*).
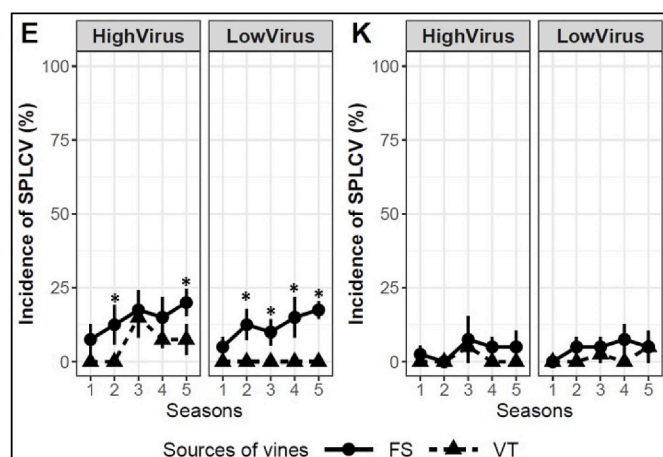

Fig. 5. Trend in sweet potato leaf curl virus (SPLCV) incidences for farmer-sourced (FS) and virus-tested (VT) planting material of the susceptible cv. Ejumula (E) and the more resistant cv. Kabode (K) recycled over five seasons in low- and high-virus-pressure environments. (* *means statistically different at P < 0.05*).

Ejumula increased gradually across the seasons. Higher incidences were recorded for farmer-sourced material compared with virus-tested material. Cv. Kabode had lower SPFMV incidences compared to cv. Ejumula at both sites.

3.4.3. Incidences of sweet potato leaf curl virus

Higher incidences of sweet potato leaf curl virus (SPLCV) were recorded at the high-virus-pressure site for cv. Ejumula compared with the low-virus-pressure site (Fig. 5). Farmer-sourced planting material of both varieties was more infected with SPLCV compared with clean virus-tested seed. Virus-tested seed of cv. Ejumula grown at the low-virus-pressure site was not infected by SPLCV throughout the experiment.

3.4.4. Incidences of dual infection with SPFMV and SPCSV

Some plants were infected with both SPFMV and SPCSV. When this happens, the plant is considered to have the disease complex known as sweet potato virus disease (SPVD). Highest incidences of dual infection with SPFMV and SPCSV, i.e., SPVD were recorded at the high-virus-pressure site for farmer-sourced planting material of cv. Ejumula
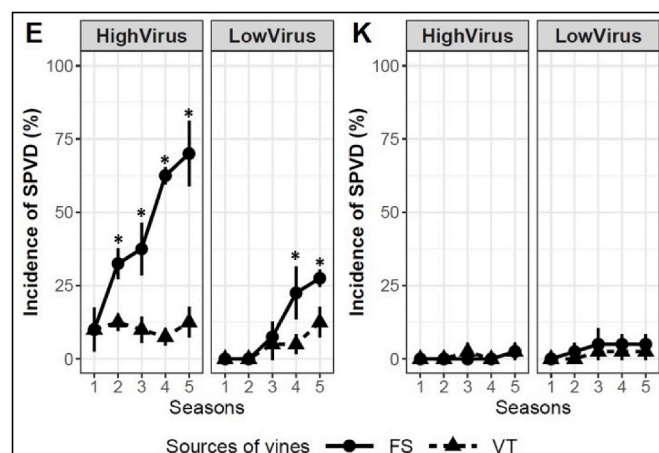
(Fig. 6). This increased gradually with season and was significantly higher compared with SPVD incidences on clean virus-tested planting material of the same variety (p < 0.05). Kabode variety recorded very low SPVD incidences at both sites regardless of the source of planting material.

3.4.5. Incidences for the potyviruses SPV2, SPVC and SPVG

Sweet potato virus G (SPVG) was absent at both sites during the entire experiment. Sweet potato virus 2 (SPV2) and sweet potato virus G (SPVG) were also absent at the low-virus-pressure site, Bunda, during the entire period of the experiment. The two viruses (SPV2 and SPVG) were present in very low incidences at the high-virus-pressure site, Geita, in Seasons 4 and 5. The incidence for SPV2 was 5% in Season 4 and 8% in Season 5. SPVG was only recorded in Season 4 and at 4% incidence. These viruses were mostly recorded on the susceptible cv. Ejumula.

4. Discussion

4.1. Cleaned-up, virus-tested seed as a control strategy against sweetpotato viruses

Use of cleaned-up, virus-tested planting material is one of the best strategies in reducing degeneration in sweetpotato and has proven effective in several countries (Bryan et al., 2003; Milgram et al., 1996). The data from this research has shown that this approach has more advantage for susceptible varieties especially in areas with high virus pressure. Virus-tested seed of the more susceptible variety, Ejumula, had higher vine and root yields compared with farmer-sourced material (Figs. 1 and 2). This directly correlated with virus incidences whereby farmer-sourced planting material of the variety had higher SPCSV, SPFMV and SPLCV incidences compared with virus-tested material. The farmer-sourced material of this variety also had higher incidences of dual infection with SPCSV and SPFMV, i.e., the sweet potato virus disease complex. Okpul et al. (2011) reported that susceptible varieties are more likely to show improved vigour and performance following virus cleaning. They observed a 148% increase in total root yields in some Australian cultivars following virus cleaning. In China, Feng et al. (2000) reported a 224% increase in yields when using cleaned-up, virus-tested seed for susceptible varieties but also noted a gradual decline in yield with successive plantings. A gradual decline in yield was also observed for cv. Ejumula planted in the high-virus-pressure area in this study. This correlated with the virus incidences which increased

with progressive cycles of field propagation.

The use of virus-tested seed did not seem to have an advantage over farmer-sourced planting material for the relatively tolerant cv. Kabode. Despite virus-tested material producing higher yields than farmer-sourced material the differences were not significant. The virus incidences recorded on this variety were also lower compared to cv. Ejumula (Figs. 3–6). This confirms that the cv. Kabode is somewhat tolerant to viruses. This differed between the different viruses with the variety recording more SPCSV incidences than SPFMV and SPLCV. Genetic makeup of a variety has been reported to influence susceptibility to viruses (Mwanga et al., 2002). Varieties that have a level of resistance to sweet potato viruses have been reported to have minimal yield increase from clean seed (Valverde et al., 2007). Carey et al. (1999) reported no root yield benefits on the use of virus-tested seed for several Ugandan landraces. They attributed this to the relatively high levels of tolerance to viruses in the local landraces used in their study. The initial farmer-sourced material used in our study was also carefully selected to ensure that only asymptomatic plants were used as seed. The symptomless plants might have been healthy especially for cv. Kabode which had very low levels of virus incidences in the first season. Cultivar resistance and selection of healthy-looking plants (positive selection) as sources of planting material are also important strategies that can complement use of virus-tested seed in limiting degeneration in sweetpotato (Gibson et al., 2004; Gibson and Kreuze, 2015; Thomas-Sharma et al., 2016).

4.2. Virus incidences

Contrary to a previous report by Tairo et al. (2004), SPCSV incidences were higher than those of SPFMV at both agroecologies. Farmer-sourced planting material of cv. Ejumula had more than 70% SPCSV incidences at the end of the first season at both sites. This was significantly higher than virus-tested seed indicating that the farmer-sourced material might have already been infected with the virus. The high incidences of SPCSV on virus-tested seed of cv. Ejumula at the end of Season 1 also indicates the possibility of external sources of virus infection. Isolation of production fields from potential external sources of infection can limit virus infection. Wosula et al. (2013) recommended locating seed beds away from sweetpotato plants of unknown sources to avoid infection from external inoculum. Distances as low as 15 m have been shown to limit infection in Uganda (Gibson et al., 2004). A study conducted by Aritua et al. (1999) also showed that SPVD incidences had a direct relation with proximity to external inoculum sources. In addition, isolating clean planting material using a physical barrier such as insect-proof nets has been shown to reduce virus infections (Ogero et al., 2019).

The begomovirus, SPLCV, had the lowest incidences with higher prevalence in the high-virus-pressure area. Despite the low incidences our results indicate that SPLCV is likely an important virus threat to sweetpotato production at the Lake Zone Tanzania. Previous studies did not include SPLCV and related sweepoviruses as threats in the Lake Zone (Ndunguru and Kapinga, 2007; Tairo et al., 2004). This is because they used serological assays which did not include begomoviruses. The challenge posed by sweepoviruses to sweetpotato production is increasingly getting global recognition (Clark and Hoy, 2006; Kim et al., 2015; Wanjala et al., 2020; Wasswa et al., 2011). These viruses can cause considerable yield losses even without symptom expression on the foliage. In addition, SPLCV has been shown to amplify the effect of SPVD resulting in significant losses. Wanjala et al. (2020) reported highest severity scores and low root yields in treatments inoculated with SPVD and SPLCV for cv. Ejumula and cv. Kakamega in Kenya. The lack of symptoms can make on-farm management practices such as those suggested by Thomas-Sharma et al. (2016) difficult to implement. The symptomless nature of SPLCV and SPFMV (in tolerant varieties) may lead to spread and prevalence of the viruses because farmers are more likely to use infected material. For efficient control of sweetpotato viruses it is important to come up with affordable diagnostics that can be deployed at all stages of the seed system. Currently, seed inspections at the downstream stages are based on visual symptoms therefore mostly effective for SPVD-infected plants. This poses a risk of leaving a high reservoir of symptomless viruses in the seed multiplication plots, which can later cause SPVD (Kreuze et al., 2021). The Loop-mediated Isothermal Amplification (LAMP) is among the technologies that can be deployed easily for field-based detection of SPCSV, SPFMV and SPLCV whose assays have already been developed (Wanjala et al., 2021).

4.3. Agroecology and virus incidences

There were higher virus incidences at the high-virus-pressure site compared with the low-virus-pressure site indicating the influence of agroecological conditions and cultivation patterns on virus infections. Geita, the high-virus-pressure area, has two distinct rainfall seasons leading to continuous sweetpotato cultivation throughout the year. Being a few metres from the lake shore, the experimental site was also in a location where farmers usually conserve their planting material. Continuous sweetpotato cultivation leads to virus accumulation in an area and has also been reported in Uganda for sweetpotato (Adikini et al., 2015) and in Tanzania for cassava (Shirima et al., 2019). Moreover, it is important to consider soil characteristics in a certain area when studying the effect of viruses on root yields. The differences in physical and mineralogical properties and nutrient status of soils can also influence yields. Mineral nutrition in the soil may slow or exacerbate the effect of viruses on yields (Barker, 2009). Adding NPK fertilizer to soil has been shown to increase the rate of reversion in sweetpotato (Ssamula et al., 2019). Better fertility and mineralogical properties of the soil in Geita might have contributed to the higher yields in Season 1 compared with the Bunda site despite the high virus incidences in the former.

5. Conclusion

This study has shown that using cleaned-up, virus-tested seed is an important strategy in limiting degeneration in sweetpotato especially for susceptible varieties grown in high-virus-pressure areas. In addition, the lack of a clear yield decline when using farmer-sourced planting material of the tolerant cv. Kabode shows why farmers may be reluctant to regularly replace preferred varieties if they are tolerant to viruses. This may have a negative implication on sustainability of seed systems to deliver cleaned-up, virus-tested seed because farmers may opt to recycling after the initial purchase. It is important to continue creating awareness among farmers that different varieties behave differently in different agroecologies and that tolerance to viruses may vary. Our findings also indicate that the prevalence of SPCSV and SPLCV at the Lake Zone Tanzania might be higher than previously thought. Surveys using sensitive molecular-based diagnostic procedures may help provide more insight on the current prevalence of various sweetpotato viruses in Tanzania. Clear mapping of high- and low-virus-pressure areas can help in targeting control strategies.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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