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DEVELOPMENT OF A MOLECULAR-BASED DETECTION TOOL FOR SWEET POTATO LEAF CURL VIRUSES AND DETERMINATION OF THEIR INCIDENCE LEVELS IN TANZANIA

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ABSTRACT

Sweet potato (*Ipomoea batatas* L.) is an important food security crop in sub-Saharan Africa, where its production is constrained by devastating diseases; including those caused by sweet potato leaf curl viruses (SPLCVs; *Begomovirus; Geminiviridae*). The objective of this study was to develop a molecular-based diagnostic detection tool for SPLCVs, as well as to generate information on their geographical distribution and incidence in Tanzania. A comprehensive survey of SPLCVs was done in all major sweet potato growing areas in Tanzania. Incidences of SPLCVs and their causative diseases were determined visually and by polymerase chain reaction (PCR), using primers designed, optimised and validated. DNA was extracted from 4166 sweet potato leaf samples and detection of SPLCVs was done. Visual incidence of disease symptoms ranged from 0 to 100%; while PCR-based incidence of SPLCVs ranged from 0 to 60%. The highest mean PCR-based incidence of SPLCVs was 32%. SPLCVs occurred in all sweet potato growing areas. There was a negative correlation between the visually assessed incidence of disease symptoms and PCR-based incidence of SPCLVs ($r = -0.122$ and $R^2 = 0.012$). A weak positive correlation between altitude and PCR-based incidence of SPLCVs was also found. In ten-fold serially diluted sweet potato DNA samples, using our new primer pair d1-SPLCVF/d1-SPLCVR, the detection limit of SPLCVs was at the dilution of 10^{-3}. The youngest fully expanded leaf of the sweet potato plant was the best for PCR detection of SPLCVs. These findings will be useful for strategic deployment of planting material and conducting sweet potato breeding experiments for resistance against SPLCVs.

Key Words: *Begomoviruses*, detection primers, *Ipomoea batatas*

RÉSUMÉ

La patate douce (*Ipomoea batatas* L.) est une culture importante pour la sécurité alimentaire en Afrique subsaharienne, où sa production est limitée par des maladies dévastatrices ; y compris ceux causés par les virus de l'enroulement des feuilles de la patate douce (SPLCV ; *Begomovirus* ; *Geminiviridae*). L'objectif de cette étude était de développer un outil de détection de diagnostic moléculaire pour les SPLCV, ainsi que de générer des informations sur leur répartition géographique et leur incidence en Tanzanie. Une enquête complète sur les SPLCV a été réalisée dans toutes les principales zones de culture de la patate douce en Tanzanie. Les incidences des SPLCV et de leurs maladies causales ont été déterminées visuellement et par réaction en chaîne par polymérase (PCR), à l'aide d'amorces conçues, optimisées et validées. L'ADN a été extrait de 4166 échantillons de feuilles de patates douces et la détection des SPLCV a été effectuée. L'incidence visuelle des symptômes de la maladie variait de 0 à 100 % ; tandis que l'incidence des SPLCV basée sur la PCR variait de 0 à 60 %. L'incidence moyenne la plus élevée des SPLCV basée sur la PCR était de 32 %. Des SPLCV se sont produits dans toutes les zones de culture de la patate douce. Il y avait une corrélation négative entre l'incidence évaluée visuellement des symptômes de la maladie et l'incidence des SPCLV basée sur la PCR ($r = -0,122$ et $R^2 = 0,012$). Une faible corrélation positive entre l'altitude et l'incidence des SPLCV basée sur la PCR a également été trouvée. Dans des échantillons d'ADN de patate douce dilués en série dix fois, en utilisant notre nouvelle paire d'amorces d1-SPLCVF/d1-SPLCVR, la limite de détection des SPLCV était à la dilution de 10^{-3}. La plus jeune feuille entièrement développée de la plante de patate douce était la meilleure pour la détection par PCR des SPLCV. Ces résultats seront utiles pour le déploiement stratégique du matériel de plantation et la conduite d'expériences de sélection de patates douces pour la résistance aux SPLCV.

Mots Clés : *Bégomovirus*, amorces de détection, *Ipomoea batatas*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) production is constrained by viral diseases which belong to nine families; namely *Betaflexiviridae, Bromoviridae, Bunyaviridae, Caulimoviridae, Closteroviridae, Comoviridae, Potyviridae, Geminiviridae,* and *Luteoviridae* (Clark *et al.,* 2012). In Tanzania, sweet potato viral infections have been confirmed for viruses which belong to five families; namely *Geminiviridae, Closteroviridae, Flexiviridae, Caulimoviridae, and Potyviridae (*Tairo *et al*., 2005; Ndunguru *et al*., 2009; Mbanzibwa *et al*., 2014). Currently, sweepoviruses are classified into fourteen recognised species, which include *Sweet potato golden vein Korea virus, Sweet potato leaf curl Canary virus, Sweet potato leaf curl China virus, Sweet potato leaf curl Georgia virus, Sweet potato leaf curl Sao Paulo virus, and Sweet potato leaf curl virus* (Walker *et al*., 2020). Viruses in these species are herein collectively referred to as sweet potato leaf curl viruses (SPLCVs). SPLCVs have single-stranded DNA genomes and belong to the genus *Begomovirus* in the family *Geminiviridae.* They are transmitted persistently by the insect vector, *Bemisia tabaci* Gennadius (Valverde *et al.,* 2004; Simmons *et al*., 2009), as well as by grafting. SPLCVs have been detected in sweet potato plants in different parts of the world such as in Taiwan and Japan (Onuki and Hananda, 1998), Israel (Cohen and Loebenstein, 1991), and in USA and Latin America (Lotrakul *et al*., 1998). In Africa, SPLCVs have been detected in sweet potato plants in Uganda (Wasswa *et al*., 2011), Tanzania (Ndunguru *et al*., 2009; Mbanzibwa *et al*., 2014), Kenya (Miano *et al*., 2006), South Africa (Esterhuizen *et al.,* 2012), and Sudan (Mohammed *et al.,* 2017). SPLCVs cause yield losses in susceptible sweet potato of up to 47% (Bramwel *et al*., 2020).

The increasing spread of sweet potato begomoviruses globally, has been attributed to the increase in population and distribution of their insect vectors (Valverde *et al.,* 2004; Simmons *et al*., 2009). Disease symptoms caused by begomoviruses, including SPLCVs, are leaf curl, vein yellowing, stunted growth, and vein swelling (blistering) (Arkorful and Addae-Frimpomaah, 2015). However, monitoring and surveillance of diseases caused by SPLCVs based on disease symptoms may be difficult, as only a few symptoms remain in mature plants, and most plants become symptomless probably due to a phenomenon known as reversion (Kim *et al*., 2015; Ssamula *et al*., 2019). Moreover, symptoms of diseases caused by SPLCVs are similar in many aspects to those caused by other plant viruses. According to Gibson *et al*. (2000) and Fuentes (2010), most viruses cause diseases with the symptoms: vein clearing, interveinal chlorosis, chlorotic mosaic, leaf curling, stunted growth, leaf narrowing, and distortion. Therefore, while leaf curl disease symptoms may indicate infections by SPLCVs, the identity of infecting virus must be confirmed using detection techniques other than visual assessment. This makes it necessary to develop accurate and cost-effective viral diagnostic tools; in this particular case for SPLCVs. Therefore, the present study, aimed to develop a molecular-based diagnostic tool for the detection of SPLCVs and generate information on the incidence and distribution of the same in Tanzania.

MATERIALS AND METHODS

Study areas. A comprehensive survey of SPLCVs was conducted from 2018 to 2020 in seven agricultural research zones in Tanzania as described in Bachwenkizi *et al.* (2022).

Sample collection. From each visited field, sweet potato vines were collected from 10 to 20 randomly selected sweet potato plants. In total, 4190 sweet potato vines were collected and replanted in insect-proof screen-houses at the Tanzania Agricultural Research Institute-Mikocheni Centre (TARI-Mikocheni). The coordinates of surveyed sweet potato fields were recorded using a Global Positioning System (GPS 72H; Garmin, Taiwan).

DNA extraction. Out of 4190 vines collected, 4166 vines sprouted and leaf samples were available for laboratory analysis. Therefore, DNA was extracted from 4166 sweet potato samples using a modified CTAB protocol described by Allen *et al*. (2006). DNA was re-suspended in DEPC water and stored at -80 °C. The concentration and purity were determined using a Nanodrop 2000/2000C Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The integrity of DNA in the samples was assessed visually by agarose gel (1.2%) electrophoresis stained with 0.1 μg ml^{-1} of ethidium bromide.

Designing primers. Primers were designed to the sequence of sweet potato leaf curl Sao Paulo virus isolate from Tanzania (Accession No. KF836891) and sequences of other SPLCVs retrieved from the National Center for Biotechnology Information (NCBI) GenBank. The sequences were aligned using Mega X software (Kumar *et al*., 2018); and the conserved sites in genomic sequences of SPLCVs were identified and primers designed manually. The primers used in this study were a forward primer d1-SPLCVF 5'-GATTGCAGAGGAAGATAGTGGG-3' and a reverse primer d1-SPLCVR 5'-ACGGAAGA TCTGCTAGAGGA-3'. The expected PCR product band size in gels was 558bp. Another primer pair designed in this study was a forward primer SPLCVcpF1 (5'-GAATGCTG TCCCAATTGCTGC-3') and a reverse primer SPLCVcpR1 (5'- CCTGCAACGCAGGGTCT G-3). This primer pair was used to cross-check the specificity of the previously mentioned primer pair.

Validation of the new primers. The sensitivity of the primer pair d1-SPLCVF/d1-SPLCVR in amplification of SPLCVs was evaluated using total genomic DNA extracted from two plants infected by two different isolates. The DNA samples were initially diluted to a final concentration of 50 ng μl^{-1} and then the sensitivity of the primers evaluated by determining their detection limits in polymerase chain reaction using ten folds serially diluted DNA. Ampliqon IIII PCR kit (Odense, Denmark) was used according to the manufacturer's instructions. The amplicons were analysed on a 1.2% agarose gel and visualised using UV light (BioDoc-It^{Tm} Imaging system, Cambridge, UK). To confirm that the observed bands were due to amplification of SPLCVs DNA, PCR products were excised from agarose gel and purified using Zymoclean™ Gel DNA Recovery kit (Cambridge Biosciences), following manufacturer procedures. Thereafter, purified PCR products were sequenced on both strands, using their corresponding primer pairs, at Inqaba (South Africa).

Titers of SPLCVs in different parts of plants. DNA was extracted from leaves collected from three different positions of sweet potato plants; namely top (fully opened young leaves), middle (estimated middle position), and bottom (bottom-most leaves, which had not undergone senescence). Two improved varieties, Ejumula and Jewel; and a local cultivar (Isakalyabasiani) were used in this study. These varieties were graft-inoculated and confirmed to be infected by SPLCSPV isolate TZ:shMVR7-5:2019 (Accession no. MW811206). The DNA samples were diluted to the concentrations of 2, 5, 10 ng and 50 ng. Virus detection was done using diagnostic primer pair d1-SPLCVF/d1-SPLCVR.

Data analysis. Computation of incidence and prevalence of SPLCVs, and the diseases they cause was done as follows:

Incidence of disease symptoms of SPLCVs (%) = (ni/Ni) x 100 ..... Equation 1

Where:

ni = number of sweet potato plants showing disease symptoms of SPLCVs in the field; and

Ni = total number of sweet potato plants on which observations were made.

PCR-based incidence of SPLCVs (%) = (nc/Nc) x100 ..... Equation 2

Where:

nc = total number of samples, which tested positive for SPLCVs in PCR reactions; while

Nc refers to the total number of samples analysed using PCR.

PCR-based prevalence of SPLCVs (%) = (Fw/Ft) x100 ..... Equation 3

Where:

Fw refers to the total number of sweet potato fields determined to contain at least one infected sweet potato plant; while Ft is the total number of sweet potato fields surveyed in a specified domain.

The Pearson bivariate was used to determine correlation coefficients between different variables, namely PCR-based incidence *versus* visually assessed incidence, PCR-based incidence *versus* altitude, and visually assessed incidence *versus* altitude. Then, analysis of incidence, prevalence and correlations, administrative districts were considered as sampling domains.

RESULTS

SPLCVs detection primers. In order to establish the incidence and distribution of SPLCVs in Tanzania, several pairs of primers

were developed. The primer pair d1-SPLCVF/ d1-SPLCVR, which targets the replication-associated protein (CI), consistently detected SPLCVs in both symptomatic and asymptomatic sweet potato leaf samples collected from all surveyed zones. In PCR, this primer pair yielded negative and positive results, in samples previously determined using next-generation sequencing technique to be non-infected and infected, respectively (data not shown).

Sensitivity of detection primers. The sensitivity of the primers, d1-SPLCVF/d1-SPLCVR, was evaluated by determining their detection limit by performing PCR on ten-fold serially diluted DNA (10^0 to 10^{-10}). Strong PCR product bands (558 bp) were observed on gel electrophoresis in dilutions of up to 10^{-3} (Fig. 1). Faint bands were observed at a dilution of 10^{-4}. In all experiments, no PCR product bands were observed on gel electrophoresis at the dilutions beyond 10^{-4}.

Specificity of detection primers. To confirm the specificity of the primer pair d1-SPLCVF/ d1-SPLCVR on amplification of SPLCVs, 32 amplified PCR products were purified, sequenced on both strands, and blast to NCBI database. All sequences were confirmed to be of SPLCVs (Bachwenkizi *et al.,* 2022). Furthermore, this primer pair and another pair of primer (SPLCVcpF1 5'-GAATGCTGT CCCAATTGCTGC-3'/SPLCVcpR1 (5'-CCTGCAACGCAGGGTCTG-3'), were used to detect SPLCVs on the same DNA samples. The samples, which tested positive for SPLCVs using d1-SPLCVF/d1-SPLCVR primers, also tested positive for primers SPLCVcpF1/ SPLCVcpR1. Both primer pairs did not amplify any DNA from known non-infected sweet potato plant samples (data not shown). This

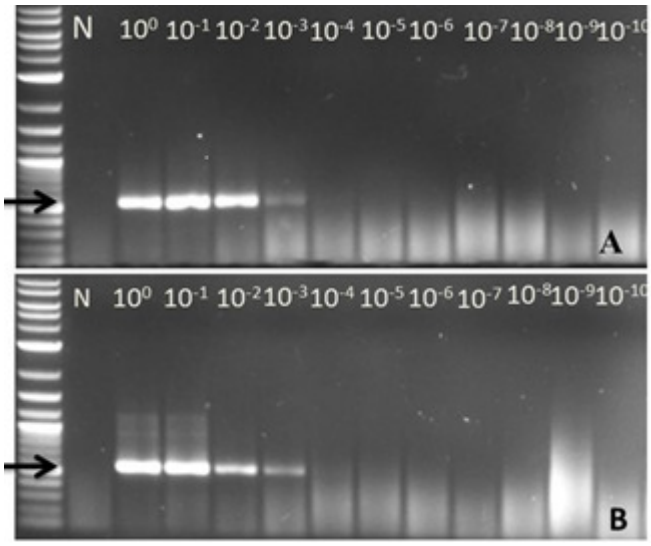

Figure 1. Determination of detection limits of primers pair d1-SPLCVF/d1-SPLCVR. (A) DNA for PCR was extracted from a plant infected with SPLCVSPV isolate TZ:shMVR7-5:2019 (Accession no. MW811206) collected from eastern Tanzania (Morogoro Region). (B) DNA for PCR was extracted from a plant infected with an isolate TZ:ARM2-4:2019 (Accession no. MW820907), which was collected from northern Tanzania (Arusha Region). Lanes labeled with an 'N' letter are for PCR products for DNA extracted from a known non-infected sweet potato sample. The arrows point to PCR product bands of the size 558bp. The DNA marker used was a 1kb Plus DNA Ladder (New England BioLabs). The other lanes are for PCR products resulting from tenfold serially diluted DNA.

indicated that our diagnostic primers specifically detected SPLCVs in sweet potato samples.

Plant part suitable for SPLCVs detection. Using graft inoculated plants of three genotypes; namely Local (Isakalyabasiani), Jewel, and Ejumula, which had been inoculated and confirmed to be infected by SPLCVs, SPLCVs were detected in all parts of sweet potato plants, which were considered (top, middle and bottom). However, detection of SPLCVs was erratic when a low concentration of DNA was used, from the bottom and middle parts of the sweet potato plants (Fig. 2). The SPLCVs were readily detected in top leaves for all genotypes and at all amounts of DNA used in PCR (2 to 50 ng per PCR reaction). Therefore, in detection works that followed, DNA was extracted from top leaves, that is, the youngest fully opened leaves.

Visually assessed incidence of disease symptoms. Visually assessed incidence of disease symptoms of SPLCVs, at field level, ranged from 0 to 100% in LZ and NZ; 0 to 95% in SHZ, 0 to 60% in WZ, 0 to 50% in EZ, 0 to 15% in SZ and 0% in CZ (Table 1). In the Coast Region, all sweet potato plants that were assessed were asymptomatic. In Mbeya Rural (SHZ), Muheza, Lushoto (EZ), Korogwe (NZ), Misungwi (LZ), Newala (SZ), Nzega (WZ) and Ikungi (CZ) districts, there were no observable virus disease symptoms on plants in all fields. Briefly, under visual assessment, all sweet potato plants in 15 out of 36 (41.6%) districts covered under this study, were asymptomatic (Table 1).

PCR-based incidence of SPLCVs. At field level, the PCR-based incidence of SPLCVs ranged from 0 to 60% (Table 1). The highest PCR-based incidence was observed in a field in Misungwi district (60%). The next highest incidence of SPLCVs was 53.3% for samples collected from Muleba district in LZ. In five districts; namely Morogoro Rural (EZ), Pangani (EZ), Bagamoyo (EZ), Karagwe (LZ) and Missenyi (LZ) districts, all analysed samples tested negative for SPLCVs. PCR detection revealed viral infections in some plants collected from fields, which were otherwise visually free from leaf curl diseases (Table 1). However, some symptomatic plant samples tested negative for SPLCVs.

Prevalence of SPLCVs. The prevalence of SPLCVs, which was computed using PCR results, ranged from 0 to 100%. The prevalence of SPLCVs was higher than 50%

Figure 2. Detection of sweet potato leaf curl viruses at three different positions in graft-inoculated sweet potato plants.

TABLE 1. Visually assessed and PCR-based incidence and prevalence of SPLCVs determined from 2018 to 2020

Zones	Regions	Districts	TS[1]	Visual based disease incidence at field level (%) SPLCVs	PCR based diseases incidence range at field level (%)	PCR based prevalence (%)	Mean altitude
EZ	Morogoro	Gairo	200	0-20	0-45	30	205.2
	Morogoro	Mvomero	200	0-15	0-20	30	440.3
	Morogoro	Morogoro Rural	40	0-15	0	0	436.3
	Dar es Salaam	Kigamboni	200	0-50	0-35	100	213.3
	Coast	Mukuranga	50	0	0-40	20	112.8
	Coast	Kisarawe	50	0	0-20	40	263.4
	Coast	Kibaha	30	0	0-50	50	174
	Coast	Chalinze	30	0	0-20	33	236
	Coast	Bagamoyo	40	0	0	0	31.5
	Tanga	Handeni	80	0-100	0-25	75	4290.1
	Tanga	Pangani	20	0	0	0	1001.7
	Tanga	Muheza	120	0	0-15	50	566
	Tanga	Lushoto	140	0	0-50	29	1524.8
NZ	Arusha	Arumeru	160	0-65	0-20	13	1127.1
	Manyara	Monduli	180	0-60	0-20	56	971.7
	Manyara	Karatu	60	0-25	0-20	67	1473
	Tanga	Korogwe	60	0	0-5	33	810
LZ	Kagera	Muleba	200	0-10	0-53.3	100	1348.3
	Kagera	Biharamulo	200	0-15	0-50	60	1379.4
	Kagera	Karagwe	100	0-50	0	0	1644
	Kagera	Missenyi	100	0-30	0	0	1175.6
	Geita	Chato	160	0-20	0-11	25	1054.4
	Geita	Geita	90	0-100	0-50	22	1251.4

TABLE 1. Contd.

Zones	Regions	Districts	TS[1]	Visual based disease incidence at field level (%) SPLCVs	PCR based diseases incidence range at field level (%)	PCR based prevalence (%)	Mean altitude
	Mwanza	Misungwi	100	0	0-60	80	1049.2
	Mwanza	Sengerema	100	0-10	0-20	20	1193.6
SHZ	Mbeya	Rungwe	200	0-55	0-21.4	20	1344.3
	Mbeya	Kyela	200	0-95	0-15	40	508.1
	Mbeya	Mbeya R	60	0	0-5	33	2064.3
	Songwe	Tunduma	40	0-10	0-15	50	1521.5
SZ	Lindi	Mtama	200	0-15	0-40	50	604.7
	Mtwara	Newala	180	0	0-10	10	700.5
WZ	Tabora	Nzega	200	0	0-35	30	1223.31
	Tabora	Urambo	140	0-20	0-20	83.3	1122.79
	Tabora	Kaliua	60	0-60	0-15	66.7	1130.29
CZ	Singida	Singida M.	60	0	0-15	66.7	1540.25
CZ	Singida	Ikungi	140	0	0-20	85.7	1558.04

[1]Indicates the total number of sweet potato samples collected from each district and on which PCR was performed

TABLE 2. Correlations between the visually assessed SPLCVs, PCR-based incidence, PCR based prevalence and altitude

	Visually assessed incidence of SPLCVs (district level)	PCR-based incidence of SPLCVs (district)	PCR-based prevalence of SPLCVs	Altitude (m)
Visually assessed incidence of SPLCVs (district level)	1			
PCR-based incidence of SPLCVs (district	-0.122 (0.515)	1		
PCR- based prevalence of SPLCVs	-0.067 (0.720)	0.813**(0.000)	1	
Altitude(m)	0.070 (0.707)	0.169 (0.362)	0.050 (0.790)	1

**Correlation was significant at the 0.05 level (2-tailed), means of parameters (altitude, prevalence, and incidence) for correlation analysis were computed at the district level (n = 36). Shown in the brackets are the P-values

in 15 districts. In five districts, all plants were free of SPLCVs. The districts without any field with infected plants were located in the coastal lowlands (2), at mid-altitude (1), and at high altitude (2) (Table 1).

Correlations between different variables. Correlation analysis was done between the visually assessed incidence of disease symptoms of SPLCVs and PCR-based incidence, visually assessed incidence of disease symptoms of SPLCVs and altitude, and PCR-based incidence and altitude (Table 2). The results showed that there was a weak positive correlation between altitude and PCR-based incidence of SPLCVs ($r = 0.169$ and $R^2 = 0.029$) (Table 2; Fig. 3). Also, there was a positive correlation between PCR-based incidence and PCR-based prevalence and between altitude and visually assessed incidence of disease symptoms of SPLCVs. However, in all three cases, the coefficient of determination (R^2) was very small (Fig. 3) indicating that the observed linear relationships were explained by other factors, which were not investigated in this study. Negative correlations were observed between PCR-based incidence of SPLCVs and visually assessed incidence of disease symptoms of SPLCVs and between PCR-based prevalence of SPLCVs and visually assessed incidence of disease symptoms of SPLCVs.

DISCUSSION

Reliability of detection primers. This work was focused on the determination of the incidence and distribution of SPLCVs and their diseases in major sweet potato growing areas in Tanzania. This was the first time a focused study on the incidence of SPLCVs was conducted for samples collected from Tanzania. Therefore, it was crucial to design primers, optimise and validate them for the detection of SPLCVs. Similarly, for the reliability of detection results, it was important to establish parts of sweet potato plants where these viruses are readily detectable. A pair of

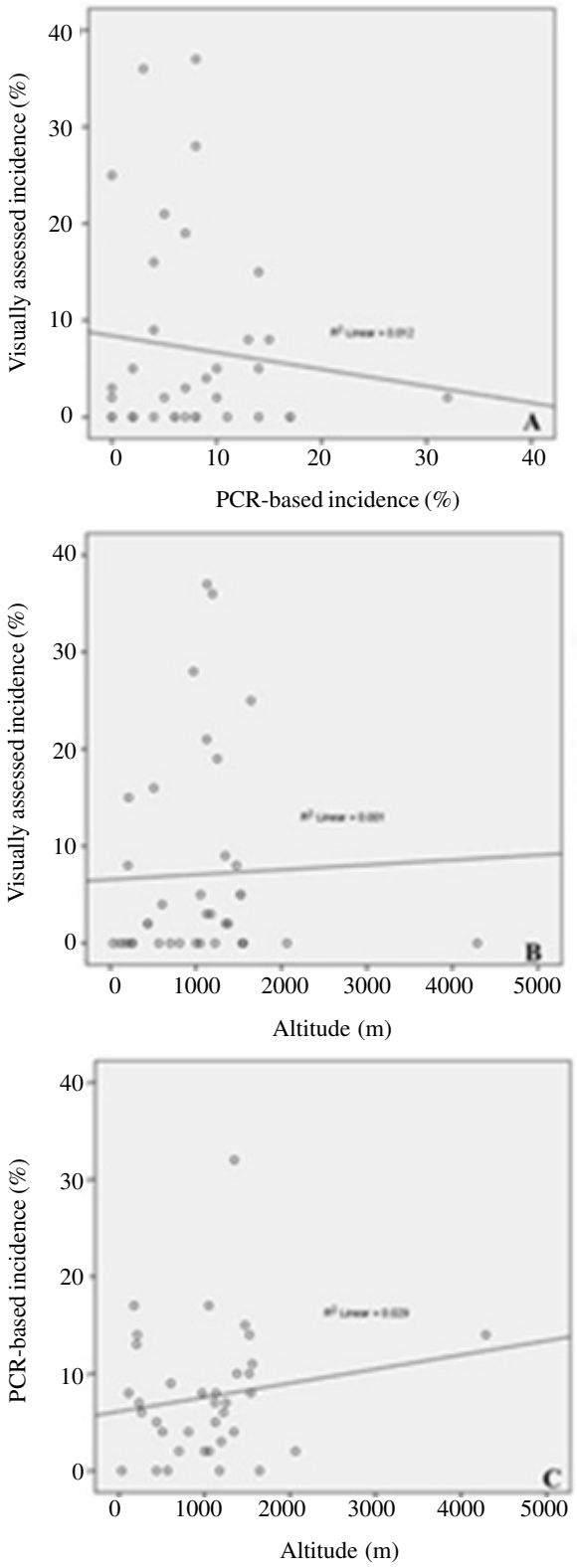

Figure 3. Scatter plots showing linear relationships between different parameters.

primers (d1-SPLCVF/d1-SPLCVR) was developed and successfully validated. Using this pair of primers, it was established that SPLCVs are reliably detected in the youngest fully opened leaves using DNA amounts of 2 to 50 ng per PCR reaction. Detection of SPLCVs using samples from the middle and bottom parts of the plants was unreliable and required at least 10 ng of genomic DNA. These results contradicted those reported by Wasswa *et al*. (2011) that SPLCUV was readily detectable in the middle and lower parts of sweet potato plants. We are, however, unable to establish the reasons for discrepancies between results from our study and those from the study by Wasswa *et al.* (2011). It is possible that the plants used in the two studies were of different genotypes, age and environmental conditions. It is likely that SPLCVs accumulate differently in different parts of plants for different genotypes. Consistent with findings from this study, the IPPC (2006) recommends sampling of young, freshly expanded leaves for detection of begomoviruses.

Sensitivity and specificity of primers. Another factor that can affect the detection of SPLCVs is the sensitivity of primers used. It was found out that the detection results, using our pair of primers, were reliable at the detection limit of up to a dilution of 10^{-3}; but amplification was also achieved at the dilution of 10^{-4}. These results were, therefore, consistent with results from another study by Aloyce *et al*. (2013), which showed that the detection limit for cassava mosaic begomoviruses was at the dilution of 10^{-3}. Therefore, the primers developed in this study can readily detect SPLCVs in sweet potato DNA samples not diluted beyond 10^{-3}. Sequencing of the PCR products obtained using the primers reported here revealed that it is only DNA of SPLCVs that was amplified (Bachwenkizi *et al.,* 2022), which confirmed specificity of the primers (IPPC, 2006). DNA from all known non-infected samples, as determined using NGS (Bachwenkizi *et al.,* 2022), did not yield any bands on gel electrophoresis; confirming the primers were specific for SPLCVs.

Incidence and distribution of SPLCVs. Understanding incidence and distribution of viruses and their diseases is the first step towards their control and management. This study has revealed for the first time the incidence and distribution of SPLCVs since their first detection in sweet potato plants in Tanzania (Ndunguru *et al*., 2009) and has confirmation through NGS sequencing (Mbanzibwa *et al.*, 2014). Our results have shown that SPLCVs are dispersed in all major sweet potato growing areas, but the levels of incidence vary significantly from one location to the other. SPLCVs have been reported on all continents where sweet potato is grown (Chung *et al*., 1985; Liang *et al*., 1990; Cohen and Loebenstein, 1991). In East Africa, SPLCVs have been detected in Kenya (Miano *et al*., 2006), Uganda (Wasswa *et al*., 2011) and Sudan (Mohammed *et al*., 2017). Indeed, in Kenya, SPLCVs have been shown to cause significant loss of root tuber yield in orange-fleshed sweet potato plants (Bramwel *et al*., 2020), which is yet to be investigated in Tanzania.

Visually assessed incidence of disease symptoms was generally higher than PCR-based incidence, which is probably due to confusion of disease symptoms in fields. However, there were many symptomless plants, which were confirmed in the laboratory to be infected by SPLCVs. This indicated that visual assessment was not a reliable method for assessing the incidence of SPLCVs in sweet potato fields. Different viruses do generally cause similar symptoms; while nutrient deficiency and edaphic factors may cause symptoms that can be confused for virus disease symptoms (Kennelly *et al.,* 2012; Arkorful and Addae-Frimpomaah, 2015). In Tanzania, it is well known that viruses, other than SPLCVs abundantly infect sweet potato (Tairo *et al*., 2005; Ndunguru *et al*., 2009; Mbanzibwa *et al*., 2014).

Based on PCR results, the incidence of SPLCVs disease was generally high in EZ and LZ compared to the other five zones. Apart from this study, many other studies done in Tanzania have reported a high incidence of other viral diseases in EZ; for instance, cassava mosaic disease (Legg and Raya, 1998) and common bean viral disease (Mwaipopo *et al*., 2018). A recent study has also shown that this zone (EZ) has the highest number of viruses in wild plants, some of which are apparently sweet potato viruses (Mwaipopo *et al*., 2021). On the other hand, the LZ borders Uganda where the occurrence of SPLCVs has been reported (Wasswa *et al*., 2011; Ssamula *et al*., 2019).

Visual assessment *versus* PCR-based determination of incidence. There was a negative correlation between the visually assessed incidence of disease symptoms and PCR-based incidence of SPCLVs (r = -0.122 and R^2 = 0.012). This is consistent with the observation that there were many symptomless plants, which were shown to be infected by SPLCVs through the use of PCR (Table 1). This piece of information is important because pathologists may wish to reduce laboratory costs by simply assessing the symptoms on plants visually. However, from the results of this study, PCR detection is an unavoidable method when assessing the status of diseases caused by SPLCVs. Similarly, correlations analysis showed that PCR-based incidence of SPLCVs could be increasing with altitude. This was in agreement with observations that generally, incidence was low in the coastal with the exception of the Kigamboni district where the incidence of SPLCVs was high. Most of the districts with high incidences were generally found in mid to high altitude (>1000 meters from sea level) areas, while districts with low incidence were generally located in low altitudes (< 1000 meters from the sea level) areas. These results contradict the previous study done by Hillocks *et al.* (1999), where high virus incidence of cassava brown streak disease was observed in low altitude. However, the results were similar to those on the incidence of Africa cassava mosaic virus (ACMV; *Begomovirus*), which was high at high elevation (Bisimwa *et al*., 2012). In other surveys, Myers *et al.* (2000) showed that incidences of bean common mosaic necrosis virus (BCMNV; *Potyvirus; Potyviridae*) were low in Southern Tanzania (high altitude), but high in Northern Tanzania and even higher in samples collected from Uganda and Kenya, suggesting that the distribution of BCMNV was related to altitude.

Visual assessment of viral disease symptoms incidence revealed that disease symptoms of SPLCVs were rare in the coastal belt; while PCR results confirmed a low incidence of SPLCVs in the areas, but with exceptions. Low incidence of SPLCVs in low altitude areas could be due to a phenomenon called reversion, which has been demonstrated in sweet potato genotypes (Ssamula *et al*., 2019). Some East African sweet potato genotypes, when exposed to high-temperature conditions, undergo reversion from viral infections including those of SPLCVs (Ssamula *et al*., 2019). According to Ssamula *et al*. (2019), reversion generally increases with increasing temperature and by improved soil nutrition. In Tanzania, high temperatures are experienced in the coastal belt where the altitudes are low and decrease towards the west where altitudes are medium (EZ) to high (SHZ, NZ, LZ and WZ).

CONCLUSION

In order to develop measures for protecting the sweet potato crop from damage caused by SPLCVs, this study developed reliable diagnostic tools and map their incidence and distribution in Tanzania. Results consistently show a readily detection of SPLCVs in the topmost parts of graft-inoculated plants, which is probably due to the higher titer of the viruses there. It is, therefore recommended that in the detection of the SPLCVs, leaf samples should

be taken from the topmost parts of the sweet potato plants. However, further studies need to be done to establish factors, which affect detection of SPLCVs in different tissues of sweet potato plants.

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