Research Article

Nadia Kèmi Assana Chabi, Pakyendou Estel Name, Ezechiel Bionimian Tibiri*, Ismail Moumouni-Moussa, Rachidatou Sikirou, Nicolas Desoignies, Valérien Amégnikin Zinsou, Fidèle Tiendrebeogo, Chaffara Léonard Antoine Afouda

Identification of viruses infecting sweetpotato (*Ipomoea batatas* Lam.) in Benin

https://doi.org/10.1515/opag-2022-0403
received January 13, 2024; accepted November 22, 2024

Abstract: Sweetpotato (*Ipomoea batatas*) production in sub-Saharan Africa is affected by viral diseases caused by several interacting viruses, including Potyvirus and Sweepovirus. This research was conducted with the aim of determining the incidence and characterizing sweetpotato viruses in Benin. A field survey was conducted in 16 districts of Benin, and 138 plant samples were collected with symptoms from 35 fields. Viruses were identified using molecular diagnoses and Sanger sequencing. The symptoms of the detected viruses were then evaluated by grafting infected sweetpotato scions onto healthy *Ipomoea setosa* plants, using two scions per plant. Eight viruses were detected from samples: cucumber mosaic virus (CMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato virus G, sweet potato feathery mottle virus, sweet potato chlorotic fleck virus, sweet potato latent virus (SPLV) with sweet potato leaf curl virus (SPLCV) and sweet potato symptomless virus 1 (SPSMV-1) predominating at 70 and 13% incidence, respectively. Co-infections and mixed infections, such as SPSMV-1 + CMV, SPLCV + CMV, SPSMV-1 + SPLCV, CMV + SPCSV, SPSMV-1 + CMV + SPLCV, and SPSMV-1 + CMV + SPLCV + SPLV + SPLCV, have been observed. This study is the first to document the significant presence of SPSMV-1 and SPLCV in sweetpotato fields in Benin. The findings could provide a valuable foundation for further research into the impact of these viruses on sweetpotato productivity in Benin. Additionally, the findings of this study could assist agricultural policymakers in developing strategies to control sweetpotato viruses in the region.

Keywords: PCR, multiplex RT–PCR, sweetpotato, viral diseases, SPSMV-1, Benin

1 Introduction

Sweetpotato (*Ipomoea batatas* Lam) is a dicotyledonous and perennial tuberous crop of the *Convolvulaceae* family. It is the third most important root and tuber crop in the world, with a production of 88,867,913 tons globally compared to 29,972,001 tons in Africa in 2021 [1]. It is a staple crop in many countries throughout the world, third after rice and wheat [2], and is known for its nutritional, medicinal, and industrial benefits [3]. Similarly, new opportunities for the crop's commercialization have been created by the creation of several functional foods and beverages via sweetpotato bioprocessing [4]. Due to its high nutritional content and exceptional adaptability to poorly fertile soils, sweetpotato has significant potential for preventing malnutrition and enhancing food production in developing countries [2]. Additionally, cultivating sweetpotato is more efficient in terms of energy and economic productivity compared to growing rice, maize, or potatoes [5].

In Benin, it is the third most eaten food after cassava and yam and is a subsistence crop during the welding period. It is farmed across the country, but mainly in the southeast and southwest regions, which produce more than half of the national output [6]. However, sweetpotato productivity has recently dropped, with values in 2020 and 2021 being 56,923 and 59,400 tons, respectively [1]. This

*** Corresponding author: Ezechiel Bionimian Tibiri,** Laboratoire de Virologie et de Biotechnologie Végétale (LVBV), Institut de l'Environnement et de Recherches Agricoles (INERA), 04 BP 8645, Ouagadougou, Burkina Faso, e-mail: ezechiel.tibiri@ujkz.bf
Nadia Kèmi Assana Chabi, Ismail Moumouni-Moussa, Valérien Amégnikin Zinsou, Chaffara Léonard Antoine Afouda: Faculty of Agronomy, University of Parakou, BP 123, Parakou, Benin
Pakyendou Estel Name, Fidèle Tiendrebeogo: Laboratoire de Virologie et de Biotechnologie Végétale (LVBV), Institut de l'Environnement et de Recherches Agricoles (INERA), 04 BP 8645, Ouagadougou, Burkina Faso
Rachidatou Sikirou: Laboratoire de Défense des Cultures (LDC), Institut National des Recherches Agricoles du Bénin (INRAB), 01BP 884, Cotonou, Bénin
Nicolas Desoignies: Phytopathology, Microbial and Molecular Farming Lab (PMMF), Centre D'Etudes et Recherche Appliquée-Haute Ecole Provinciale du Hainaut Condorcet, Ath, Belgium; University of Liege - Gembloux Agro-Bio Tech, TERRA – Teaching and Research Center, Plant Sciences Axis, Gembloux, Belgium

Open Access. © 2024 the author(s), published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.

decrease could be due to several biotic and abiotic factors, such as insect pests, virus infections, and weeds [7–9].

More than 30 viruses infect sweetpotato, and the most frequently identified are DNA viruses from the *Geminiviridae* and *Caulimoviridae* families [10,11]. Moreover, these viruses are capable of inducing both single and mixed infections [11]. The most severe viral disease globally, known as sweet potato virus disease (SPVD), arises from co-infection and synergistic interaction between the sweet potato chlorotic stunt virus (SPCSV) and the sweet potato feathery mottle virus (SPFMV) [12], with production losses ranging from 60 to 98% [13].

Viruses affecting sweetpotato plants have previously been identified in Nigeria and Ethiopia [14–16], Kenya [17], Uganda [7], Korea [18], and Burkina Faso [11]. Therefore, accurate identification and early detection of pathogens is the cornerstone of disease management for many crops. It is also known that some viruses may not show symptoms, making it difficult to identify them by visual observation of infected plants. However, molecular detection methods can provide rapid results, making them valuable for informed decision-making in disease management [19]. Multiplex PCR is highly useful in plant pathology, as multiple bacteria or viruses frequently infect the same crop or host. This methodology has proven to be a valuable tool for the detection and identification of pathogens [20].

In this study, we used molecular approaches, such as PCR, multiplex RT-PCR, and Sanger sequencing, to analyze samples collected in the main sweetpotato-producing districts of Benin. This study aims to provide preliminary results of a nationwide survey on sweetpotato viruses and their distribution in Benin, and the results are critical in establishing a national key for sweetpotato virus hotspot infections and guiding decision makers on seed distribution strategies for sustainable sweetpotato production.

2 Materials and methods

2.1 Sample collection and preparation

Surveys were conducted in the main area of sweetpotato production in Benin during the cropping season from September 2019 to April 2021. A total of 35 sites belonging to 16 districts in five agroecological zones (AEZs) were surveyed. The sampled fields were selected with the help of local traditional authorities and agricultural extensionists. In each field, two to seven symptomatic cuttings were sampled. Information such as the origin of the planting material, chemical treatment, and plant characteristics were collected. A total of 138 stem cuttings (10–15 cm long) from plants showing typical symptoms of viral disease (vein clearing, leaf curling, and stunting) were collected (Table 1). The cuttings were then grown in plastic pots containing an autoclaved mixture of soil, sand, and compost (1:1:1) and stored in the greenhouse under protection from insect vectors. Two weeks after planting, young leaves from the cuttings were collected in paper envelopes, dried at 37°C, and stored in the laboratory for virus disease detection.

Table 1: Regions, districts, number of fields investigated, number of sweetpotato samples collected, and their symptoms of viral disease analyzed in this study

AEZs	Districts	No. of fields investigated	No. of samples collected	Symptoms
AEZ II (North Benin cotton zone)	Banikoara	2	4	Vc
	Gogounou	2	2	Vc
	Kandi	1	2	Vc
AEZ IV (West atacora zone)	Matéri	1	2	Vc
	Toucoutouna	1	2	Vc; Ic; Cp
AEZ V (Central cotton zone)	Bakpérou	2	7	Vc
	Parakou	2	9	Vc; Ic
AEZ VI (bar land zone)	Abomey-Calavi	2	20	Vc; Ic; Lc
	Akpo-Missrété	2	5	Vc; Ic
	Allada	2	8	Vc; Ic
	So-Ava	3	11	Vc; M; Lc; stunting
	Bonou	2	3	Vc; Lc
AEZ VIII (fishing zone)	Come	2	6	Vc; Ic
	Klouekanme	1	2	Vc; Ic
	Dangbo	5	20	Vc; Ic
	Adjohoun	5	33	Vc; Ic; Lc; stunting

M: mosaic, Ic: interveinal chlorotic, Lc: leaf curl, Cp: chlorotic points, Vc: vein clear, S: stunting.

2.2 PCR and multiplex RT-PCR detection of viruses in sweetpotato plants

Total DNA extraction was conducted from sweetpotato upper leaves following the cetyltrimethylammonium bromide method as described by Doyle and Doyle [21]. Similarly, total RNA extraction was carried out using RNA Extracol reagent (EURx, Poland) in accordance with the manufacturer's instructions. Subsequently, the detection of ten viruses was conducted using a PCR and multiplex RT-PCR approach following the method described by Kwak et al. [22]. In this process, eight viruses were targeted via multiplex RT-PCR, with sweet potato leaf curl virus (SPLCV), SPFMV, sweet potato virus G (SPVG), and sweet potato latent virus (SPLV) in one set, as well as sweet potato chlorotic fleck virus (SPCFV), sweet potato virus C (SPVC), sweet potato virus 2 (SPV2) and sweet potato symptomless virus 1 (SPSMV-1) in a separate set. Cucumber mosaic virus (CMV) and SPCSV were identified independently using a single RT-PCR, as described by Kwak et al. [22], the primer pairs CMV-DP u and CMV-DP d2 were used for CMV, and SPCSV-uni-f1 and SPCSV-uni-r1 were employed for SPCSV (Table 2).

Furthermore, RT reactions were carried out at 42°C for 30 min in a final 5 µl reaction obtained by combining 0.5 µl of total RNA, 0.5 µl of a mixture of equal amounts of 32 µM reverse primers for four viruses, 1 µl of 5× RT buffer, 0.5 µl of 2.5 µM dNTP, 0.1 µl of BSA (10 mg/ml), 8U RNase inhibitor, 0.5U Avian Myeloblastosis Virus Reverse Transcriptase and sufficient water (H_2O) to bring the total volume to 5 µl. RT reactions were terminated by heating at 95°C for 5 min. When RT was completed, 20 µl of a solution comprising 0.5 µl of a mixture of equal amounts of 32 µM forward primers for four viruses, 5 µl of 5× PCR buffer, 2.5 µl of 25 mM $MgCl_2$, 0.4 µl of BSA (10 mg/ml), 1U Go Taq polymerase (Promega, USA), and H_2O was added to the tube containing RT products. PCR was performed in a thermal cycler (Bio-Rad, USA) with the following conditions: pre-denaturing step at 94°C for 5 min; 35 cycles of a denaturing step at 94°C for 30 s; an annealing step at 55°C for 30 s; an extension step at 72°C for 60 s; and a final extension at 72°C for 10 min. The diagnosis of the SPLCV was performed using PCR under identical conditions, as described by Kwak et al. [22]. PCR products were analyzed by electrophoresis on a 1.5% agarose gel at 100 V for 90 min and stained with ethidium bromide, and DNA bands were visualized using an ultraviolet transilluminator. The positive PCR products were sent to Macrogen Company for sequencing using the Sanger method.

2.3 Grafting based on the PCR and multiplex RT-PCR results

The results of the PCR and multiplex RT-PCR tests were used to select samples of sweetpotato grafted onto healthy *Ipomea setosa*. Grafts were derived from five samples of each plant that was individually infected with SPLCV, SPSMV-1, SPLV, SPFMV, SPCFV, SPVG, CMV, and SPCSV.

Table 2: Primers used in this study for the detection of sweetpotato infecting viruses

Virus	Primers	Sequence (5′–3′)	Loci	Size (nt)
SPLCV	SPLC-u1	TCTGCCGTCGATCTGGAACTC	2315–2335	507
	SPLC-d1	GTGCCCGCTTTGGTGGAC	2821–2803	
SPFMV	SPFMV 1-F	TACACACTGCTAAAACTAGG	9073–9092	356
	SPFMV 1-R	AGTTCATCATAACCCCATGA	9428–9409	
SPVG	SPG 3-F	CAATGCCAAATGGAAGAATAG	9945–9965	286
	SPG 3-R	GCATGATCCAATAGAGGTTTTA	10230–10209	
SPLV	SPLV 1-F	GGAGTCAGTTCAATCAATGGTA	9340–9361	184
	SPLV 1-R	AGTGGCTTTATTGGGTATGAT’	9523–9503	
SPCFV	SPCFV 2F	AGCTGCTCAAACAAGCAAGAGG	8526–8547	579
	SPCFV 2R	GCTCAAAAGTACTTTAAAACATGC	9104–9081	
SPVC	SPVC-F	ATTCTTGAATGGGATAGATCACATG	9353–9377	447
	SPVC-R	AGCTTCACGAAGCGCAGC	9799–9782	
SPV2	SPV2-F1	ATGTGTTGAACCATCAGCTGAA	9414–9435	369
	SPV2-R1	GTAACTTGCCTTGGGCTACG	9782–9763	
SPSMV-1	SPSMV-1 F1	ACCGTGTATTTGATGACGATGTAC	352–375	230
	SPSMV-1 R	GGGAAGTTCTGGTAGAACGTATC	581–559	
SPCSV	SPCSV-uni-f1	GGGAAGAMGAGAYATGGAGTTAA	4484–4506	583
	SPCSV-uni-r1	CCTTGTTACAAAGAGCGTTCCT	5066–5045	
CMV	CMV-DP u	CGTCGTGGTTCCCGCTCCG	1309–1327	474
	CMV-DP d2	AGCGCGCATCGCCGAAAGAT	1782–1763	

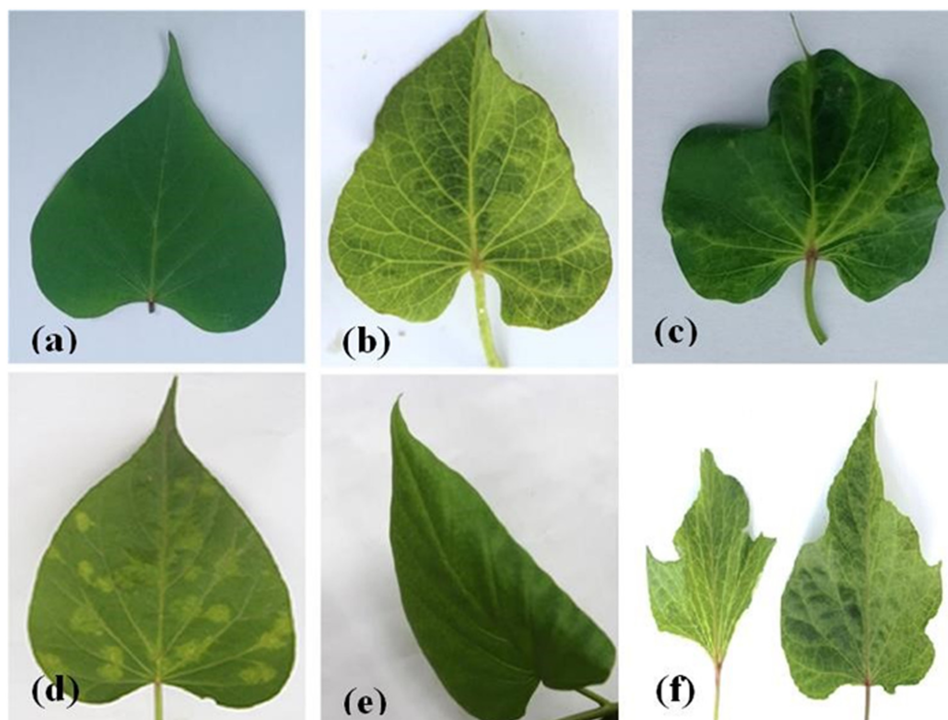

Figure 1: Symptoms observed in sweetpotato plants in the field: (a) healthy plant, (e) leaf curl downwards, (d) chlorotic spots, (b and c) vein clear, and (f) stunting.

Additionally, grafts were obtained from five samples of each plant that was coinfected with SPSMV-1 + CMV, SPLCV + CMV, SPSMV-1 + SPLCV, CMV + SPCSV, SPSMV-1 + CMV + SPLCV, and SPSMV-1 + CMV + SPLCV + SPLV + SPLCV. We also chose five plants grown from sweetpotato samples that were negative in all molecular assays. Two successfully side-grafted *I. setosa* plants were selected from each set and grown in an insect-proof, air-conditioned glasshouse at a temperature range of 25–27°C for further observation. The symptoms were recorded weekly, and *I. setosa* leaf samples were collected at 25, 45, and 60 days after grafting and subjected to the same molecular assays as the sweetpotato samples.

2.4 Sequences analysis

Sequences were cleaned and assembled *de novo* using Geneious Prime 2022 (Biomatters Ltd). The contigs obtained were submitted to BLASTn search tools in NCBI, and the pairwise sequence comparison was performed [23]. The homologous sequences were retrieved for phylogenetic analysis. Using the Muscle Algorithm in MEGA v.11.0.13, the sequences were aligned with sequences from other parts of the world and West Africa retrieved from GenBank. The evolutionary history was inferred using maximum likelihood with the Tamura-Nei model [24]. Among several models, the Tamura-Nei model provided the best nucleotide substitution fit for our dataset. Phylogenetic reconstruction was performed with bootstrap support values of 1,000. The trees were visualized and edited using Figtree v1.4.4. Maize streak virus (MSV) (AF003952.1) and tomato curly stunt virus (ToCSV) (NC_004675.1) were used to root the SPSMV-1 and SPLCV trees, respectively.

3 Results

3.1 Symptoms observed in the field

Most of the symptoms observed during the survey were vein clear (Figure 1b and c) and chlorotic spot (Figure 1d) in AEZ II and IV, leaf curling (Figure 1e), and stunting (Figure 1f) in AEZ VI and VIII.

3.2 Detection of viruses and incidence of the disease

Molecular detection revealed that 89.13% of the samples were infected with the above-mentioned viruses. These

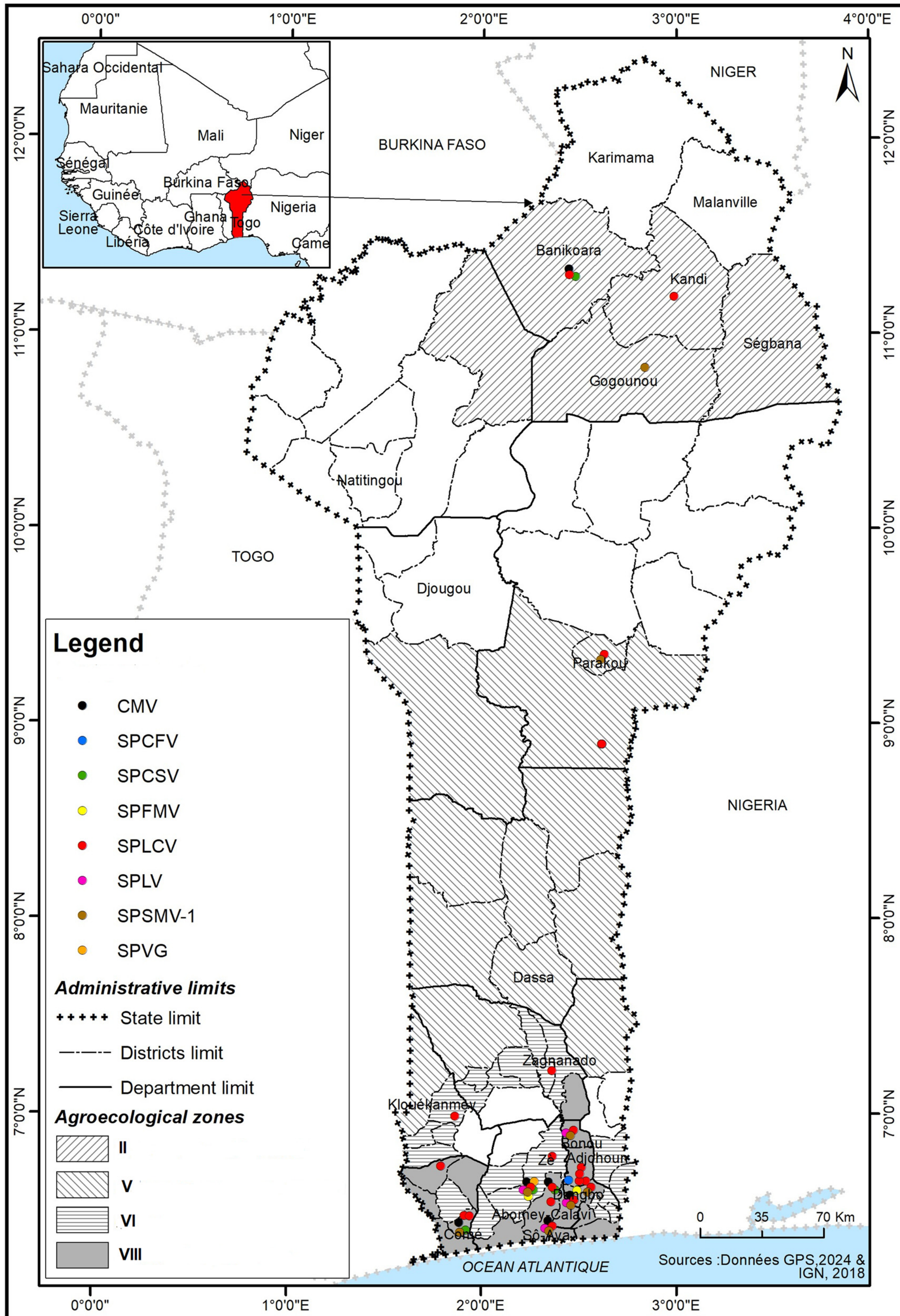

Figure 2: Map of Benin, showing the locations where viruses were detected in this study. Created using QGIS software (v3.26.3).

samples were infected with SPLCV (69.91%), SPSMV-1 (13%), CMV (8.13%), SPLV (4.06%), SPFMV (2.43%), SPVG (0.81%), SPCFV (0.81%), and SPCSV (0.81%). SPLCV was identified in all AEZs under investigation, whereas SPSMV-1 was only present in AEZ VI (12.5%) and AEZ VIII (6.25%) (Figure 2). The SPLV infection occurred at 20% in AEZ II and 40% in AEZ VIII. SPFMV was detected in 33.3% of the samples from AEZ VIII. SPCFV and SPVG were detected only in AEZ VI and VIII. Co-infections such as SPSMV-1 + CMV and SPSMV-1 + CMV + SPLCV were detected in AEZ VI at a rate of 8.33%. In AEZ VIII, co-infections detected were SPSMV-1 + SPLCV and SPLCV + CMV at a rate of 5.26%. The following viruses were detected in AEZ VI (3.03%): CMV + SPCSV, SPSMV-1 + CMV + SPLCV, and SPSMV-1 + CMV + SPLCV + SPLV + SPLCV.

3.3 Transmission of virus to *Ipomea setosa*

Grafting samples with unique infections or co-infections (as detected by PCR and RT-PCR) onto *I. setosa* enabled the successful transmission of three viruses, namely SPLCV, SPSMV-1, and SPFMV, as detected by molecular assays. This allowed us to assess the plants for viral symptoms. Five of the SPLCV-positive samples (RB025, RB036, RB068, RB084, and RB123) and three SPSMV-1-positive samples (RB022, RB032, and RB061) confirmed positivity for *I. setosa*, whereas two samples (RB017 and RB095) previously positive for these two viruses displayed no symptoms on *I. setosa*. Two cases of co-infections, SPSMV-1 + SPLCV, were confirmed to be positive for *I. setosa*. Sample RB062, positive for SPLCV + CMV, revealed the additional presence of SPFMV after grafting, while sample RB115, initially positive for SPSMV-1 + CMV + SPLCV, confirmed positivity only for SPLCV after grafting.

Twenty-five days after inoculation, symptoms became visible on all healthy *I. setosa* plants (Figure 3) that were successfully grafted. Symptoms ranged from mild to severe deformation. The most severe symptoms observed in cases with SPLCV infection were related to leaf curling (Figure 3b) and vein clearing (Figure 3d). The majority of SPLCV-infected plants stopped growing after 45 days. Plants infected with SPSMV-1 only showed symptoms of chlorosis (Figure 3c), but when co-infected with SPLCV, symptoms ranged from vein clear (Figure 3d) to severe leaf streak (Figure 3e). After 60 days, one of the SPSMV1 + SPLCV co-infected plants had more severe leaf curl symptoms (Figure 3f).

3.4 Sequence analysis

The BLASTn search with 18 SPLCV contigs of 900 nucleotides in length and 8 SPSMV-1 contigs of 200 nucleotides in length from Benin (Table 3) were compared, respectively,

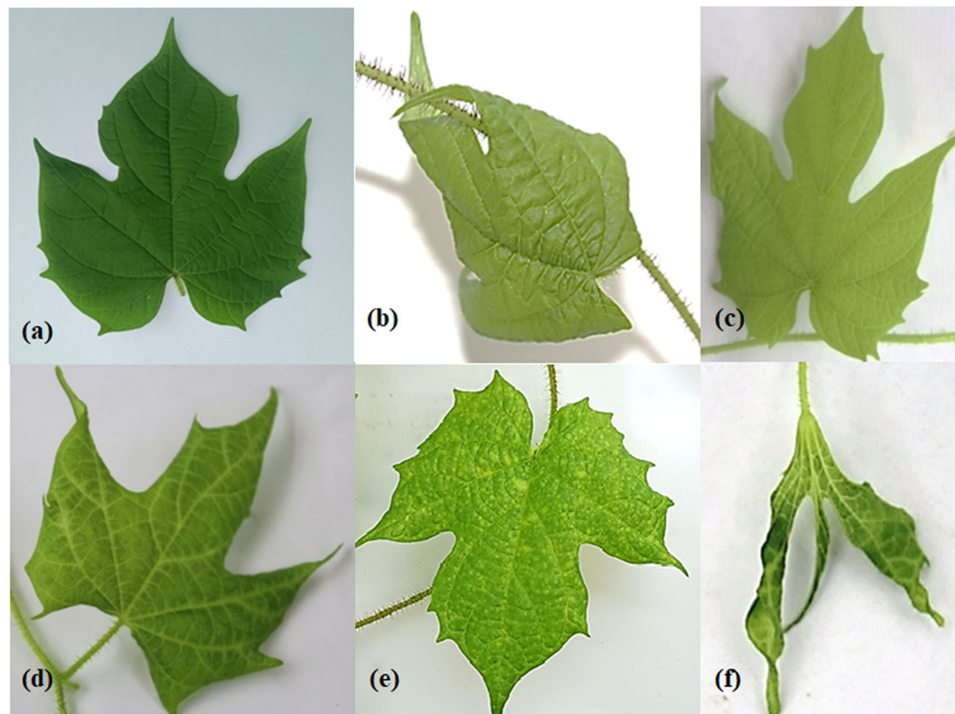

Figure 3: Foliar symptoms of *Ipomoea setosa* infected with (a) healthy plant; (b) SPLCV, (c) SPSMV-1; (d) SPLCV + SPSMV-1; (e) SPLV + SPLCV + SPSMV-1 + SPFMV; and (f) SPSMV-1 + SPLCV + SPVG + SPLV + SPFMV.

with 24 SPLCV and 13 SPSMV-1 isolates from other geographical locations in the phylogenetic analysis. The phylogenetic tree revealed three main groups in SPLCV sequences. The isolates from China (MH602269) and USA (AF104036) share 99% nucleotide identity with the isolates RB025, RB123, RB091, RB133, RB020, and RB063 from Benin, which are classified as Group 1. In Group 2, isolates from USA (HQ333138.1), Argentina (JQ349087), Puerto-Rico (DQ644562), Burkina-Faso (LS990769), South-Korea (KT992055.1), Japan (AB433786), United Kingdom (AJ586885.2), Brazil (HQ393473.1), Spain (MW574050), Portugal (MG254543.1), Greece (KF697071.1), Peru (KC253234.1 and KC253235.1), India (KU32597), Venezuela (KF716172.1), Brazil, Sudan (KY270782), and Uganda (FR751068.1) share the same clade with isolates from Benin (RB051, RB053, RB049, RB067, RB065, RB045, and RB084).

However, the third group includes the following isolates from Benin (RB003, RB0113, RB004, RB006, and RB033), which share 97% of nucleotide identity (Figure 4).

In the case of SPSMV-1, the sequences are divided into two groups. The first group includes isolates from Brazil (MG680260.1), Uruguay (KY565234.1), Taiwan (KY565233.1), Kenya (KY565231.1), USA (KY565232.1), USA (Q44429), and the isolates RB014, RB023, RB114, and RB124 from Benin; while the second group includes the isolates from China (KC155631.1), Spain (ON527002.1), Brazil (MH375686), Portugal (KY585237.1), and Korea (MF148248.1), which constitute a clade with the following isolates RB026, RB033, RB098, and RB138 from Benin.

These sequences are also closely related, as shown by the length of the branch linking them and the high bootstrap value of this branch (96%). This suggests that they originate from the same SPSMV-1 strain (Figure 5).

4 Discussion

Sweetpotatoes are a subsistence crop that could help reduce poverty in underdeveloped countries like Benin. However, viral infections can seriously limit the economic growth of this crop. Unlike previous studies, which were limited to visual observations in a single district of Benin, this study is the first to cover five AEZs of sweetpotato production in Benin, to use molecular tools and Sanger sequencing to identify viruses, resulting in the first virus database in the country. This study identified eight viruses, three of which

Table 3: Isolates, origin, and accession numbers of SPLCV and SPSMV-1 from Benin

Virus	Isolates	Locality	Abbreviation	Accession numbers
SPLCV	RB003	Abomey-calavi	SPLCV[RB:Ab:RB003:21]	This study
	RB004	Dangbo	SPLCV[RB:Dgbo:RB004:21]	This study
	RB006	Dangbo	SPLCV[RB:Dgbo:RB006:21]	This study
	RB020	So-ava	SPLCV[RB:Sv:RB020:21]	This study
	RB025	Adjohoun	SPLCV[RB:Adj:RB025:21]	This study
	RB035	Dangbo	SPLCV[RB:Dgbo:RB035:21]	This study
	RB045	Comè	SPLCV[RB:Co:RB045:21]	This study
	RB049	Bonou	SPLCV[RB:Bn:RB049:21]	This study
	RB051	Bonou	SPLCV[RB:Bn:RB051:21]	This study
	RB053	Dangbo	SPLCV[RB:Dgbo:RB053:21]	This study
	RB063	Comè	SPLCV[RB:Co:RB063:21]	This study
	RB065	Gogounou	SPLCV[RB:Gog:RB065:21]	This study
	RB067	Parakou	SPLCV[RB:Par:RB067:21]	This study
	RB084	Allada	SPLCV[RB:Alla:RB084:21]	This study
	RB091	Comè	SPLCV[RB:Co:RB091:21]	This study
	RB113	Dangbo	SPLCV[RB:Dgbo:RB113:21]	This study
	RB123	Toviklin	SPLCV[RB:Tv:RB123:21]	This study
	RB133	Comè	SPLCV[RB:Co:RB133:21]	This study
SPSMV-1	RB014	Adjohoun	SPSMV-1[RB:Adj:RB014:21]	OQ832050
	RB023	Allada	SPSMV-1[RB:Alla:RB023:21]	OQ832051
	RB026	Abomey-calavi	SPSMV-1[RB:Sv:RB026:21]	OQ832052
	RB033	Akpo-Missrete	SPSMV-1[RB:Akp:RB033:21]	OQ832053
	RB098	Comè	SPSMV-1[RB:Co:RB098:21]	OQ832057
	RB114	Allada	SPSMV-1[RB:Alla:RB114:21]	OQ832058
	RB0124	Abomey-calavi	SPSMV-1[RB:Ab:RB124:21]	This study
	RB138	Allada	SPSMV-1[RB:Alla:RB138:21]	OQ832059

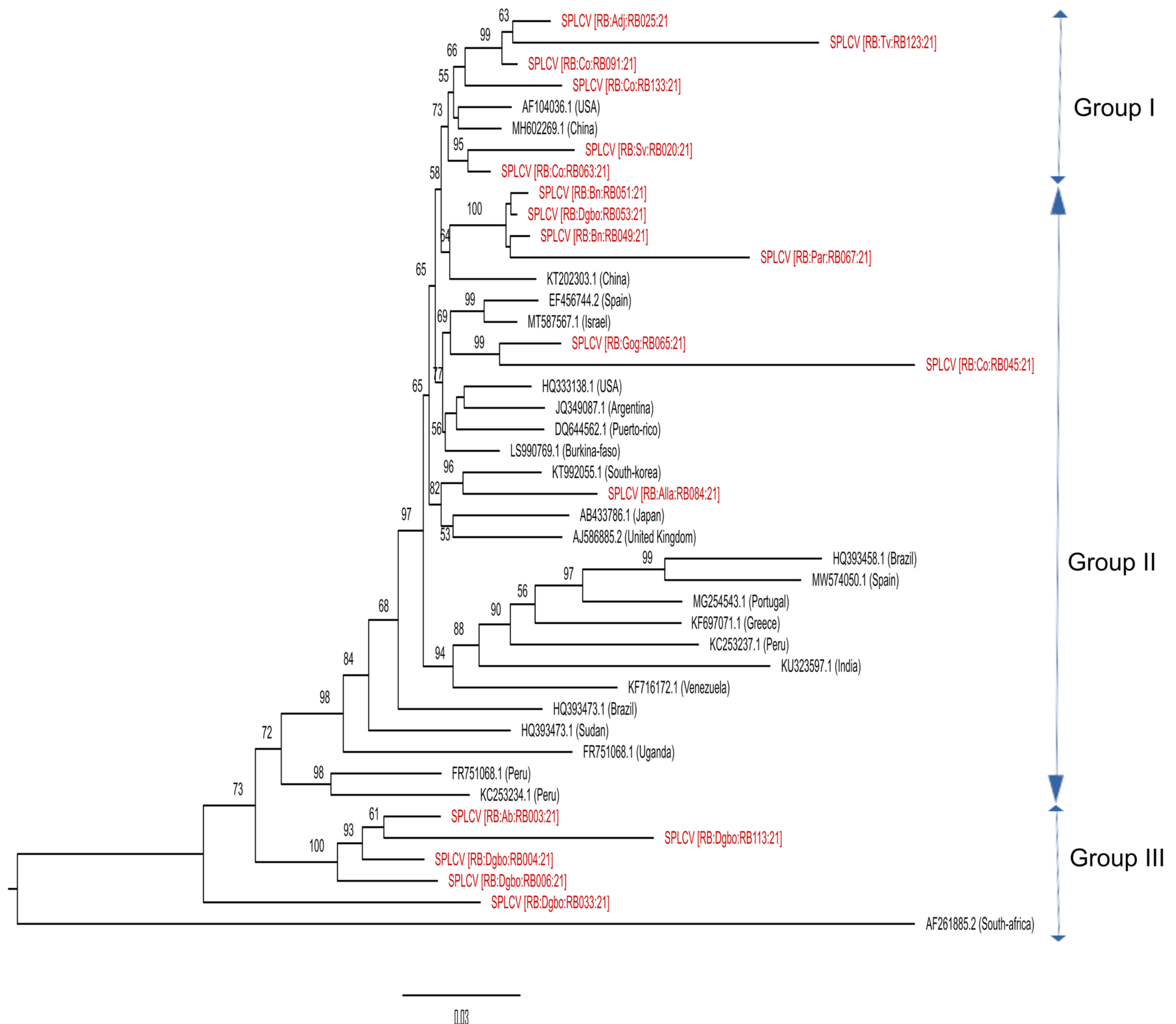

Figure 4: Maximum likelihood phylogenetic trees obtained from alignment of 24 sequences obtained from GenBank (in black) and 18 sequences obtained in this study (in red). The tree was made MEGA 11 using *ClustalW* with 1,000 replicates. Bootstrap values are percentages with values shown at the branch and rooted with the ToCSV (Genbank accession number: AF261885).

belong to the *Potyviridae* family (SPLV, SPVG, and SPFMV), two to the *Geminiviridae* family (SPSMV-1 and SPLCV), and the remaining three to the *Betaflexiridae* (SPCFV), *Bromoviridae* (CMV), and *Closteroviridae* (SPCSV) families.

SPLCV is one of the most widespread viruses discovered in sweetpotato. Around the world, it has been reported in the USA, Taiwan, Korea, Argentina, Japan, Brazil, China [25], Indonesia [26], and Spain [27]. In East Africa, it is reported in Uganda [7,28], Sudan [29], and Tanzania [17]. In West Africa, the presence of this virus was reported in Burkina Faso [11,30], and it was recorded as the second most prevalent virus detected in sweetpotatoes. In Benin, SPLCV was the predominant virus detected in key sweetpotato production regions, where local farmers cultivate the crop year-round. These regions also serve as key seed production sites for growers in other parts of Benin. This may account for the widespread distribution of SPLCV across the country. Then, Sweepovirus can cause an impact on root yields of various sweetpotato cultivars resulting in potential losses of up to 80% [31,32].

We also identified SPSMV-1 in this study, which has been documented as the second most important virus. It has been identified in countries such as China, Spain, Peru, and Brazil [17,27,33–35]. In Africa, SPSMV-1 has only been detected in sweetpotato in Tanzania [17].

Viruses such as CMV, SPLV, SPFMV, SPCSV, SPVG, and SPCFV were detected in the samples tested. Three of these

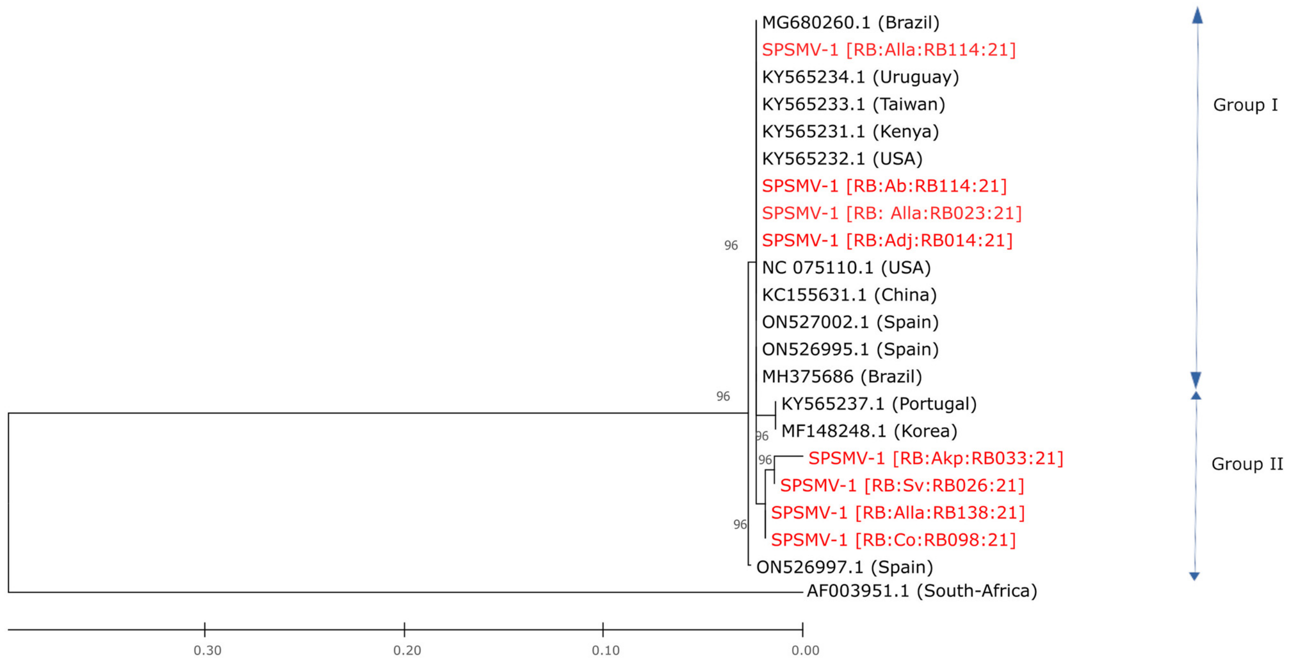

Figure 5: Maximum likelihood phylogenetic trees obtained from alignment of 13 sequences obtained from GenBank (in black) and 08 sequences obtained in this study (in red). The tree was made MEGA 11 using *ClustalW* with 1,000 replicates. Bootstrap values are percentages with values shown at the branch and rooted with the *Maize Streak Virus* (MSV) (Genbank accession number: AF003952).

viruses (SPFMV, SPVG, and SPLV) have also been detected on sweetpotato in Korea [22]. Nevertheless, according to Clark et al. [10], these viruses cause less damage in single infections than in co-infections with other viruses. Infections caused by SPLV and SPCFV could cause synergistic disease and distinct characteristics when sweetpotato is co-infected with SPFMV or a co-infection between SPVG and other *Potyviruses*, such as SPFMV [36].

Co-infection and mixed infection such as SPLCV + SPSMV-1, SPLCV + CMV, SPSMV-1 + CMV, CMV + SPCSV, SPLCV + SPSMV-1 + CMV, SPLCV + SPSMV-1 + CMV + SPLV, and SPLCV + SPSMV-1 + CMV + SPLV + SPFMV + SPVG were also detected. Tibiri et al. [11] also reported co-infections and mixed infections in their work in Burkina Faso. However, these authors did not observe any co-infections involving SPSMV-1. Co-infections of SPSMV-1 + SPLCV and SPLCV + CMV were the most frequently observed in sweetpotato plants tested in the districts of Adjohoun and Gogounou within AEZ VIII. The SPFMV + SPCSV co-infection, responsible for SPVD and widely reported globally, including in several African countries such as Burkina Faso, was not detected in this study despite the similarity of the observed symptoms. Recently, David et al. [37] reported that SPVD is not solely the result of SPFMV + SPCSV co-infection but can also involve SPCSV + SPVC.

Sequence analyses suggest that SPSMV-1 isolates RB014 and RB114 from Benin are closely related to isolates from Brazil (MG680260), Kenya (KY565232.1), and Spain (ON527002.1 and ON52002.1) and that they come from the same virus strain and can be used to study the geographical distribution and evolution of this virus. For SPLCV, there are two main groups. Those with isolates RB003 and RB113 from Benin and those with isolates from China (MH602269) and the USA (AF104036) belong to two distinct groups. These groups could represent distinct species or lineages.

The presence of three virus species was confirmed by grafting sweetpotato scions onto *I. setosa* and then using diagnostic methods; *I. setosa* also showed the symptoms associated with each virus (Figure 3). The samples identified as infected by SPLCV showed symptoms such as vein clear, leaf curling, and stunting after grafting. Tibiri et al. [30] reported similar symptoms on sweetpotato plants, respectively, in Burkina Faso.

Despite the leaf curl symptoms observed in the tested *I. setosa* plants, some samples tested negative upon PCR analysis. These symptoms could be caused by nutrient deficiency, and edaphic factors can cause symptoms that can be mistaken for a viral disease [22]. Two samples that initially tested negative for SPLCV subsequently tested positive after grafting onto *I. setosa*. This may be attributed to the increased susceptibility of this indicator plant to sweetpotato viruses, as it tends to show more pronounced symptoms due to higher virus titers, as explained by Sivparsad and Gubba [38].

In most cases, SPSMV-1 exhibited mild to moderate symptoms. This observation aligned with the results obtained by Souza et al. [35] and concluded that SPSMV-1 does not induce

significant symptoms in sweetpotato plants. This result was corroborated by grafting onto *I. setosa*, which exhibited chlorosis and mild interveinal symptoms, as observed by Wang et al. [33] following grafting with SPSMV-1-infected sweetpotato plants (Figure 3c). Consequently, farmers can plant cuttings infected with asymptomatic SPSMV-1 as propagation material for subsequent production and spread phytosanitary problems at the next harvest. In the case of co-infections, SPSMV-1 + SPLCV and SPLCV + CMV were the most common combinations, with severe symptoms of vein clearing, leaf curling, and stunting. Leaf curling was the most common symptom in SPLCV + SPSMV-1 positive samples. This suggests that symptoms caused by SPLCV may have masked those of the other two viruses. Symptoms may not reflect the necessary link between the different viral infections. Symptoms caused by SPLCV may have masked those of the other two viruses.

5 Conclusion

The presence of viruses such as SPLCV, SPCFV, SPVG, SPLV, SPFMV, and SPSMV-1 was established in Benin, with SPLCV being the most prevalent. SPSMV-1, identified to our knowledge for the first time in West Africa in this study, was also recorded in the main seed production areas of local sweetpotato accessions. This suggests that the distribution system of sweetpotato planting material may be affected by the presence of these viruses. Furthermore, symptoms induced by these viruses vary depending on the presence of single or co-infections and range from mild chlorosis or vein thinning to leaf curling or even stunting. The presence of these viruses in the main producers' area of cuttings used as seed in Benin raises concerns for sweetpotato cultivation and food security in the region. Urgent measures must be taken to limit the spread of viruses in Benin and neighboring countries.

6 Recommendations

These results suggest that there is a significant production barrier for sweetpotato, but they can be overcome with the help of researchers and stakeholders in agricultural development. As a result, the research evaluated the field effects of SPLCV and SPSMV-1, the two viruses most frequently detected in sweetpotato in Benin during this investigation. Additionally, we propose obtaining the genome sequences of these viruses to gain comprehensive insights into the isolates from Benin. We also propose obtaining the genome sequences of these viruses to gain detailed information about the isolates from Benin.

Acknowledgements: We extend our appreciation to the producers who facilitated sample collection, as well as to the members of the Laboratoire de Virologie et de Biotechnologie Végétale (LVBV) in Burkina Faso, without whom this study would not have been feasible.

Funding information: This work was funded by the Orange Fleshed Sweetpotato Project (Grant number AURG-II-2-247).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and consented to its submission to the journal. All the authors have read and approved the final manuscript. N.K.A.C.: conceptualization, investigation, data curation, and writing – original draft; P.E.N.: conceptualization, methodology, and partial analysis; E.B.T.: conceptualization, methodology, formal analysis, and writing – review and editing; F.T.:–resources, validation, visualization, review, and editing; I.M.-M.: project administration; R.S.: resources and writing – review and editing; V.A.Z.: resources and writing – review and editing; N.D.: writing – review and editing; and C.L.A.A.: supervision and validation.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] FAOSTAT. https://www.fao.org/faostat/en/#data/QCL. [Accessed 21 December 2023]. 2023. Available: http://www.fao.org/faostat.
[2] Akomeah B, Quain MD, Ramesh SA, Anand L, Rodríguez López CM. Common garden experiment reveals altered nutritional values and DNA methylation profiles in micropropagated three elite Ghanaian sweet potato genotypes. PLoS One. 2019;14:e0208214. doi: 10.1371/journal.pone.0208214.
[3] Tadda SA, Kui X, Yang H, Li M, Huang Z, Chen X, et al. The response of vegetable sweet potato (Ipomoea batatas lam) nodes to different concentrations of encapsulation agent and ms salts. Agronomy. 2022;12:19. doi: 10.3390/agronomy12010019.
[4] El Sheikha AF, Ray RC. Potential impacts of bioprocessing of sweet potato: Review. Crit Rev Food Sci Nutr. 2017;57:455–71. doi: 10.1080/10408398.2014.960909.
[5] Tang C, Lu Y, Jiang B, Chen J, Mo X, Yang Y, et al. Energy, economic, and environmental assessment of sweet potato production on plantations of various sizes in South China. Agronomy. 2022;12:1290. doi: 10.3390/agronomy12061290.
[6] Batcho A, Nwogwugwu JO, Adeyinka SO, Mohsin A, Murtaza S. Evaluation of the agronomic characteristics of 16 varieties of sweet

potato (ipomea batatas) grown in the agro-ecological conditions of Southern Benin. J Agric Sci. 2019;9:20–8. doi: 10.31901/24566535.2018/09.1-2.121.
[7] Wasswa P, Otto B, Maruthi MN, Mukasa SB, Monger W, Gibson RW. First identification of a sweet potato begomovirus (sweepovirus) in Uganda: characterization, detection and distribution. Plant Pathol. 2011;60:1030–9. doi: 10.1111/j.1365-3059.2011.02464.x.
[8] Adjatin A, Aboudou R, Loko LY, Bonou-gbo Z, Sanoussi F, Orobiyi A, et al. Ethnobotanical investigation and diversity of sweet potato (Ipomea batatas L.) landraces grown in Northern Benin. Int J Adv Res Biol Sci. 2018;5:59–73. doi: 10.22192/ijarbs.2018.05.08.008.
[9] Abrham T, Beshir HM, Haile A. Sweetpotato production practices, constraints, and variety evaluation under different storage types. Food Energy Secur. 2021;10:e263. doi: 10.1002/fes3.263.
[10] Clark CA, Davis JA, Abad JA, Cuellar WJ, Fuentes S, Kreuze JF, et al. Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. Plant Dis. 2012;96:168–85.
[11] Tibiri EB, Pita JS, Tiendrébéogo F, Bangratz M, Néya JB, Brugidou C, et al. Characterisation of virus species associated with sweetpotato virus diseases in Burkina Faso. Plant Pathol. 2020;69:1003–17. doi: 10.1111/ppa.13190.
[12] Valverde RA, Clark CA, Valkonen JPT. Viruses and virus disease complexes of sweetpotato. Plant Viruses. 2007;1:116–26.
[13] Abidin PE, Akansake DA, Asare KB, Acheremu K, Carey EE. Effect of sources of sweetpotato planting material for quality vine and root yield. Open Agric. 2017;2:244–9. doi: 10.1515/opag-2017-0026.
[14] Untiveros M, Fuentes S, Kreuze JF. Molecular variability of sweet potato feathery mottle virus and other potyviruses infecting sweet potato in Peru. Arch Virol. 2008;153:473–83. doi: 10.1007/s00705-007-0019-0.
[15] Adams MJ, Carstens EB. Ratification vote on taxonomic proposals to the ICTV (2012). Arch Virol. 2012;157:1411–22.
[16] Buko DH, Gedebo A, Spetz C, Hvoslef-Eide AK. An update of sweet potato viral disease incidence and spread in Ethiopia. Afr J Agric Res. 2020;16:1116–26. doi: 10.5897/ajar2020.14918.
[17] Mbanzibwa DR, Tairo F, Gwandu C, Kullaya A, Valkonen JPT. First report of Sweetpotato symptomless virus 1 and Sweetpotato virus A in Sweetpotatoes in Tanzania. Plant Dis. 2011;95:224. doi: 10.1094/PDIS-10-10-0707.
[18] Kim J, Yang J, Kwak H-R, Kim M-K, Seo J-K, Chung M-N, et al. Virus incidence of sweet potato in Korea from 2011 to 2014. Plant Pathol J. 2017;33:467–77.
[19] El Sheikha AF, Ray RC. Is PCR-DGGE an innovative molecular tool for the detection of microbial plant pathogens?. Vol. 6. Chichester: John Wiley & Sons Ltd; 2014. p. 409–33. doi: 10.1002/9781118533024.ch17.
[20] López MM, Llop P, Olmos A, Marco-Noales E, Cambra M, Bertolini E. Are molecular tools solving the challenges posed by detection of plant pathogenic bacteria and viruses? Curr Issues Mol Biol. 2009;11:13–46. doi: 10.21775/cimb.011.013.
[21] Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11–5.
[22] Kwak H-R, Kim M-K, Shin J-C, Lee Y-J, Seo J-K, Lee H-U, et al. The current incidence of viral disease in Korean sweet potatoes and development of multiplex RT-PCR assays for simultaneous detection of eight sweet potato viruses. Plant Pathol J. 2014;30:416–24. doi: 10.5423/PPJ.OA.04.2014.0029.
[23] Bao Y, Chetvernin V, Tatusova T. Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. Arch Virol. 2014;159:3293–304. doi: 10.1007/s00705-014-2197-x.
[24] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4. doi: 10.1093/molbev/msw054.
[25] Loebenstein G. Control of sweet potato virus diseases. Adv Virus Res. 2015;91:33–45. doi: 10.1016/bs.aivir.2014.10.005. 1st edn.
[26] Listihani L, Yuniti IGAD, Lestari PFK, Ariati PEP. First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia. Indian Phytopathol. 2022;75:595–8. doi: 10.1007/s42360-022-00489-6.
[27] Fiallo-Olivé E, García-Merenciano AC, Navas-Castillo J. Sweet potato symptomless virus 1: First detection in europe and generation of an infectious clone. Microorganisms. 2022;10:1736. doi: 10.3390/microorganisms10091736.
[28] Ssamula A, Okiror A, Avrahami-Moyal L, Tam Y, Gaba V, Gibson RW, et al. Factors influencing reversion from virus infection in sweet-potato. Ann Appl Biol. 2020;176:109–21. doi: 10.1111/aab.12551.
[29] Mohammed HS, El Siddig MA, El Hussein AA, Ibrahim FA, Navas-Castillo J, Fiallo-Olivé E. First report of sweet potato leaf curl virus infecting sweet potato in Sudan. Plant Dis. 2017;101:8–9.
[30] Tibiri EB, Tiendrébéogo F, Pita JS, Somé K, Bangratz M, Néya JB, et al. Molecular and biological features of sweet potato leaf curl virus in Burkina Faso. Acta Sci Microbiol. 2019;2:170–7.
[31] Ling K-S, Jackson DM, Harrison H, Simmons AM, Pesic-VanEsbroeck Z. Field evaluation of yield effects on the U.S.A. heirloom sweetpotato cultivars infected by sweet potato leaf curl virus. Crop Prot. 2010;29:757–65. doi: 10.1016/j.cropro.2010.02.017.
[32] Andreason SA, McKenzie-Reynolds P, Whitley KM, Coffey J, Simmons AM, Wadl PA. Tracking sweet potato leaf curl virus through field production: implications for sustainable sweetpotato production and breeding practices. Plants. 2024;13:1267. doi: 10.3390/plants13091267.
[33] Wang Y-J, Zhang D-S, Zhang Z-C, Wang S, Qiao Q, Qin Y-H, et al. First Report on Sweetpotato symptomless virus 1 (Genus Mastrevirus, Family Geminiviridae) in Sweetpotato in China. Plant Dis. 2015;99:1042. doi: 10.1094/PDIS-12-14-1358-PDN.
[34] Cao M, Lan P, Li F, Abad J, Zhou C, Li R. Genome characterization of sweet potato symptomless virus 1: a mastrevirus with an unusual nonanucleotide sequence. Arch Virol. 2017;2404:2881–4. doi: 10.1007/s00705-017-3396-z.
[35] Souza CA, Rossato M, Melo FL, Boiteux LS, Pereira-Carvalho RC. First report of sweet potato symptomless virus 1 infecting Ipomoea batatas in Brazil. Plant Dis. 2018;102:2052. doi: 10.1094/PDIS-01-18-0083-PDN.
[36] Li F, Zuo R, Abad J, Xu D, Bao G, Li R. Simultaneous detection and differentiation of four closely related sweet potato potyviruses by a multiplex one-step RT-PCR. J Virol Methods. 2012;186:161–6. doi: 10.1016/j.jviromet.2012.07.021.
[37] David M, Kante M, Fuentes S, Eyzaguirre R, Diaz F, De Boeck B, et al. Early-stage phenotyping of sweet potato virus disease caused by sweet potato chlorotic stunt virus and sweet potato virus C to support breeding. Plant Dis. 2023;107:2061–9. doi: 10.1094/PDIS-08-21-1650-RE.
[38] Sivparsad BJ, Gubba A. Identification and distribution of viruses infecting sweet potato (Ipomoea batatas L.) in KwaZulu-Natal province, South Africa. S Afr J Plant Soil. 2013;30:179–90. doi: 10.1080/02571862.2013.854415.