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Characterization of virus species associated with sweetpotato virus diseases in Burkina Faso

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Abstract
Sweetpotato (Ipomoea batatas) production in sub-Saharan Africa is severely affected by viral diseases caused by several interacting viruses, including sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), and sweet potato leaf curl virus (SPLCV). However, the aetiology of viral symptoms on sweetpotato is rarely established in most countries in Africa. Here, we aimed to investigate and characterize the incidence of sweetpotato viruses in Burkina Faso. We performed a countrywide survey in 18 districts of Burkina Faso and collected 600 plants, with and without symptoms, from 80 fields. Viral strains were identified using nitrocellulose membrane-ELISA, PCR, and reverse transcription-PCR. Three scions from each of 50 selected plants with symptoms were grafted to healthy Ipomoea setosa and then serological and molecular tests were performed on the 150 recorded samples. Three viruses were detected: 24% of samples were positive for SPFMV, 18% for SPLCV, and 2% for SPCSV. Across all diagnostic tests, 40% of all plant samples were virus-negative. Coinfections were found in 16% of samples. Partial sequences were obtained, including 13 that matched SPFMV, one that matched SPLCV, and one that matched SPCSV. All identified SPFMV isolates belonged to either phylogroup B or A-II. The SPCSV-positive isolates had 98% gene sequence homology with SPCSV-West Africa for the coat protein. Begomovirus-positive isolates clustered with SPLCV-United States. This first study of sweetpotato viral diseases in Burkina Faso indicates widespread occurrence and suggests a need for further epidemiological investigations, breeding programmes focused on virus-resistant varieties, and improved farming practices to control disease spread.

KEYWORDS
diagnostic, grafting, Ipomoea batatas, Ipomoea setosa, SPVD, sweetpotato

1 INTRODUCTION

In sub-Saharan Africa, sweetpotato (Ipomoea batatas) is the third most important root and tuber crop after cassava (Manihot esculenta) and yam (Dioscorea spp.). This highly productive crop performs well in poor soils and is relatively drought-insensitive (Tairo et al., 2005). However, sweetpotato productivity is constrained by both biotic and abiotic factors; of these, viral diseases and weevil damage are
by far the most important (Valverde et al., 2007). More than 30 viruses have been reported to infect sweetpotato, and several of these are recently described DNA viruses belonging to the families *Geminiviridae* and *Caulimoviridae* (Clark et al., 2012; Cuellar et al., 2015).

In Burkina Faso, sweetpotato is the most important root crop and ranks third, after cereals and legumes, among all crops (Dabiré and Belem, 2001). Sweetpotato production in Burkina Faso increased from 13,618 t in 1998 to 167,550 t in 2013 (FAOSTAT, 2013). In western Burkina Faso, sweetpotato is grown as a cash crop. However, because sweetpotato is propagated vegetatively, it is prone to the accumulation of viruses and other pathogens (Souto et al., 2003; Cuellar et al., 2015). Farmers can lose up to 100% of the crop yield; losses differ depending on the cultivar, the infecting virus, the stage of infection, and whether the crop is infected with one or more viruses (Valverde et al., 2007; Rey et al., 2012; Loebenstein, 2015). Given the susceptibility of sweetpotato to virus infection, coinfections are common and cause a generalized disease known as sweet potato virus disease (SPVD; Valverde et al., 2007).

Sweet potato feathery mottle virus (SPFMV; family *Potyviridae*, genus *Potyvirus*) is one of the most damaging viruses infecting sweetpotato worldwide (Moyer et al., 1980; Untiveros et al., 2008; Loebenstein, 2015). The virus is transmitted by aphids, including *Aphis gossypii*, *A. craccivora*, *Myzus persicae*, and *Lipaphis erysimi*, in a nonpersistent manner (Ward and Shukla, 1991; Loebenstein, 2015). Like many sweetpotato viruses, SPFMV can also be mechanically transmitted through grafting to *Ipomoea* spp., such as *I. batatas*, *I. setosa*, *I. nil*, *I. incarnata*, and *I. purpurea*, as well as to *Nicotiana benthamiana* and *Chenopodium amaranticolor* (Clark et al., 2012). Transmission of SPFMV by seed, pollen, or contact between plants has not been proven (Clark et al., 2012; Loebenstein, 2015). SPFMV can be diagnosed by grafting infected scions onto *I. setosa*, on which it causes vein-clearing and vein-banding (Rännäli et al., 2009; Loebenstein, 2015).

The SPFMV genome consists of a single positive-sense RNA strand with a poly(A) tail at its 3′ terminus and a genome-linked protein (VPg) at its 5′ terminus (Wylie et al., 2017). The genome contains a large open reading frame (ORF) that encodes a polyprotein and the PIPO ORF (Wylie et al., 2017). Previous studies on the diversity of the coat protein (CP) genomic region revealed four phylogenetic lineages: East African (EA) from Tanzania, Uganda, and Kenya; rust-set crack (RC) from Australia, Africa, Asia, and North America; ordinary (O) from Japan, China, Korea, Niger, Nigeria, and Argentina; and sweet potato virus C (as renamed by Adams and Carstens, 2012) from the USA, China, Australia, Africa, East Africa, and Argentina (Untiveros et al., 2008; Adams and Carstens, 2012). With increased understanding of SPFMV and the biosafety implication of its nomenclature, a neutral classification system has been proposed that uses Latinized numerals to take account of biological and geographical considerations (Jones and Kehoe, 2016). Consequently, SPFMV EA became A-I, O became A-II, and RC became B (Maina et al., 2018). SPFMV has been characterized in East Africa (Kenya, Tanzania, and Uganda) and West Africa (Nigeria and Niger), where it is a known threat to sweetpotato production (Karyeija et al., 2001; Untiveros et al., 2008).

The begomoviruses known to infect sweetpotato (“sweepoviruses”) are all monopartite and phylogenetically distinct from both the Old and New World begomovirus species (Fauquet and Stanley, 2005). In Africa, sweepoviruses have been reported only in Kenya, Uganda, South Africa, Tanzania and, more recently, in Sudan (Miano et al., 2006; Wasswa et al., 2011; Esterhuizen et al., 2012; Mbanzibwa et al., 2014; Mohammed et al., 2017). Sweepoviruses are transmitted by the whitefly *Bemisia tabaci* (Trenado et al., 2011).

Sweet potato chlorotic stunt virus (SPCSV) is a bipartite member of the family *Closteroviridae*, genus *Crinivirus*. Like other criniviruses, it is phloem-limited (Cohen et al., 1992; van Regenmortel et al., 2000; Loebenstein, 2015). SPCSV has a worldwide distribution (Karyeija et al., 1998) and is present in the main sweetpotato production areas in Africa, and usually coinfects its host plant with other viruses in the field (Mukasa et al., 2003; Tairo et al., 2004). Coinfection makes separation and purification of SPCSV difficult, thus determination of the SPCSV genomic sequence has been greatly constrained (Qin et al., 2014). To date, only two SPCSV strains have been identified: SPCSV-East African (SPCSV-EA) and SPCSV-West African (SPCSV-WA). The SPCSV-EA strain is more widespread and more studied than SPCSV-WA. Studies have shown that most sweetpotato-infecting viruses can cause severe synergistic disease complexes when they coinfect with SPCSV, leading to increased yield losses. These synergistic disease complexes may include RNA viruses of genera *Potyvirus*, *Ipomovirus*, *Carlavirus*, and *Cucumovirus*, as well as DNA viruses of genera *Begomovirus*, *Covemovirus*, and *Solodovirus*, that are symptomless when they infect singly (Karyeija et al., 2000; Mukasa et al., 2006; Untiveros et al., 2007; Cuellar et al., 2011; 2015). Thus, SPCSV can increase the susceptibility of sweetpotato to a wide range of viruses (Cuellar et al., 2009; 2015).

In coinfection with SPCSV, SPFMV can cause SPVD, which is responsible for yield losses of 70%-100% (Milgram et al., 1996; Gutiérrez et al., 2003; Ateka et al., 2004; Mukasa et al., 2006; Untiveros et al., 2008; Loebenstein, 2015). Additionally, molecular studies showed that infection with SPCSV enhances SPFMV RNA viral titres at least 600-fold, whereas SPCSV titres remain equal or less compared with single infection (Karyeija et al., 2000; Kokkinos and Clark, 2006; Mukasa et al., 2006).

As Burkina Faso is now actively including orange-fleshed sweetpotato in its food security strategy, there is increased focus on major threats to sweetpotato production, particularly from viruses. Symptoms resembling those induced by SPVD have been observed in sweetpotato fields in the main production areas in Burkina Faso, but the viruses associated with SPVD have never been confirmed. A study in 2010 by the Pan-African Sweet Potato Virome project (http://bioinfo.bti.cornell.edu/virome) aimed to understand the diversity, distribution, and evolution of sweetpotato viruses across Africa. However, only a few samples from one site in Burkina Faso...
were available to that project. Nevertheless, this small sample identified the presence of sweet potato leaf curl virus (SPLCV), SPCSV, and SPFMV.

The aim of our countrywide study in Burkina Faso was to assess the presence of viruses in sweetpotato-growing areas and to establish the aetiology of the symptoms observed in the sweetpotato fields surveyed. The knowledge generated will contribute to the current body of knowledge of sweetpotato viruses and will inform decisions concerning production of this crop in Burkina Faso.

2 | MATERIALS AND METHODS

2.1 | Plant material collection

From March 2015 to October 2017, 600 young (top) leaves and stem cuttings exhibiting typical symptoms as well as symptomless ones were collected from 80 farms in 18 provinces in Burkina Faso (Figure 1a, Table 1). The leaf samples were immediately put into paper envelopes, dried at 37 °C, and stored at the Environmental and Agricultural Research Institute laboratory located at Kamboinsé Research Station. Cuttings (10–15 cm length) were grown in plastic pots containing an autoclaved mixture of soil, sand, and peat moss (equal volumes) and maintained in an insect-proof greenhouse.

2.2 | Nitrocellulose membrane ELISA for virus diagnosis

Fresh leaf samples from stem cuttings growing in the insect-proof greenhouse were subjected to a nitrocellulose membrane ELISA (NCM-ELISA) to test for presence of viruses. The NCM-ELISA test kit with polyclonal antibodies was used according to the manufacturer's instructions and the protocol by Lizarraga and Fernandez-Northcote (1989). The kit was supplied by the International Potato Center (CIP; Lima, Peru), and can detect the following 10 viruses: SPFMV, SPCSV, sweet potato mild mottle virus, sweet potato latent

FIGURE 1 Sweetpotato survey 2015–2017: (a) Map of Burkina Faso showing the locations of samples in this study. Areas sampled for sweetpotato leaves and cuttings are listed in Table 1. (b) Locations with samples positive for SPCSV, SPFMV, and SPLCV by molecular diagnostic methods. The smallest, single-colour circles represent one specific virus identified at one farm; medium single-colour circles indicate one specific virus identified at more than one farm; largest segmented circles indicate the detection of more than one virus at more than one farm. Viruses detected in each location are shown in Table 1. (c) Locations with samples positive for mixed infection of SPFMV + SPCSV by molecular diagnostic methods. The smallest circles represent one farm with SPFMV + SPCSV infections; medium circles indicate >1 farm with SPFMV + SPCSV infections. Locations with samples positive for mixed infections of SPFMV + SPCSV are shown in Table 1. (d) Locations with samples positive for mixed infection of SPCSV, SPFMV, and SPLCV by molecular diagnostic methods. Within the areas sampled, mixed infections were recorded. The smallest, single-colour circles represent one specific virus combination identified at one farm; the smallest, segmented circles represent detection of more than one virus combination at one farm; larger single-colour circles indicate one specific virus combination found at more than one farm; larger segmented circles indicate the detection of more than one virus combination at more than one farm. Mixed infections detected in each location are shown in Table 1.
virus, sweet potato chlorotic flecks virus, sweet potato mild speckling virus, sweet potato C6 virus, sweet potato collusive virus, sweet potato virus G (SPVG), and cucumber mosaic virus. The membranes were prespotted with negative and positive controls of each virus; viral presence was indicated by a colour shift in the membrane of virus-positive samples.

2.3 Reverse transcription PCR and PCR for amplification of CP genes

2.3.1 SPFMV and SPCSV

Total RNA was extracted from leaf samples using a RNeasy Plant Mini Kit (Qiagen) according to the manufacturer’s instructions. Reverse transcription (RT) was performed on extracted RNA using MMLV reverse transcriptase (Promega) and random hexamers (Promega) as primers at 42 °C for 1 hr (Prasanth and Hegde, 2008) to produce cDNA.

Thereafter, PCR was carried out for the amplification of the SPFMV CP gene. The primers used for this step were CP1A (5′-GCAGAGGATGTCTCTGACACC-3′) and CP1S (5′-AGTGGAAGGCACCATCAG-3′), previously described by Prasanth and Hegde (2008), together with Maximo Taq DNA polymerase (GeneON). The PCR was carried out in 50 µl reaction volumes using 2.5 µl cDNA and 0.2 µM each of primers CP1A/CP1S. The conditions were 94 °C for 3 min; then 30 cycles of 94 °C for 30 s, 56.3 °C for 30 s, and 72 °C for 1 min; and a final cycle of 72 °C for 10 min.

Primers CP-F (5′-ATGGCTGATAGCACTAAAGTCGA-3′) and CP-R (5′-TCAACAGTGAAGACCTGTTCCAG-3′) were used to amplify the CP gene of SPCSV according the protocol of Qin et al. (2014). The PCR was carried out in 50 µl reaction volumes using 3 µl cDNA, and 0.2 µM of primers CP-F/CP-R using the LA Taq DNA polymerase (TaKaRa). The conditions were 94 °C for 3 min; then 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s; and a final cycle of 72 °C for 10 min.

2.3.2 SPLCV

The 600 dried young leaves collected from the field were processed for DNA extraction. Leaf samples (30 mg) were ground using the TissueLyser II (Qiagen) and DNA was extracted using the CTAB method (Doyle and Doyle, 1987). Degenerate primers SPG1 5′-CCCCKGTGCWGRAATCCAT-3′ and SPG2 5′-ATCCVAAYWTCGGAGCTAA-3′ developed by Li et al. (2004) were used to amplify the CP gene of sweetpotoviruses that infect sweetpotato. The optimized amplification was performed in 50 µl reaction volumes containing 2 µl of the extracted DNA, 1 µl of each primer, and 2.5 µl 10× Taq DNA polymerase buffer (GeneON). The conditions were 94 °C for 3 min; then 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s; and a final cycle of 72 °C for 10 min.

### TABLE 1
Results of nitrocellulose membrane-ELISA, reverse transcription-PCR, and PCR on sweetpotato showing SPFMV, SPLCV, and SPCSV in single infection and SPFMV + SPCSV (sweet potato virus disease, SPVD), SPFMV + SPLCV, SPCSV + SPLCV, and SPFMV + SPCSV + SPLCV in mixed infection by locality in Burkina Faso

| Province   | SPFMV | SPLCV | SPCSV | SPFMV + SPCSV (SPVD) | SPFMV + SPLCV | SPCSV + SPLCV | SPFMV + SPCSV + SPLCV | Plants uninfected |
|------------|-------|-------|-------|-----------------------|----------------|----------------|-----------------------|-----------------|
| Sourou     | 4     | 8     | 0     | 2                     | 1              | 0             | 0                     | 2               |
| Banwa      | 2     | 0     | 0     | 0                     | 0              | 0             | 0                     | 5               |
| Mouhoun    | 7     | 0     | 0     | 0                     | 0              | 0             | 0                     | 7               |
| Kénédougou | 32    | 36    | 1     | 6                     | 8              | 0             | 9                     | 2               |
| Houet      | 23    | 10    | 4     | 10                    | 4              | 1             | 0                     | 11              |
| Léraba     | 8     | 0     | 0     | 1                     | 0              | 0             | 0                     | 61              |
| Comoé      | 15    | 0     | 0     | 2                     | 1              | 1             | 0                     | 2               |
| Poni       | 6     | 0     | 0     | 0                     | 0              | 0             | 0                     | 7               |
| Noumblie   | 2     | 0     | 0     | 0                     | 0              | 0             | 0                     | 47              |
| Bougouriba | 5     | 0     | 0     | 0                     | 2              | 0             | 0                     | 1               |
| Ioba       | 3     | 0     | 1     | 0                     | 0              | 0             | 0                     | 9               |
| Sissili    | 9     | 0     | 2     | 7                     | 1              | 0             | 0                     | 5               |
| Nahouri    | 12    | 41    | 4     | 3                     | 10             | 12            | 2                     | 6               |
| Kadiogo    | 4     | 2     | 1     | 1                     | 0              | 0             | 0                     | 44              |
| Boulgou    | 5     | 7     | 0     | 5                     | 1              | 1             | 0                     | 0               |
| Kouritenga | 0     | 2     | 0     | 1                     | 1              | 0             | 0                     | 1               |
| Koumpélogo | 1     | 0     | 0     | 0                     | 0              | 0             | 0                     | 21              |
| Gourma     | 5     | 1     | 0     | 2                     | 2              | 0             | 1                     | 8               |
primer (10 µM), 1 µl of 10 mM dNTP mix, 5 µl of 10 x Taq DNA polymerase reaction buffer, 0.2 µl (1 U) of Maximo Taq DNA polymerase and 39.8 µl of water. The PCR conditions used were as follows: 94 °C for 5 min; then 30 cycles of 90 °C for 40 s, 60 °C for 40 s, and 72 °C for 1 min; and a final cycle of 72 °C for 10 min. To confirm amplification, 10 µl of PCR products were electrophoresed in 1% agarose gel in Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), stained with ethidium bromide and viewed under UV transillumination.

2.4 | Sequencing and sequence analysis

PCR products from RT-PCR and PCR were sequenced by the Sanger method by Genewiz Company. Contigs obtained were cleaned and assembled de novo using Geneious v. 8.1.7 (Biomatters Ltd). All the sequences were subjected to the BLAST search tools in NCBI using Geneious and subsequently to pairwise sequence comparison (Altschul et al., 1990; Bao et al., 2014). The homologous sequences were retrieved for phylogenetic analysis. Consensus sequences were MAFFT-aligned using T-coffee v. 11.00.8 tools (Chang et al., 2014).

Using ClustalW in MEGA v. 7.0.14, the sequences were aligned with sequences from other parts of the world retrieved from GenBank (Kumar et al., 2016). Evolutionary history was inferred using maximum likelihood with the Tamura–Nei model (Kumar et al., 2016). Among a number of models, the Tamura–Nei model provided the best nucleotide substitution fit for our data. Phylogenetic reconstruction was performed with bootstrap support values of 1,000. The trees were visualized and edited using FigTree v. 1.4.3.

2.5 | Virus transmission by grafting

Sweetpotato samples to be used in grafting to I. setosa were selected on the basis of the NCM-ELISA, PCR, and RT-PCR results. For each of the three virus species detected, scions were taken from 10 sweetpotato plants known to be infected with a single virus. Grafts were also taken from 10 samples each from plants co-infected with SPFMV + SPCSV, SPFMV + SPLCV, SPCSV + SPLCV, or SPFMV + SPCSV + SPLCV. Additionally, we selected five plants grown from symptomless samples and five plants grown from samples with symptoms that gave negative results in all molecular tests. In total, three scions from each of 50 sweetpotato plants were side-grafted to a healthy I. setosa plant. All samples grafted were grown in an air-conditioned, insect-proof glasshouse maintained at 25–27 °C. The symptoms were recorded weekly for 60 days.

The I. setosa leaf samples were harvested 25, 45, and 60 days after grafting. All plants were subjected to the same serological and molecular tests described previously for the sweetpotato samples.

3 | RESULTS

3.1 | Symptoms observed in fields

The most frequently observed viral symptoms on field plants were stunting, leaf curling, vein-clearing, and leaf distortion. Of these, the most severe were stunting and leaf curling. Chlorotic spots and purpling were also observed on older leaves. Leaf symptoms among the collected samples were generally mild regardless of geographical

FIGURE 2  Leaf symptoms on sweetpotato plants affected by viruses. (a, b) Mild mosaic and vein-clearing. (c) Leaf curling upward at the margin with vein-clearing. (d) Symptomless plant (extreme left) with severe stunting of growth and leaf curling (extreme right). (e) Chlorotic spotting in systemically infected leaves. (f) Leaf narrowing, stunting of growth, mosaic and mottling symptoms
TABLE 2  Isolates and strains of sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), and sweet potato leaf curl virus (SPLCV) from Burkina Faso detected or used for phylogenetic analysis in this study

| Virus       | Isolate | Strain    | Accession no. |
|-------------|---------|-----------|---------------|
| SPFMV       | BFA1    | B (RC)    | LT977076      |
|             | BFA1b   | B (RC)    | LT977262      |
|             | BFA3    | B (RC)    | LT977176      |
|             | BFA3b   | B (RC)    | LT977180      |
|             | BFA3c   | B (RC)    | LT977174      |
|             | BFA3d   | B (RC)    | LT977172      |
|             | BFA3e   | B (RC)    | LT977182      |
|             | BFA79   | A-II (O)  | LT977186      |
|             | BFA148  | A-II (O)  | LT977184      |
|             | BFA169  | A-II (O)  | LT977170      |
|             | BFA173  | B (RC)    | LT977178      |
|             | BFA412  | B (RC)    | LT977264      |
|             | BFA465  | B (RC)    | LT977266      |
| SPCSV       | BFA1279 | WA        | LT993430      |
| Sweepeviruses | BFA271 | SPLCV-US  | LS991866      |

Note: SPFMV phylogroups are B (RC), A-II (O), A-I (EA); SPCSV strains are West Africa (WA) and East Africa (EA); and sweepeviruses are sweet potato mosaic virus (SPMV), sweet potato leaf curl Virus (SPLCV), sweet potato leaf curl Canary virus (SPLCSCV), sweet potato leaf curl South Carolina virus (SPLCCSV), sweet potato leaf curl Georgia virus (SPLCGV), sweet potato leaf curl Henan virus (SPLCHnV), sweet potato leaf curl Sao Paulo virus (SPLCSPV), sweet potato leaf curl Sichuan virus (SPLCSIV), sweet potato leaf curl South Carolina virus (SPLCSCV), sweet potato leaf curl Uganda virus (SPLCUC), sweet potato leaf curl virus-China (SPLCVCN), sweet potato leaf curl virus-Brazil (SPLCV-BR), sweet potato leaf curl Uganda virus (SPLCUC), sweet potato leaf curl virus-Italy (SPLCV-IT), sweet potato leaf curl virus-Japan (SPLCV-JP), sweet potato leaf curl virus-Paralba (SPLCV-PB), sweet potato leaf curl virus-Pernambuco (SPLCV-PE), sweet potato leaf curl virus-Puerto Rico (SPLCV-PR), sweet potato leaf curl virus-Rondonia (SPLCV-RO), sweet potato leaf curl virus-Sao Paulo (SPLCV-SP), sweet potato leaf curl virus-South Carolina (SPLCV-SC), sweet potato leaf curl virus-United States (SPLCV-US), sweet potato leaf curl virus-Spain (SPLCV-ES), and sweet potato virus C (SPVC).

3.2  Detection of viruses

The NCM-ELISA revealed the presence of only two virus species: SPFMV and SPCSV. Of the 600 sweetpotato field samples tested, 45% tested positive for viruses: 34% positive for SPFMV and 11% positive for SPCSV (6% of all plants tested were coinfected with both viruses). About half (55%) of the plants tested negative for all 10 of the virus species that the NCM-ELISA could detect. Approximately 4% of the 113 symptomless plants were SPFMV-positive. However, 22% of the 487 plants with symptoms that tested negative for all 10 antisera had leaf curling and leaf distortion symptoms (Figure 2c,d). Using RT-PCR, we found that 48% gave negative results for the two viruses identified through NCM-ELISA, 38% were SPFMV-positive, 14% were SPCSV-positive (Figure 1b, Table 1), and 7% showed SPFMV + SPCSV coinfection (Figure 1c, Table 1).

Next, PCR was performed on all 600 extracted DNA samples to check for presence of SPLCV; 28% of the sweetpotato plants were positive for SPLCV. Using SPG1 and SPG2 primers to test for SPLCV, 72% of plants were virus-negative.

The main virus-detection results from the 600 plants processed are reported in Table 1. Single infections were as follows: 24% plants were singly infected with SPFMV, 18% with SPLCV, and 2% with SPCSV (Table 1). Taking into account all sweetpotato samples tested, we found the following instances of coinfections: 7% with SPFMV + SPCSV, 5% with SPFMV + SPLCV, 3% with SPCSV + SPLCV, and 2% with SPFMV + SPCSV + SPLCV (Table 1). Both the PCR and RT-PCR results showed that 40% of the samples were virus-negative.

Plants collected from all areas surveyed showed SPFMV symptoms, mild mosaic, and vein-clearing (Figure 1b). The plant samples with the most severe symptoms (leaf distortion and stunting) were those coinfected by SPFMV and SPCSV (Figure 1d). SPFMV was the most prevalent virus in all surveyed areas (Figure 1b). In contrast, coinfections of SPLCV + SPCSV (Figure 1d) were found only in areas where sweetpotato production is high.

3.3  CP gene analysis

Using de novo assembly in Geneious, 15 partial sequences were obtained from RT-PCR and PCR products. From these partial sequences, 13 CP gene sequences were obtained for SPFMV, one for SPCSV and one for SPLCV (Table 2).

The SPFMV amplicons, approximately 945 nucleotides in length and corresponding to the CP-encoding region, were successfully amplified and sequenced from 13 selected samples using the primer pair CP1A and CP1S. The CP sequences from the 13 isolates obtained from plants in Burkina Faso shared more than 99% nucleotide identity with one another. The BLASTn searches of sequences from other studies resulted in three SPFMV groupings, highlighting the identity of Burkina Faso SPFMV sequences with those SPFMV sequences from other geographical locations. Eight Burkina Faso CP sequences (LT977172, LT977174, LT977176, LT977180, LT977262, LT972763, LT977264, and LT977265) showed 98% nucleotide identity with SPFMV isolates from Egypt and Korea (GenBank accession numbers AJ515379 and AF015540, respectively). The second group showed Burkina Faso CP sequences (LT977178 and LT977266) with 94% nucleotide identity with SPFMV isolates from East Timor and the USA (MF572055 and S43450, respectively). The last group, the CP sequence LT977186 showed...
96% nucleotide identity with SPFMV isolates from East Timor and Japan (MF572056 and AB465608, respectively). The corresponding translated CP protein sequences from all 13 Burkina Faso sequences contained 315 amino acids with the DAG tripeptide motif at the N-terminus (amino acid positions 9–11).

A SPCSV amplicon of approximately 774 nucleotides in length and corresponding to the CP-encoding region was amplified using primers CP-F and CP-R and sequenced. The BLASTn search revealed that Burkina Faso sequence LT993430 showed 98% nucleotide identity with two SPCSV isolates from Spain (KU511274 and FJ807785).

A SPLCV amplicon of approximately 792 nucleotides in length, corresponding to the CP-encoding region, was amplified from selected samples using primers SPG1 and SPG2 (Li et al., 2004) and successfully sequenced. The BLASTn search revealed that sequence LS991866 had 98% nucleotide identity with SPLCV-US isolate FJ76701 from China.

The CP genes from the 13 SPFMV isolates from Burkina Faso were compared with 18 SPFMV isolates from other geographical locations in the phylogenetic analysis, revealing three major phylogroups: A-I, A-II, and B (Figure 3). In phylogroup B, sequences of isolates from Burkina Faso (LT977176, LT977172, LT977182, LT977262, LT977180, LT977076, LT977174, LT977264, LT977178, and LT977266) clustered with sequences AJ515379 (Egypt), EU021065 (Peru), S43450 (USA), and AF015540 (Korea).

Phylogroup A-II clustered Burkina Faso isolate sequences LT977186, LT977184, and LT977170 with sequences AY459598 (Tanzania), AO10699 (Nigeria), AO10705 (Niger), AB465608 (Japan), and Z98942 (China).

The CP gene from the partially sequenced SPLCV isolate from Burkina Faso was compared with 24 CP genes in SPLCV isolates from around the world. Our phylogenetic analysis clustered the Burkina Faso isolate BFA271 with SPLCV-US sequences AF104036 (USA) and HQ393473 (Brazil) (Figure 4).

The CP gene from the partially sequenced SPCSV isolate from Burkina Faso was compared with eight CP genes in SPCSV isolates from around the world. Our phylogenetic analysis showed that isolate BFA1279 (sequence LT993430) from Burkina Faso belonged to strain WA together with Spanish sequences KU511274 and FJ807785 (Figure 5).

3.4 | Grafting on I. setosa

The only samples selected for grafting to I. setosa were those that showed single as well as coinfections as detected through NCM-ELISA, RT-PCR, and PCR.

Grafting allowed us to successfully transmit all three viruses identified by molecular analysis to I. setosa (Table 3) and evaluate...

**FIGURE 3** Maximum-likelihood phylogenetic trees obtained from alignment of nucleotide sequences of coat protein (CP) genes from sweet potato feathery mottle virus (SPFMV). The comparisons made were between phylogroup C (three) and CP gene (nine) sequences obtained from GenBank, strain O, one new CP gene sequence obtained plus the five CP gene sequences from GenBank, and phylogroup RC, 10 new CP gene sequences obtained plus four CP genes sequences from GenBank. The tree was created in MEGA v. 7.0.14 using ClustalW with 1,000 replicates. Bootstrap values are percentages with values shown at the nodes. The tree was rooted with sweet potato virus C (JX489166). Sequence labels: from this study (red), from GenBank (black).
plants for viral symptoms (Figure 6). Of the five symptomless sweetpotato samples that were virus-negative according to the PCR and NCM-ELISA results, two samples—BFA383 from Di and BFA340 from Bagré—were positive for SPFMV on the grafted *I. setosa* plants (according to RT-PCR).

Samples BFA199 (from Tiébélé) and BFA496 (from Sikorola-Dierikandougou), with symptoms, tested positive for SPFMV and SPCSV based on RT-PCR and NCM-ELISA, whereas both samples tested negative for SPLCV by PCR. Only one plant sample (BFA428 from Yoro) showed leaf distortion and vein-clearing symptoms (Table 3) after grafting but was virus-negative according to all three diagnostic tests. Eight of the 10 sweetpotato samples tested positive for single SPFMV infection in our molecular tests; this result was supported by the grafting results on *I. setosa*, which exhibited typical SPFMV vein-clearing symptoms (Figure 6a). The other two samples (BFA1284 from Boura and BFA1473 from Matourkou) showed SPFMV + SPCSV coinfection.

For those sweetpotato samples initially found by PCR and RT-PCR to be singly infected by SPCSV (Figure 6c), the grafting results confirmed SPCSV infection in 9 of 10 samples. Sample BFA1282 (from Boura) showed coinfection of SPFMV + SPCSV (Figure 6k). No other virus was found in sample BFA289 (from Tiébélé) after grafting onto *I. setosa*.

In the cases of those sweetpotato samples determined through molecular analysis to be infected singly by SPLCV, the diagnosis was confirmed by the *I. setosa* grafting results that showed SPLCV symptoms (Figure 6b). Mixed infection was found as follows: SPFMV + SPCSV + SPLCV was detected in the plant planted with BFA372 (Nakalbo; Figure 6d,e), SPFMV + SPLCV was detected in the plant planted with BFA322 (Bonsrima; Figure 6g), and SPCSV + SPLCV was detected in the plant planted with BFA774 (Kotoura; Figure 6h,i).

Twenty-one days after grafting scions on *I. setosa*, viral symptoms were visible on 5 out of 150 grafted plants. After 60 days, symptoms varied from moderate to severe. The major symptoms were vein-clearing, leaf curling, interveinal chlorosis, and chlorosis. The most severe symptoms were in cases of coinfection, typically involving SPFMV + SPCSV (Figure 6j–l), SPFMV + SPLCV, and SPCSV + SPLCV. Surprisingly, coinfections involving all three viruses (SPFMV + SPCSV + SPLCV) only produced moderate or mildly severe symptoms (Figure 6d,e). These symptoms included leaf distortion, vein-clearing, chlorotic spots, and necrotic spots. After 60 days, three of the five plants grafted with symptomless sweetpotato scions were still symptomless, whereas the other two plants exhibited vein-clearing and interveinal chlorosis symptoms (Table 3).
DISCUSSION

Despite studies on virus accumulation in sweetpotato and on the presence of sweet potato viruses in East Africa, the incidence of viruses is poorly documented in many regions and countries of Africa. For our work, we conducted a baseline survey to determine the status of viral diseases in sweetpotato across Burkina Faso. Extensive field surveys (2015–2017) were carried out to assess the prevalence and distribution of viral diseases on sweetpotato plants in the major sweetpotato-growing areas of Burkina Faso.

Some samples that tested negative using NCM-ELISA were found to be positive for SPFMV and SPCSV when using RT-PCR, thus highlighting the importance of using multiple assays for detecting viruses. Because of the known synergy between SPCSV and some DNA viruses (Cuellar et al., 2015), we also checked for the presence of DNA viruses belonging to the genus Begomovirus using molecular tools.

To determine which strains of the detected viruses are present in Burkina Faso, we sequenced PCR products for SPFMV, SPCSV, and SPLCV. These results demonstrated that SPFMV A-II and B phylogenroups, SPCSV-WA, and SPLCV-US were all present in sweetpotato fields in Burkina Faso. Samples BFA340 from Bagré and BFA383 from Di were negative for viruses in all our diagnostic tests; however, after grafting to I. setosa, these two plants showed positive results for at least one of SPFMV, SPCSV, and SPLCV. Similar results were obtained by Abad and Moyer (1992) and Valverde et al. (2007) who proposed that the phenolics and latex found in some sweetpotato cultivars might compromise nucleic acid extraction.

The most widespread virus on sweetpotato crops around the world is SPFMV (Ateka et al., 2004; Njeru et al., 2008; Maina et al., 2018), and it was also the most prevalent virus detected (24%) in our Burkina Faso samples. Leaf vein-clearing is not specific to SPFMV (Moyer and Salazar, 1989) but can indicate the presence of other viral infections. Because mosaic and streak-like symptoms were also prevalent in our sweetpotato samples from fields, it is likely that other viruses in the family Potyviridae were also present (Moyer and Salazar, 1989; Brunt et al., 1996).

FIGURE 5 Maximum-likelihood phylogenetic trees obtained from alignment of nucleotide sequences of coat protein (CP) genes from sweet potato chlorotic stunt virus (SPCSV). The comparisons made were between 15 CP gene sequences obtained from GenBank and one new CP gene sequence obtained in this study. The tree was created in MEGA v. 7.0.14 using ClustalW with 1,000 replicates. Bootstrap values are percentages with values shown at the nodes. The tree was rooted with lettuce infectious yellows virus (LIYV) (U15440). Sequence labels: from this study (red), from GenBank (black)
| Locality       | Sweetpotato sample | Symptoms  | NCM-ELISA | PCR/RT-PCR |
|---------------|--------------------|-----------|-----------|------------|
|               |                    |           | l. batatas | l. setosa  | SPF MV     | SPF CSV    |
| Bama          | BFA44<sup>a</sup>  | A         | A         | -          | -          | -          |
| Di            | BFA383<sup>a</sup> | A         | A         | -          | -          | +          |
| Douna         | BFA1055<sup>a</sup>| A         | A         | -          | -          | -          |
| Bagré         | BFA340<sup>a</sup> | A         | I         | -          | -          | +          |
| Matourkou     | BFA1486<sup>a</sup>| A         | Vc        | -          | -          | -          |
| Bama          | BFA49<sup>b</sup>  | Cp        | A         | -          | -          | -          |
| Sikorola      | BFA96<sup>b</sup>  | Vc        | Vc, Vn    | +          | -          | +          |
| Sokouraba     | BFA160<sup>b</sup> | C         | A         | -          | -          | -          |
| Tiébélé       | BFA199<sup>b</sup> | Ld, Vc, Lc| Ld, B     | +          | +          | +          |
| Yoro          | BFA428<sup>b</sup> | Ld, Vc, Lc| Vn, Np, C | -          | -          | -          |
| Mahan         | BFA173<sup>f</sup> | Vc, Lc    | Vc, Lc    | +          | +          | +          |
| Sokouraba     | BFA169<sup>e</sup> | Vc, C     | Vc, Ld, Vn| +          | +          | +          |
| Samogohiri    | BFA148<sup>e</sup> | Vc, Cp    | Ld, C     | +          | -          | +          |
| Bagré         | BFA79d<sup>d</sup>| Vc, Ld, B | Np, Cp, Ld| +          | +          | +          |
| Yoro          | BFA40d<sup>d</sup> | Vc        | Vc, Ld    | +          | -          | -          |
| Bamako        | BFA1208<sup>e</sup>| Ld, Cp    | Lc, S     | +          | +          | -          |
| Di            | BFA384<sup>e</sup> | Vc, Ld,   | Vc, Lc    | +          | -          | +          |
| Bama          | BFA69<sup>f</sup>  | C         | C, Ld     | -          | -          | -          |
| Guénon        | BFA307<sup>f</sup>| Lc, Cp, I | Lc         | -          | -          | -          |
| Banfora       | BFA1092<sup>f</sup>| I, Vc     | Vc, I     | +          | -          | -          |
| Batié         | BFA1163<sup>f</sup>| Vc        | I, Vn     | +          | -          | -          |
| Boura         | BFA1284<sup>d</sup>| S         | Ld, S     | +          | -          | +          |
| Di            | BFA381<sup>e</sup> | Vc, Cp    | C, Np     | -          | -          | +          |
| Farakoba      | BFA1473<sup>e</sup>| Vc, Ld,   | I, C      | +          | +          | -          |
| Sikorola      | BFA87<sup>e</sup>  | Vc, Np    | Vc        | +          | -          | -          |
| Kalwatenga    | BFA489<sup>e</sup> | C         | A         | -          | -          | -          |
| Ouargaye      | BFA1388<sup>g</sup>| Lc        | A         | +          | -          | -          |
| Sanké         | BFA523<sup>e</sup> | Vc        | VC, B     | +          | -          | -          |
| Sao           | BFA1308<sup>e</sup>| C         | Vc, B     | +          | -          | -          |
| Bama          | BFA50<sup>h</sup>  | Vc, Cp    | Lc, Cp    | -          | -          | +          |
| Bama          | BFA62<sup>h</sup>  | Vc        | Lc        | -          | +          | -          |
| Bonsrima      | BFA321<sup>h</sup>| Cp        | A         | -          | -          | +          |
| Boura         | BFA1282<sup>h</sup>| S         | Cp, Ld    | +          | +          | -          |
| Bonsrima      | BFA1286<sup>h</sup>| Cp        | Vn        | -          | +          | -          |
| Dano          | BFA1298<sup>h</sup>| C         | C         | -          | +          | -          |
| Guénon        | BFA311<sup>h</sup>| Cp        | Vn, Cp    | -          | -          | +          |
| Sourou        | BFA917<sup>h</sup> | C         | Cp, Ld    | -          | -          | +          |
| Tiébélé       | BFA289<sup>h</sup> | Cp        | A         | -          | -          | -          |
| Bonsrima      | BFA322<sup>h</sup> | C, Lc     | S         | -          | +          | -          |

(Continues)
The second most prevalent virus that we detected was SPLCV (18%). As a single infection, SPCSV was also present among samples collected but was not very common in the areas surveyed. However, we did observe instances of SPFMV + SPCSV + SPLCV coinfection, a cause of SPVD. This viral disease has been reported as having the greatest impact on sweetpotato yield worldwide (Valverde et al., 2007; Rey et al., 2017). Few reports exist on the synergistic interactions between RNA and DNA viruses. However, previous studies, such as Cuellar et al. (2015), showed that SPCSV can interact synergistically with sweepoviruses.

Grafting sweetpotato scions onto I. setosa, and subsequent use of diagnostic tests, confirmed the presence of the three virus species and of coinfections; the I. setosa also displayed the symptoms associated with each virus (Table 3). Two sweetpotato samples with symptoms gave negative results in the diagnostic tests, but I. setosa plants grafted with these samples produced clear symptoms of viral infection. The possible interaction of these viruses with SPFMV has been shown in the work of Clark et al. (2012) and Mulabisana et al. (2018).

The majority of symptoms observed in this study are in accordance with the typical SPFMV symptoms described by Moyer and Salazar (1989) and Gibson et al. (1997). Previous research confirmed that SPFMV on its own causes mild or no symptoms in some sweetpotato cultivars (Gibson et al., 1997; Ateka et al., 2004), suggesting that the SPFMV-positive, symptomless samples in our study may have been singly infected with SPFMV. The most severe symptoms were observed for coinfections, mainly involving SPFMV + SPLCV but sometimes SPCSV + SPLCV. These results corroborate those of previous studies using grafting on I. setosa (Maina et al., 2018). In addition, our study showed that the accumulation of three or more viruses does not necessarily have a more devastating effect than a coinfection involving only two viruses. Indeed, the symptoms observed in double coinfections (SPFMV + SPCSV) were more severe than those resulting from multiple coinfection with SPFMV + SPCSV + SPLCV, for which foliar symptoms were moderate.

Symptomless infections present a challenge for SPFMV management. Fortunately, this virus can be diagnosed by grafting on I. setosa, even if viral titre is very low and the virus is undetectable by NCM-ELISA or RT-PCR. Our study corroborates the results of Gibson et al. (1997). They showed that although about 95% of symptomless plants were virus-negative when NCM-ELISA was used, in the remaining 5% the most prevalent virus was SPFMV. Moreover,

### TABLE 3 (Continued)

| Locality   | Sweetpotato sample | Symptoms | NCM-ELISA | PCR/RT-PCR |
|------------|--------------------|----------|-----------|------------|
|            |                    | I. batatas | I. setosa | SPFMV | SPCSV | SPLCV |
| Di         | BFA3881            | Cp        | S         | -       | -      | +      |
| Kotoura    | BFA7741            | C, Lc     | Lc, Cp, Np| -       | -      | +      |
| Louanga    | BFA3661            | Lc        | Lc        | -       | -      | +      |
| Mahon      | BFA1741            | C, Lc     | A         | -       | -      | +      |
| Nakalbo    | BFA3721            | Lc        | S         | -       | -      | +      |
| Pampangou  | BFA4961            | Cp        | C         | -       | -      | +      |
| Tiébély    | BFA1931            | Lc        | C         | -       | -      | +      |

Abbreviations: A, symptomless; B, blistering; C, chlorosis; Cp, chlorotic points; I, interveinal chlorosis; Lc, leaf curling; Ld, leaf distortion; Np, necrotic points; S, stunting; Vc, vein-clearing; Vn, vein necrosis.

1Symptoms on both I. batatas and on grafted I. setosa.
2Coinfection with SPFMV + SPCSV + SPLCV.
3Coinfection with SPFMV + SPCSV.
4Coinfection with SPFMV + SPLCV.
5SPFMV single infection confirmed by RT-PCR.
6SPCSV single infection confirmed by RT-PCR.
7SPLCV single infection confirmed by PCR.
they found that approximately 15% of the symptomless plants tested induced viral symptoms when grafted on *I. setosa* (Moyer and Salazar, 1989; Gibson et al., 1997).

Although symptom scoring, serology, and grafting are useful for the detection of SPFMV, SPCSV, and SPLCV, they are not suitable for virus strain demarcation (Ryu and Choi, 2002; Prasanth and Hegde, 2008; Qin et al., 2014). Previous studies showed that SPFMV phylogroup B and phylogroup A are unrestricted geographically (Kreuze et al., 2000; Untiveros et al., 2008; Tugume et al., 2010). Phylogroup B is also known to infect sweetpotato in Asia (Japan, Korea, and China) and North Africa (Egypt) (Tairo et al., 2005). To avoid geographical and biological confusion, Jones and Kehoe (2016) proposed the current approach in which phylogroup nomenclature is based on letters of the alphabet and Latinized numbers.

Our study shows that all SPFMV isolates from Burkina Faso can be assigned to phylogroups A-II and B. Phylogroup A-I was not detected among our samples, even though some sweetpotato cultivars have been introduced from East Africa (Somé et al., 2015). Another finding from our examination of CP amino acid sequences of all isolates from Burkina Faso is the presence of the DAG motif that, according to other research (Shukla et al., 1994; Ateka et al., 2017), is involved in virus transmission by aphids. The presence of the DAG motif in the CP, together with the presence of aphids in the environments sampled, suggests that these insects may play a role in SPFMV transmission.

**FIGURE 6** Virus-associated symptoms in *Ipomoea setosa* plants infected by grafting with *I. batatas*. (a) Vein-clearing like that seen in SPFMV infection; (b) upward leaf curling due to SPLVC; (c) leaf chlorosis due to SPCSV; (d, e) leaf curling, interveinal chlorosis, and blistering due to SPFMV + SPCSV + SPLCV coinfection; (f) symptomless leaf; (g) downward leaf curling and vein-clearing due to SPFMV + SPLCV coinfection; (h, i) downward leaf curling, chlorosis, and light vein-clearing due to SPCSV + SPLCV; (j–l) chlorosis, vein-clearing, severe leaf distortion, blistering, and stunting due to SPFMV + SPCSV (sweet potato virus disease).
on sweetpotato in Burkina Faso. We did not investigate viral transmission by aphids, but suggest that this should be considered in future surveys.

Of the samples in this study, 60% were infected with viruses that lead to SPVD, either as single infections or as coinfections, on cultivated sweetpotato in Burkina Faso. These viruses do not seem to be widespread in the surveyed areas; however, their presence—together with that of other viral diseases—could compromise the health of sweetpotato crops in Burkina Faso. Our work confirms that of Ryu and Choi (2002) on the importance of using multiple methods for virus screening, such as NCM-ELISA, RT-PCR, and PCR for the detection of SPFMV, SPCSV, and SPLCV.

In view of our results, we conclude that management approaches for SPVD should continue to include monitoring sweetpotato crops in Burkina Faso and should be performed more widely in West Africa. Furthermore, we advocate the setting up of management plans to address the development of resistant varieties and to disseminate information about good farming practices to better control spread of viral diseases. The next step in this project is to obtain the full genomes of SPFMV, SPLCV, and SPCSV. Then we will investigate the mechanisms of mixed infection and test for evidence of synergy to better understand the interactions between DNA and RNA viruses and between viruses and plants.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
Abad, J.A. and Moyer, J.W. (1992) Detection and distribution of sweet-potato feathery mottle virus by in vitro-transcribed RNA probes (riboprobes), membrane immunobinding assay, and direct blotting. *Phytopathology*, 82, 300–305.

Adams, M.J. and Carstens, E.B. (2012) Ratification vote on taxonomic proposals to the ICTV (2012). *Archives of Virology*, 157, 1411–1422.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.

Ateka, E.M., Njeru, R.W., Kibarу, A.G., Kimenu, J.W., Barg, E., Gibson, RW et al. (2004) Identification and distribution of viruses infecting sweet potato in Kenya. *Annals of Applied Biology*, 144, 371–379.

Ateka, E., Alicai, T., Ndunguru, J., Tairo, F., Sseruwagi, P., Kiarie, S et al. (2017) Unusual occurrence of a DAG motif in the Ipomovirus Cassava brown streak virus and implications for its vector transmission. *PLoS One*, 12, e0187883.

Bao, Y., Chetverin, V. and Tatusova, T. (2014) Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. *Archives of Virology*, 159, 3293–3304.

Brunt, A.A., Cradtree, K., Dahlwitz, M.J., Gibbs, A.J. and Watson, L. (1996) Viruses of Plants. Description and Lists from the VIDEO Database. Wallingford, UK: CABI.

Chang, J.-M., Tommaso, P. and Notredame, C. (2014) TCS: a new multiple sequence alignment reliability measure to estimate alignment accuracy and improve phylogenetic tree reconstruction. *Molecular Biology and Evolution*, 31, 1625–1637.

Clark, C.A. and Hoy, M.W. (2006) Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease*, 90, 83–88.

Clark, C.A., Davis, J.A., Abad, J.A., Cuellar, W.J., Fuentes, S., Kreuze, JF et al. (2012) Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease*, 96, 168–185.

Cohen, J., Franck, A., Vetten, H.J., Lesemann, D.E. and Loebenstein, G. (1992) Purification and properties of closterovirus-like particles associated with a whitefly-transmitted disease of sweet potato. *Annals of Applied Biology*, 121, 257–268.

Cuellar, W.J., Kreuze, J.F., Rajama, M., Cruzado, K.R., Utriveros, M. and Valkonen, J.P.T. (2009) Elimination of antiviral defense by viral RNAse III. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 10354–10358.

Cuellar, W.J., De Souza, J., Barrantes, I., Fuentes, S. and Kreuze, J.F. (2011) Distinct cloemoviruses interact synergistically with sweet potato chlorotic stunt virus (genus Crinivirus) in cultivated sweet potato. *Journal of General Virology*, 92, 1233–1243.

Cuellar, W.J., Galvez, M., Fuentes, S., Tugume, J. and Kreuze, J. (2015) Synergistic interactions of begomoviruses with Sweet potato chlorotic stunt virus (genus Crinivirus) in sweet potato (*Ipomoea batatas* L.). *Molecular Plant Pathology*, 16, 459–471.

Dabiré, R. and Belem, J. (2001) Plantes à tubercules et racines du Burkina Faso. WASNET News. WASNET News, 8, 12–16.

Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Esterhuizen, L.L., van Heerden, S.W., Rey, M.E.C. and van Heerden, H. (2012) Genetic identification of two sweet potato infecting begomoviruses in South Africa. *Archives of Virology*, 157, 2241–2245.

FAOSTAT (2013) *Food and Agriculture Data*. Available at: http://www.fao.org/faostat/en/#data. [Accessed 6 April 2020].

Fauquet, C.M. and Stanley, J. (2005) Revising the way we conceive and name viruses below the species level: a review of Geminivirus taxonomy calls for new standardized isolate descriptors. *Archives of Virology*, 150, 2151–2179.

Gibson, R.W., Mwangi, R.O.M., Kasule, S., Mpembe, I. and Carey, E.E. (1997) Apparent absence of viruses in most symptomless field-grown sweet potato in Uganda. *Annals of Applied Biology*, 130, 481–490.

Gutiérrez, D.L., Fuentes, S., Salazar, L.F., Potato, I., Ker, I. and Roth, I.L. (2003) Sweet potato virus disease (SPVD): distribution, incidence, and effect on sweetpotato yield in Peru. *Plant Disease*, 87, 297–302.

Jones, R.A.C. and Kehoe, M.A. (2016) A proposal to rationalize with-in-species plant virus nomenclature: benefits and implications of in-action. *Archives of Virology*, 161, 2051–2057.

Karyjeva, R.F., Gibson, R.W. and Valkonen, J.P.T. (1998) The significance of sweet potato feathery mottle virus in subsistence sweet potato production in Africa. *Plant Disease*, 82, 1–15.
Mukasa, S.B., Rubaihayo, P.R. and Valkonen, J.P.T. (2003) Incidence of viruses infecting sweet potato plants. *Virology*, 36, 26–36.

Karyeija, R.F., Kreuze, J.F., Gibson, R.W. and Valkonen, J.P.T. (2001) Variability of sweet potato feathery mottle virus in Africa. *African Crop Science Journal*, 9, 293–299.

Kim, J., Yang, J., Kwak, H.R., Kim, M.K., Seo, J.K., Chung, MN et al. (2017) New viruses infecting sweet potato in Korea from 2011 to 2014. *Plant Pathology Journal*, 33, 467–477.

Kokkinos, C.D. and Clark, C.A. (2006) Interactions among sweet potato chlorotic stunt virus and different potyviruses and Potyvirus strains infecting sweetpotato in the United States. *Plant Disease*, 90, 1347–1352.

Kreuze, J.F., Karyeija, R.F., Gibson, R.W. and Valkonen, J.P.T. (2000) Comparisons of coat protein gene sequences show that East African isolates of sweet potato feathery mottle virus form a genetically distinct group. *Archives of Virology*, 145, 567–574.

Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.

Li, R., Salih, S. and Hurtt, S. (2004) Detection of geminiviruses in sweetpotato by polymerase chain reaction. *Plant Disease*, 88, 1347–1351.

Lizarraga, C. and Fernandez-Northcote, E.N. (1989) Detection of potato viruses X and Y in sap by a modified indirect enzyme linked immuno-sorbent assay on nitrocellulose membranes (NCM-ELISA). *Plant Disease*, 73, 11–14.

Loebenstein, G. (2015) Control of sweet potato virus diseases. *Advances in Virus Research*, 91, 33–45.

Maina, S., Barbetti, M.J., Edwards, O.R., de Almeida, L., Ximenes, A. and Jones, R.A.C. (2018) Sweet potato feathery mottle virus and sweet potato virus C from East Timorese and Australian sweetpotato: biological and molecular properties and biosecurity implications. *Plant Disease*, 103, 589–599.

Mbanzibwa, D.R., Tugume, A.K., Chiunga, E., Mark, D. and Tairo, F.D. (2014) Small RNA deep sequencing-based detection and further evidence of DNA viruses infecting sweetpotato plants in Tanzania. *Annals of Applied Biology*, 165, 329–339.

Miano, D.W., Labonte, D.R., Clark, C.A., Valverde, R.A. and Hoy, M.W. (2006) First report of a begomovirus infecting sweetpotato in Kenya. *Plant Disease*, 90, 832.

Milgram, M., Cohen, J. and Loebenstein, G. (1996) Effects of sweet potato feathery mottle virus and sweet potato sunken vein virus on sweet potato yields and rates of reinforcement of virus-free planting material in Israel. *Phytoparasitica*, 24, 189–193.

Mohammed, H.S., El Siddig, M.A., El Hussein, A.A., Ibrahim, F.A., Navas-Castillo, J. and Fiallo-Olivé, E. (2017) First report of sweet potato leaf curl virus infecting sweet potato in Sudan. *Plant Disease*, 101, 8–9.

Moyer, J.W., Cali, B.B., Kennedy, G.G. and Abou-Ghadr, F. (1980) Identification of two sweet potato feathery mottle virus strains in North Carolina. *Plant Disease*, 64, 762–764.

Moyer, J.W. and Salazar, L.F. (1989) Viruses and virus-like diseases of sweet potato. *Plant Disease*, 73, 451–455.

Mukasa, S.B., Rubaihayo, P.R. and Valkonen, J.P.T. (2003) Incidence of viruses and virus-like diseases of sweetpotato in Uganda. *Plant Disease*, 87, 329–335.

Mukasa, S.B., Rubaihayo, P.R. and Valkonen, J.P.T. (2006) Interactions between a crinivirus, an ipomovirus and a potyvirus in co-infected sweetpotato plants. *Plant Pathology*, 55, 458–467.

Mulabonsana, M.J., Cloete, M., Mabasa, K.G., Laurie, S.M., Oelofse, D., Esterhuizen, LL et al. (2018) Survey of common viral diseases of sweet potato in South Africa reveal novel isolates of sweet potato viruses. *South African Journal of Botany*, 114, 280–294.

Ndunguru, J. and Kapenga, R. (2007) Viruses and virus-like diseases affecting sweet potato subsistence farming in southern Tanzania. *African Journal of Agricultural Research*, 2, 232–239.

Njeru, R.W., Bagabe, M.C., Nkezabahizi, D., Kayiranga, D., Kajuga, J., Butare, L et al. (2008) Viruses infecting sweet potato in Rwanda: occurrence and distribution. *Annals of Applied Biology*, 153, 215–221.

Prasanth, G. and Hegde, V. (2008) Occurrence of sweet potato feathery mottle virus and sweet potato leaf curl Georgia virus on sweet potato in India. *Plant Disease*, 92, 311.

Qin, Y., Wang, L., Zhang, Z., Qiao, Q., Zhang, D., Tian, Y. et al. (2014) Complete genomic sequence and comparative analysis of the genome segments of sweet potato chlorotic stunt virus in China. *PLoS ONE*, 9, e106323.

Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L. et al. (2009) Molecular characterization of sweet potato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and Southern Africa. *Plant Disease*, 93, 933–939.

Regenmortel, M.H.V., Faquett, C.M., Bishop, D.H.L., Castens, E., Estes, M.K., Lemon, S., et al. (2000) *Virus Taxonomy: The Seventh Report of the International Committee on Taxonomy of Viruses*. San Diego: Academic Press.

Rey, M.E.C., Ndunguru, J., Berrie, L.C., Paximadis, M., Berry, S., Cossa, N et al. (2012) Diversity of dicotyledonous-infecting geminiviruses and their associated DNA molecules in Southern Africa, including the South-West Indian Ocean Islands. *Viruses*, 4, 1753–1791.

Ryu, K.H. and Choi, S.H. (2002) Molecular detection and analysis of sweet potato feathery mottle virus from root and leaf tissues of cultivated sweet potato plants. *Plant Pathology Journal*, 18, 12–17.

Shukla, D.D., Ward, C.W. and Brunt, A.A. (1994) The *Potyviridae*. Wallingford, UK: CAB.

Somé, K., Ouedraogo, T.J., Belem, J., Asante, K.I., Vernon, G. and Danquah, Y.E. (2015) Breeding sweetpotato for yield and beta-carotene content in Burkina Faso. *Centre Agricultural Bioscience International*, 6, 69–78.

Souto, E.R., Sim, J., Chen, J., Valverde, R.A. and Clark, C.A. (2003) Properties of strains of sweet potato feathery mottle virus and two newly recognized potyviruses infecting sweet potato in the United States. *Plant Disease*, 87, 1226–1232.

Tairo, F., Kullaya, A. and Valkonen, J.P.T. (2004) Incidence of viruses infecting sweetpotato in Tanzania. *Plant Disease*, 88, 916–920.

Tairo, F., Mukasa, S.B., Jones, R.A.C., Kullaya, A., Rubaihayo, P.R. and Valkonen, J.P.T. (2005) Unravelling the genetic diversity of the three main viruses involved in sweet potato virus disease (SPVD), and its practical implications. *Molecular Biotechnology*, 6, 199–211.

Trenado, H.P., Orilio, A.F., Marquez-Martin, B., Moriones, E. and Navas-Castillo, J. (2011) Sweepoviruses cause disease in sweet potato and related *Ipomea* spp.: fulfilling Koch’s postulates for a divergent group in the genus *Begomovirus*. *PLoS ONE*, 6, e27329.

Tugume, A.K., Cuellar, W.J., Mukasa, S.B. and Valkonen, J.P.T. (2010) Molecular genetic analysis of virus isolates from wild and cultivated plants demonstrates that East Africa is a hotspot for the evolution and diversification of sweet potato feathery mottle virus. *Molecular Ecology*, 19, 3139–3156.

Untiveros, M., Fuentes, S. and Kreuze, J.F. (2008) Molecular variability of sweet potato feathery mottle virus and other potyviruses infecting sweet potato in Peru. *Archives of Virology*, 153, 473–483.

Untiveros, M., Fuentes, S. and Salazar, L.F. (2007) Synergistic interaction of sweet potato chlorotic stunt virus (*Crinivirus*) with *carla*-, *cucumo*-, *ipomo*-, and *potyviruses* infecting sweet potato. *Plant Disease*, 91, 669–676.

Valverde, R.A., Clark, C.A. and Valkonen, J.P.T. (2007) Viruses and virus disease complexes of sweetpotato. *Plant Viruses*, 1, 116–126.

Ward, C.W. and Shukla, D.D. (1991) Taxonomy of potyviruses: current properties and some solutions. *Intervirology*, 3, 269–296.

Wasswa, P., Otto, B., Maruthi, M.N., Mukasa, S.B., Monger, W. and Gibson, R.W. (2011) First identification of a sweet potato Begomovirus (sweepovirus) in Uganda: characterization, detection and distribution. *Plant Pathology*, 60, 1030–1039.
Wylie, S.J., Adams, M., Chalam, C., Kreuze, J.F., López-Moya, J.J., Ohshima, K. et al. (2017) ICTV virus taxonomy profile: Potyviridae. Journal of General Virology, 98, 352–354.

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SUPPORTING INFORMATION
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