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Characterization of virus species associated with sweet potato virus disease in Costa Rica and riboprobe development for its rapid detection

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Abstract

Sweet potato (*Ipomoea batatas*) is a crucial crop for food security and economic stability in many regions, including Costa Rica. This study aimed to assess the prevalence and genetic diversity of two major viruses associated with sweet potato virus disease (SPVD) in Costa Rica: sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV). A total of 50 leaf samples displaying typical viral disease symptoms were collected from major sweet potato-growing regions. Using multiplex reverse transcription (RT)-PCR, SPFMV was detected in 82% of the samples and SPCSV in 52%, with a coinfection rate of 48%. Genetic analysis revealed that SPFMV isolates belonged predominantly to phylogenetic group B, while SPCSV isolates clustered with the West African (WA) strain. The low genetic diversity within these viral populations suggests a limited number of initial infection events followed by local spread. Riboprobe technology was evaluated as an alternative to RT-PCR for virus detection. While the riboprobes for SPFMV demonstrated sensitivity comparable to RT-PCR, the SPCSV riboprobes showed slightly lower sensitivity, particularly when using crude leaf extracts. Despite this, riboprobes offer significant advantages in terms of cost and ease of use for large-scale screening. Our findings underscore the need for robust diagnostic and management strategies to control SPVD and highlight the importance of continuous monitoring and research to mitigate the impact of viral diseases on sweet potato production in Costa Rica. These insights contribute to the global understanding of SPVD and support collaborative efforts in plant pathology research.

KEYWORDS
genetic diversity, sweet potato chlorotic stunt virus, sweet potato feathery mottle virus, sweet potato virus disease, viral detection methods, virus prevalence

1 | INTRODUCTION

The sweet potato (*Ipomoea batatas*) is a globally significant root crop integral to food security and economic stability in numerous regions (Qin et al., 2014; Sapakhova et al., 2023). In Costa Rica, the sweet potato is not only a staple food deeply embedded in the diet and culture but also holds substantial economic importance (León-Pacheco et al., 2021). However, the prevalent method of its vegetative propagation makes

it highly susceptible to the accumulation and dissemination of various pathogens, including viruses (Cuellar et al., 2015; Echodu et al., 2019).

Viral infections of sweet potato can have devastating consequences for farmers, with potential yield losses reaching up to 100%, depending on factors such as the cultivar, the infecting virus and the stage of infection (Clark et al., 2012; Loebenstein, 2015). Globally, over 30 viruses are known to infect sweet potato (Clark et al., 2012; Cuellar et al., 2015). However, the most severe impacts on crop yield and disease severity are typically linked to coinfection with multiple viruses, leading to the widespread manifestation of the disease complex known as sweet potato virus disease (SPVD) (Barkessa, 2018; Cuellar et al., 2015; Tibiri et al., 2020; Valverde et al., 2007). The key viral agents associated with SPVD are sweet potato feathery mottle virus (SPFMV, *Potyvirus bataplumei*) and sweet potato chlorotic stunt virus (SPCSV, *Crinivirus ipomeae*) (David et al., 2023; Tibiri et al., 2020; Zhang et al., 2020). Although other viruses, such as sweet potato mild mottle virus (SPMMV, *Ipomovirus lenisbatatae*) and sweet potato latent virus (SPLV, *Potyvirus batatalatentis*), may also occur in coinfection, the most severe effects are primarily linked to the synergistic interaction between SPFMV and SPCSV, which significantly increases disease severity and leads to substantial yield reductions (Mukasa et al., 2006; Tibiri et al., 2020).

SPFMV, a member of the *Potyviridae* family, genus *Potyvirus*, is one of the most damaging pathogens affecting sweet potatoes worldwide (Barkessa, 2018; Loebenstein, 2015). Its genome consists of a single positive-sense RNA strand with a poly (A) tail at its 3′ end and a genome-linked protein (VPg) at its 5′ end. The genome contains a large open reading frame (ORF) encoding a polyprotein and the PIPO ORF (Wylie et al., 2017). Phylogenetic studies have identified three distinct lineages, A-I, A-II and B, that differ in geographical distribution and virulence (Maina et al., 2018; Tibiri et al., 2020). Lineages A-I and A-II, predominantly found in Africa and Asia, are associated with more severe symptoms, whereas lineage B, with a broader global distribution, generally causes milder symptoms (Kwak et al., 2015; Tairo et al., 2005). These variations are driven by nucleotide sequence differences in the coat protein (CP) gene, which influence virus–host interactions and virulence (Untiveros et al., 2010).

SPCSV, a bipartite member of the *Closteroviridae* family, genus *Crinivirus*, is restricted to the phloem of the host plant (Cuellar et al., 2011; Loebenstein, 2015). SPCSV has a global distribution and often coexists with other viruses in the plant, complicating its separation and purification. To date, only two strains of SPCSV have been identified: SPCSV-Eastern Africa (SPCSV-EA) and SPCSV-Western Africa (SPCSV-WA) (Kwak et al., 2015; Tairo et al., 2005; Untiveros et al., 2010).

While SPFMV may incite mild symptoms or remain asymptomatic individually, SPCSV typically induces mild to moderate symptoms including generalized chlorosis, slight growth retardation, purple discolouration of older leaves, and mild chlorotic spots on intermediate leaves, leading to yield losses of up to 43% (Karyeija et al., 2000). However, coinfection by both viruses significantly increases SPFMV titres, exacerbating disease severity and yield losses (Adikini et al., 2016).

This study aims to assess the presence of SPFMV and SPCSV, the main viruses associated with SPVD, in sweet potato-producing regions of Costa Rica. To achieve this, a multiplex reverse transcription (RT)-PCR was conducted, followed by an evaluation of the genetic diversity of these viruses. Additionally, the potential of riboprobe technology as an alternative detection method to RT-PCR was explored due to its accuracy, sensitivity (pg/μL of viral RNA), robustness and cost-effectiveness for large-scale screening (James et al., 2006; Pallás et al., 2011, 2017).

2 | MATERIALS AND METHODS

2.1 | Plant material collection

Sampling was conducted in diverse sweet potato-producing regions across Costa Rica, including the North Huetar, Atlantic Huetar and Central regions. A total of 50 young (upper) leaves exhibiting typical symptoms of viral diseases were collected. The selection of sampling areas was based on the extent of sweet potato cultivation in each region, ensuring a proportional representation of the producing zones in the analysis (Figure 1). The samples were stored in plastic bags labelled with the collection date and location, then transported to the laboratory on ice for further processing.

2.2 | RNA extraction and multiplex RT-PCR targeting the CP genes

RNA extraction was performed using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. The purity and concentration of RNA were assessed using the NanoDrop Lite spectrophotometer (Thermo Scientific). For the multiplex RT-PCR assay targeting the CP genes of SPFMV and SPCSV, the SuperScript IV One-Step RT-PCR System (Invitrogen) was employed. CP1A and CP1S primers were used for SPFMV (Prasanth & Hegde, 2008), while CP-F and CP-R primers were chosen to amplify the SPCSV CP gene (Qin et al., 2014). Additionally, nad5 primers described by Menzel et al. (2002) were included to allow the specific amplification of the mRNA of the mitochondrial *nad5* gene. The reaction mixture comprised 1 μL of total RNA, 5 μL of 2× Platinum SuperFi RT-PCR master mix, 0.1 μL of SuperScript IV RT Mix and 0.2 μM of each primer. Cycling parameters were as follows: reverse transcription at 50°C for 15 min; RT inactivation and initial denaturation at 98°C for 2 min; then 30 cycles of denaturation at 98°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. The final extension step was 72°C for 5 min. The amplified products were analysed by electrophoresis on 2% agarose gels prestained with ethidium bromide (EtBr) in TAE buffer for 45 min at 100 V. Specific bands corresponding to SPFMV (960 bp), SPCSV (774 bp) and the mRNA of the mitochondrial *nad5* gene (181 bp) were visualized under UV light. Positive and negative (water) controls were included in each RT-PCR run to ensure result accuracy. For dot blot assays, uniplex RT-PCRs were performed using the same primers and thermocycling conditions described above.

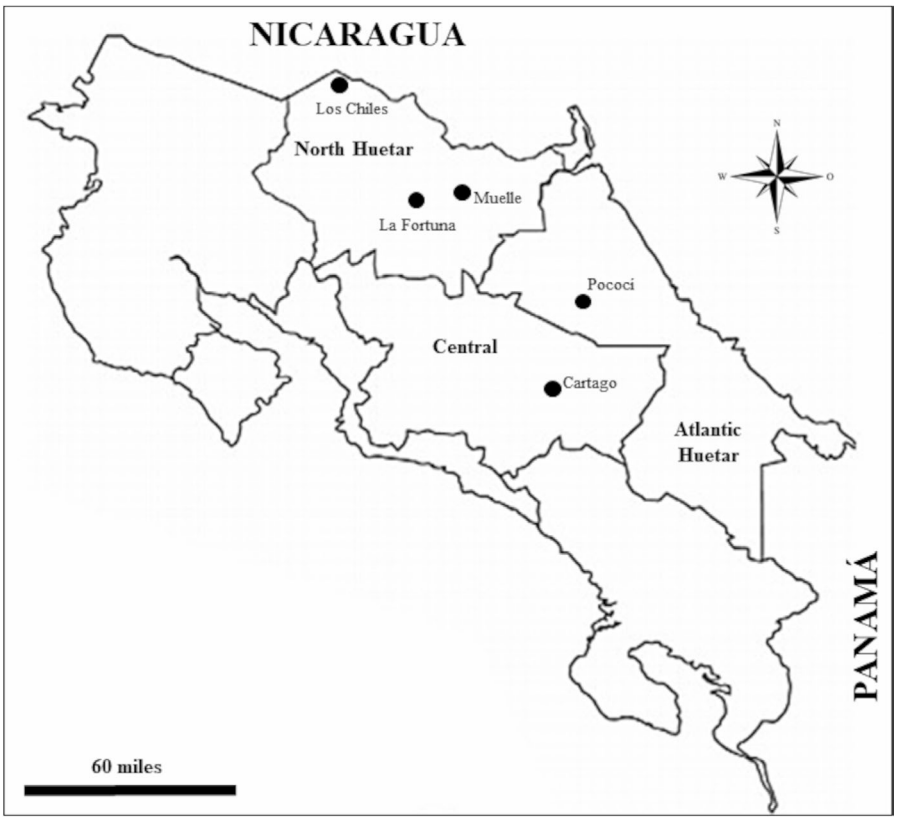

FIGURE 1 Map of Costa Rica showing the geographic locations of the samples collected for this study. According to PROCOMER (2020), approximately 527.6 ha of sweet potatoes are cultivated in Costa Rica, mainly distributed across three regions: North Huetar (70%), Atlantic Huetar (12%) and Central (6%). To ensure equitable representation of the cultivation areas, 34 samples were collected from the North Huetar region, 10 samples from the Atlantic Huetar region and six samples from the Central region, all originating from various farms.

2.3 | Sequencing and sequence analysis

The RT-PCR products were sequenced using Sanger sequencing at the Instituto de Biología Celular y Molecular de Plantas of the Universitat Politècnica de València (IBMCP-UPV). Contigs obtained were cleaned and assembled de novo using SnapGene v. 7.1.1. Subsequently, all sequences underwent nucleotide BLAST searches in the National Center for Biotechnology Information (NCBI) database to identify homologous sequences for phylogenetic analysis. Multiple sequence alignment was performed using ClustalW in MEGA v. 11.0.13 software (Tamura et al., 2021). For reconstructing phylogenetic relationships, the maximum-likelihood method with the Tamura–Nei model (Kumar et al., 2016) was employed. Phylogenetic trees were generated with bootstrap support values of 500 repetitions and were visualized and edited using MEGA v. 11.0.13 software (Tamura et al., 2021).

2.4 | Synthesis of riboprobes labelled with digoxigenin

A 300-nucleotide region of the CP gene was amplified using specific primers 4123sSPFMV9720+XhoI (5′-ataactcGAGG TCACTGACCCAGAAG-3′) and 4124AsSPFMV10036+SalI (5′-TTGAGTCGACCGGGTGTTTGC-3′) for SPFMV and 4121sSPCSV4784+XhoI (5′-cacactcGAGTATAGTTGGAAGACTC -3′) and 4122AsSPCSV5113 + SalI (5′-cacagtcgaCCTGTTCCAGT CAACTGAG-3′) for SPCSV. The RT-PCR was conducted with the SuperScript III One-Step RT-PCR System (Invitrogen) following the manufacturer's instructions. PCR products were first digested with XhoI and SalI restriction enzymes, extracted from agarose gels and then cloned individually into a pBluescript SK (+) vector (AddGene) previously digested with XhoI and dephosphorylated. Subsequently, 1 μg of each plasmid was linearized using the XhoI restriction enzyme, purified by phenol-chloroform extraction and ethanol precipitated (Herranz et al., 2005). The linearized plasmid was then used to synthesize the riboprobe as described in previous studies (Más et al., 1993; Pallás et al., 1998).

2.5 | Dot-blot assays

For each sample, 1 μL of total RNA (200–300 ng) or 4 μL of leaf-tissue homogenates in 5 volumes of cold extraction buffer (50 mM sodium citrate, 5 mM EDTA, pH 8.5) (Sánchez-Navarro et al., 1998) were directly applied to positively charged nylon membranes (Roche), air-dried and cross-linked using a UV transilluminator (exposed for 5 min). Prehybridizations and hybridizations with the probe were conducted as previously described (Pallás et al., 1998; Sánchez-Navarro et al., 1999). Chemiluminescent detection was performed using chemiluminescent alkaline phosphatase substrate (CSPD) reagent as a substrate following the manufacturer's recommendations (Roche). Films were exposed for 30 min. For end-point dilution limit assays, 1.5 ng of total RNA from infected samples was serially diluted (three times) in healthy tissue extract and transferred. Dot-blot hybridization was conducted as described previously.

3 | RESULTS

3.1 | SPFMV and SPCSV distribution in Costa Rica

The multiplex RT-PCR analysis targeting the CP genes of SPFMV and SPCSV revealed a widespread distribution of both viruses in sweet potato-producing regions in Costa Rica. Among the 50 samples analysed, 82% tested positive for SPFMV, while 52% were positive for SPCSV. Additionally, 48% of the samples displayed coinfection with both viruses, highlighting the frequent occurrence of mixed infections in sweet potato plants (Figure 2 and Table 1).

3.2 | Genetic diversity of SPVD-associated viruses in Costa Rican sweet potatoes

To evaluate the genetic diversity and phylogenetic relationships of SPFMV and SPCSV isolates in Costa Rican sweet potatoes, 14 CP amplicons from each virus were selected from SPVD-positive plants collected from the North Huetar, Atlantic Huetar and Central regions (eight, four and two sequences, respectively; Data S1). High sequence conservation was observed in CP nucleotide sequences with over 98.4% identity in SPFMV and over 99.4% in SPCSV.

Comparative analyses were conducted by juxtaposing Costa Rican isolates with 17 SPFMV CP genes collected from diverse geographic locations previously categorized into phylogenetic lineages A-I, A-II and B by Tibiri et al. (2020). Phylogenetic analyses revealed that all SPFMV isolates in Costa Rica (GenBank accession numbers PP887954–PP887967) clustered within phylogroup B (Figure 3). Additionally, pairwise comparisons of SPFMV CP gene sequences isolated in Costa Rica revealed an average similarity of 97.3% with isolates from phylogroup B, whereas sequence similarities with phylogroups A-I and A-II were 91.4% and 89.6%, respectively (Table S1).

Similarly, SPCSV CP gene sequences isolated in Costa Rica were compared with eight other SPCSV isolates, previously classified into WA and EA strains by Tibiri et al. (2020). Phylogenetic analyses revealed that all SPCSV isolates in Costa Rica (GenBank accession numbers PP887968–PP887981) clustered with accessions belonging to the WA strain (Figure 4). Furthermore, pairwise comparison analysis demonstrated that the strains isolated in Costa Rica exhibited a high average similarity of 98.7% with WA isolates, whereas the sequence similarity with EA strain members was notably lower at 66.4% (Table S2). These findings highlight the genetic homogeneity within both SPFMV and SPCSV populations in Costa Rica, providing valuable insights into the evolutionary dynamics and epidemiology of these viruses in the region.

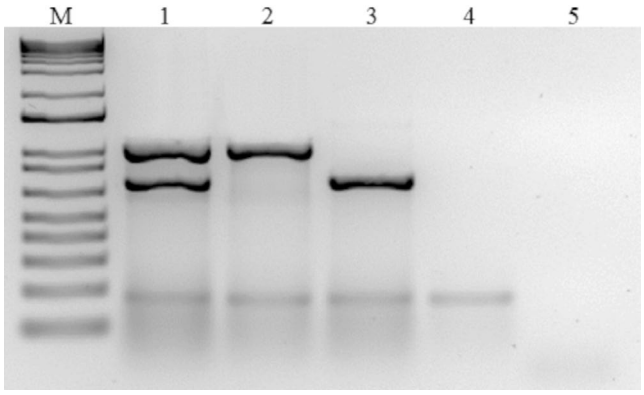

FIGURE 2 Representation of results for the detection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) by multiplex reverse transcription (RT)-PCR targeting the coat protein (CP) gene. Size marker (Lane M): 1 kb Plus DNA Ladder (Invitrogen). Lanes 1–3 show RT-PCR products from sweet potato leaf samples with different virus statuses: Infected with both SPFMV and SPCSV, infected with SPFMV only, and infected with SPCSV only, respectively. Lane 4 represents RT-PCR of a healthy control plant, and lane 5 represents the negative control.

3.3 | Application of non-isotopic molecular hybridization in SPVD diagnosis

To evaluate the end-point detection limit and specificity of synthesized riboprobes for SPFMV and SPCSV, serial three-fold dilutions of total RNA extracted from single SPVD-infected tissue were prepared in total nucleic acids from healthy tissue and applied onto two independent nylon membranes. Each membrane was individually hybridized with the corresponding riboprobe for SPFMV or SPCSV. Specific hybridization signals were detected up to dilution 3^{-9} (1:19,683) for SPFMV (Figure 5, left membrane) and 3^{-4} (1:81) for SPCSV (Figure 5, right membrane), corresponding to approximately 0.08 and 18.5 pg of total RNA of infected plant, respectively. The analysis of the same dilutions with uniplex RT-PCR using 1 μL of each dilution, revealed an endpoint detection limit of 3^{-9} (1:19,683) and 3^{-8} (1:6561) for SPFMV and SPCMV, respectively.

To simulate a routine field assay, 11 previously characterized field samples were analysed. The sensitivity of the riboprobes was compared using total RNA (200–300 ng) and 4 μL of leaf extract (equivalent to 0.8 mg of tissue) from the same sample. A 100% concordance with previous uniplex RT-PCR results was observed when using total RNA samples (Figure 6, rows labelled "RNA" and "RT-PCR" in both membranes). However, in leaf extracts, the sensitivity of the riboprobes decreased by 12.5% (seven positives out of eight) and 9.1% (10 positives out of 11) for SPFMV and SPCSV, respectively, resulting in false negatives (Figure 6, rows labelled "leaf" and "RT-PCR" in both membranes).

4 | DISCUSSION

Our results show a high prevalence of SPFMV (82%) and SPCSV (52%), with a coinfection rate of 48%, highlighting the widespread dissemination of these viruses in sweet potato crops in Costa Rica and the frequency of mixed viral infections that exacerbate

TABLE 1 Distribution of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) in sweet potato-producing regions in Costa Rica.

Region	SPFMV	SPCSV	SPFMV + SPCSV	No virus detected	Total samples
North Huertar					
La Fortuna	10	8	7	3	14
Muelle (San Carlos)	9	6	6	1	10
Los Chiles	8	5	5	2	10
Atlantic Huertar					
Pococí	8	4	3	1	10
Central					
Cartago	6	3	3	0	6
Total	41	26	24	7	50

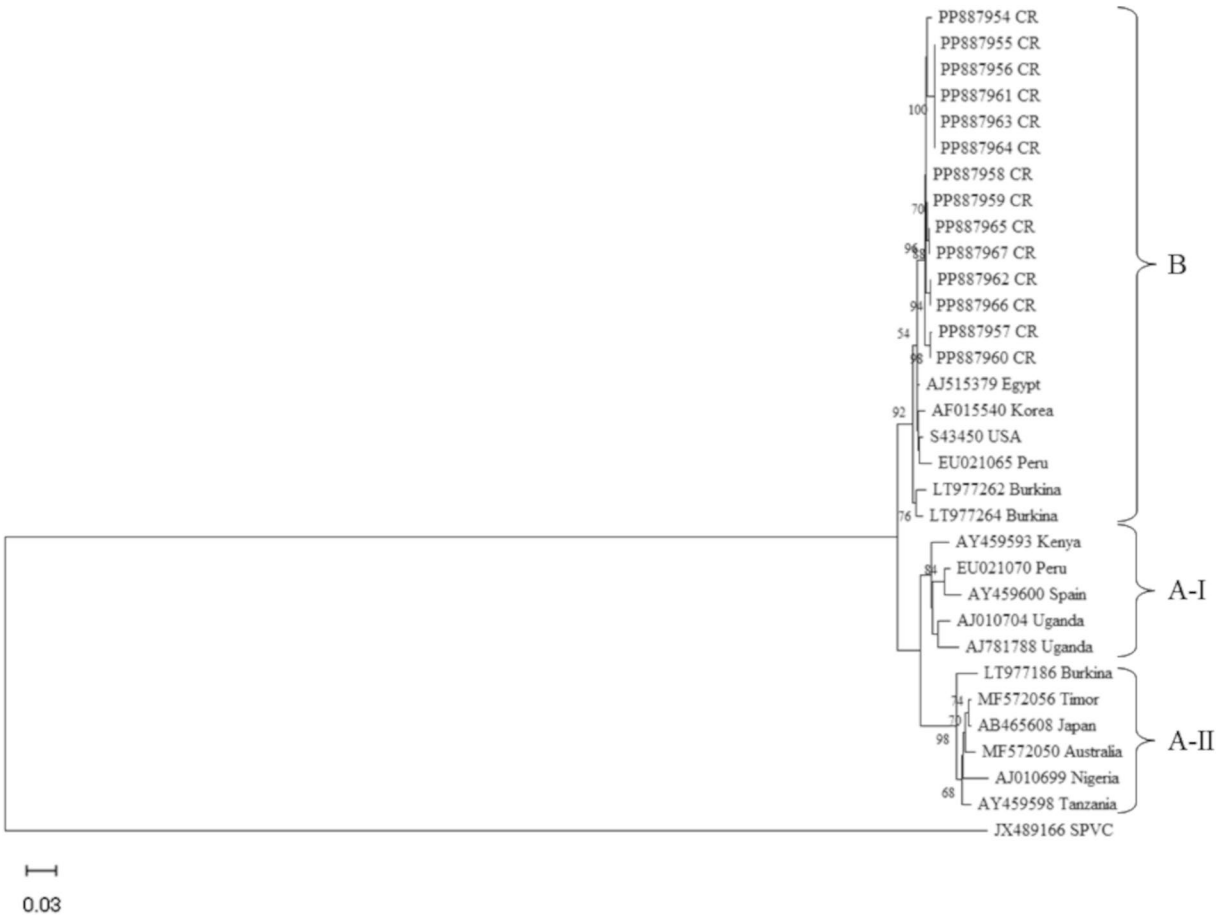

FIGURE 3 Maximum-likelihood phylogenetic tree reconstructed from the alignment of nucleotide sequences of sweet potato feathery mottle virus (SPFMV) coat protein (CP) genes. Bootstrap values, indicating the reliability of the phylogenetic groupings, are shown as percentages at the nodes of the tree. Sweet potato virus C (SPVC, GenBank ID: JX489166) was used as the root of the tree to establish the phylogenetic relationship of SPFMV isolates in Costa Rica. The three phylogenetic subgroups A-I, A-II and B, as described by Tibiri et al. (2020), are indicated. Isolates from this study (GenBank IDs PP887954 to PP887967) are labelled with the abbreviation "CR" (Costa Rica).

disease severity and increase yield losses (Adikini et al., 2016; Clark et al., 2012). Additionally, 14% of the plants tested negative for both viruses, probably due to our sampling strategy, which focused on symptomatic plants to maximize viral detection. Moreover, the presence of other viruses infecting sweet potato cannot be ruled out, as more than 30 viral species have been reported (Mukasa et al., 2006; Valverde et al., 2007). In this study, we focused on SPFMV and SPCSV due to their critical role in the SPVD complex, which causes the most severe yield losses, reaching 80%–100% in coinfected plants (Adikini et al., 2016; Cuellar et al., 2015; Karyeija et al., 2000).

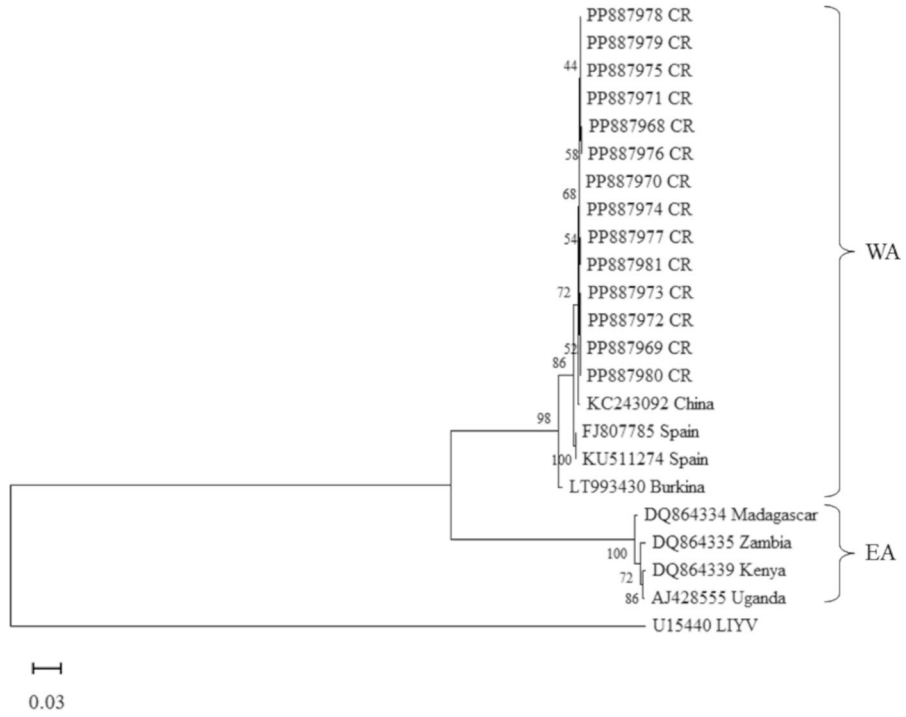

FIGURE 4 Maximum-likelihood phylogenetic tree reconstructed from the alignment of nucleotide sequences of sweet potato chlorotic stunt virus (SPCSV) coat protein (CP) genes. Bootstrap values, indicating the reliability of the phylogenetic groupings, are shown as percentages at the nodes of the tree. Lettuce infectious yellows virus (LIYV, GenBank ID U15440) was used as the root of the tree to establish the phylogenetic relationship of SPCSV isolates in Costa Rica. The two subgroups WA and EA described by Tibiri et al. (2020) are indicated. Isolates from this study (GenBank IDs PP887968 to PP887981) are labelled with the abbreviation "CR" (Costa Rica).

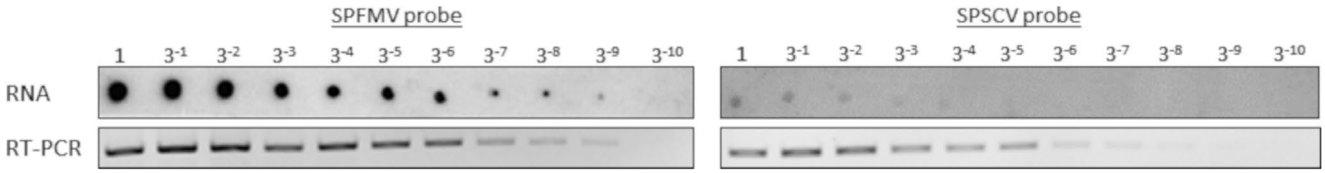

FIGURE 5 Comparative limit of detection between non-isotopic molecular hybridization using riboprobes and reverse transcription (RT)-PCR techniques for the detection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV). Serial three-fold dilutions of total RNA extracted from a single sweet potato virus disease (SPVD)-infected tissue were prepared in total nucleic acids from healthy tissue and hybridized with SPFMV (left membrane) and SPCSV (right membrane) riboprobes. Dilution factors used were: 1:3 (3^{-1}), 1:9 (3^{-2}), 1:27 (3^{-3}), 1:81 (3^{-4}), 1:243 (3^{-5}), 1:729 (3^{-6}), 1:2187 (3^{-7}), 1:6561 (3^{-8}), 1:19,683 (3^{-9}) and 1:59,049 (3^{-10}). Uniplex RT-PCRs from each dilution are shown below the membranes.

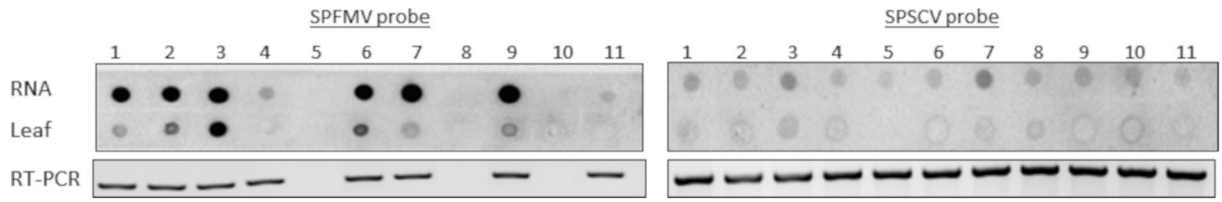

FIGURE 6 Analysis of field samples by non-isotopic molecular hybridization using riboprobes and reverse transcription (RT)-PCR techniques. A total of 11 samples were analysed by dot-blot molecular hybridization using sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) riboprobes with total RNA (RNA) or with leaf-tissue homogenates (Leaf). Uniplex RT-PCRs from total RNA of each sample are shown below each membrane.

Genetic analysis revealed that the SPFMV isolates in Costa Rica predominantly belong to phylogenetic group B, with a high sequence similarity to other isolates within this group. This is significant, as SPFMV phylogroups A-I and A-II are associated with more severe symptoms and higher incidence in various regions, including Africa and Asia, while phylogroup B is generally associated with milder

symptoms (Kwak et al., 2015; Tairo et al., 2005). Regarding SPCSV, our isolates clustered with the West African (WA) strain, known for its widespread distribution and ability to cause moderate to severe symptoms when coinfected with SPFMV (Karyeija et al., 2000; Untiveros et al., 2010).

The low genetic diversity observed in both viral populations may have several implications. First, it suggests that the introduction of these viruses in Costa Rica could have occurred through a limited number of initial infection events, followed by local spread (Tairo et al., 2005). Second, the high sequence conservation could facilitate the development of targeted diagnostic tools and management strategies, as existing methods may be effective in detecting and controlling these predominant strains (James et al., 2006). However, it also raises concerns about the potential for rapid spread and severe outbreaks if new, more virulent strains are introduced or evolve, demanding constant and adaptable monitoring (Karyeija et al., 2000).

The implementation of robust diagnostic tools is crucial for the effective management of SPVD. In this study, we evaluated riboprobe technology as an alternative to RT-PCR for the detection of SPFMV and SPCSV. Our results showed that the riboprobe for SPFMV had sensitivity comparable to RT-PCR, while the riboprobe for SPCSV showed slightly lower sensitivity. However, when using leaf extracts, we observed a decrease in riboprobe sensitivity, a common phenomenon likely due to the presence of inhibitory compounds in crude extracts, the instability of viral RNA, or a lower amount of viral RNA in crude extracts compared to total RNA extraction (Pallás et al., 2011). Despite the sensitivity differences observed between RT-PCR and molecular hybridization, the direct comparison of leaf extracts from field samples investigated using RT-PCR or riboprobes revealed a difference of only 10% fewer positives, making the riboprobes a good alternative to the RT-PCR technique.

Compared to the RT-PCR technique, riboprobes offer significant advantages in terms of cost and ease of use for large-scale sample analysis. Although RT-PCR is highly sensitive, it can be expensive due to the required reagents and equipment (Rubio et al., 2020), limiting the number of field samples that could be analysed. In contrast, riboprobes are more economical and suitable for large-scale detection, which is essential in disease monitoring and management programmes over large cultivation areas. This is particularly important in contexts where resources are limited and extensive monitoring is required (Pallás et al., 2011, 2017).

To improve the sensitivity of riboprobes, the literature suggests several strategies. One option is to perform multiple replicates of each sample, increasing the probability of detecting viruses present (Herranz et al., 2005; Sánchez-Navarro et al., 1999). Additionally, optimizing the sample preparation protocol to reduce the presence of inhibitory compounds, increase the RNA stability or concentration, and improve hybridization efficiency is recommended (Ferriol et al., 2015). These strategies would not only enhance the sensitivity of riboprobes but also maintain their economic viability, making them a powerful and practical tool for virus detection in large-scale monitoring programmes.

In conclusion, this study provides valuable insights into the prevalence and genetic diversity of viruses associated with SPVD in Costa Rica. The high rates of prevalence and coinfection observed emphasize the urgent need for improved diagnostic tools and management strategies to mitigate the impact of these viruses on sweet potato crops. Although the number of samples analysed was relatively small ($n = 50$), it is consistent with similar studies that have drawn significant conclusions on virus prevalence and distribution (Nhlapo et al., 2018; Tang et al., 2024). This research serves as a preliminary evaluation, offering a foundation for future studies with larger sample sizes. Furthermore, the synthesis and evaluation of riboprobes for SPFMV and SPCSV demonstrated their potential as cost-effective and sensitive techniques for large-scale virus detection. These findings contribute to the global effort to understand better and control viral diseases in sweet potato production, underlining the importance of continued research and international collaboration to combat these persistent threats.

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CONFLICT OF INTEREST STATEMENT

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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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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