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Research

Fruit and Ornamental Plants as Natural Hosts of Cacao Mild Mosaic Virus (CaMMV)

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Abstract

Cacao mild mosaic virus (CaMMV), a member of the Badnavirus genus (family: *Caulimoviridae*), causes an emerging disease on *Theobroma cacao*. It is associated with branch dieback and has been reported to reduce yield. As there is no treatment for infected plants, preventing transmission is the most effective strategy. However, to understand what inoculum reservoirs exist in the environment, it is necessary to determine the host range of the virus. Previously, this virus was only reported on cacao. To determine whether other species could serve as hosts, plants in the Malvaceae and other families growing near CaMMV-infected cacao were sampled and tested for the presence of the virus. Plants belonging to seven species of Malvaceae, one species of Fabaceae, and one species of Meliaceae tested positive, including widely grown ornamental plants and fruit trees such as hibiscus (*Hibiscus* sp.), maga (*Thespesia grandiflora*), and durian (*Durio* sp.). These additional hosts may contribute to disease spread by serving as inoculum reservoirs in areas where the virus is present. The CaMMV strains found on non-cacao hosts shared a high identity with cacao-infecting strains, suggesting recent movement between cacao and other species.

Keywords: alternative hosts, epidemiology, inoculum reservoir, transmission

Viruses have been confirmed in cacao on every continent where the crop is cultivated (North America, South America, Asia, and Africa) (Muller et al. 2018; Posnette 1944; Posnette and Palma 1944; Posnette et al. 1950; Probowati et al. 2019). Most of these are in the *Badnavirus* genus (Chingandu et al. 2017; Muller et al. 2018; Probowati et al. 2019), except for *Cacao yellow mosaic virus*, *Cacao necrosis virus*, and *Cacao polerovirus*, which are in the *Tymovirus*, *Nepovirus*, and *Polerovirus* genera, respectively (Ding et al. 1990; Kenten 1972; Ullah and Dunwell 2023). Most of the *Badnavirus* species (>10) known to infect cacao belong to the species complex that causes cacao swollen shoot virus disease (CSSVD), which significantly limits the production of cocoa beans (Muller et al. 2018; Ullah et al. 2021). Research efforts targeting cacao viruses focus on the most virulent species causing CSSVD, which are limited to West Africa, where over 75% of the world's cocoa beans are grown. These highly virulent species cause severe yield reduction and tree death within a few years after infection (Muller et al. 2018; Posnette 1947). Annual losses due to CSSVD were estimated at

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76,000 metric tons in 2012 (Ploetz 2016) and have led to farms in high-pressure areas being abandoned.

In the Americas, virus symptoms on cacao were reported in Trinidad and Venezuela in the 1940s (Posnette 1944; Posnette and Palma 1944). Research in Trinidad described two distinct strains, which were later named cacao mild mosaic virus (CaMMV) and cacao yellow vein banding virus (Chingandu et al. 2017). Until 2020, CaMMV had only been reported in Trinidad and was believed to have been mostly eradicated following a government tree removal program in the late 1950s. However, within the past few years, CaMMV has been detected in Puerto Rico, Brazil, Florida, Hawaii, and Indonesia and may be widespread worldwide (Kandito et al. 2022; Keith et al. 2024; Puig et al. 2020, 2021; Ramos-Sobrinho et al. 2021). The genome of CaMMV is approximately 7.5 kb and contains three open reading frames (ORFs) found in all members of the *Badnavirus* genus, plus an additional one known as ORFY (Chingandu et al. 2019). PCR primers used to detect badnaviruses in cacao are designed for the highly conserved region of ORF3 containing the putative movement protein (Chingandu et al. 2019; Puig 2021b).

Plants infected with CaMMV develop symptoms such as leaf vein banding, leaf and pod mosaic, and dwarfed pods, and yield losses are reported to be comparable to mild to moderate strains of CSSVD in West Africa, with annual yield reductions of 6.6 to 19% (Baker and Dale 1947; Cope 1953). A recent study showed that infected plants were more susceptible to fungi causing branch dieback and developed greater lesions following inoculation (Puig 2022). CaMMV is transmitted by several mealybug species, via the use of infected material during grafting, and from seeds harvested from infected mother plants (Kirkpatrick 1950, 1953; Puig 2021a). No transmission has been observed through mechanical means such as pruning tools (Posnette 1944).

Research on CaMMV in Trinidad found five species of mealybugs able to transmit the virus: *Planococcus citri*, *Dysmicoccus brevipes*, *D.* sp. near *brevipes*, *Ferrisia virgata*, and *Pseudococcus comstocki* (Kirkpatrick 1950, 1953). In Florida, *Pseudococcus jackbeardsleyi*, *Maconellicoccus hirsutus*, *Ps. comstocki*, and *F. virgata* were found feeding on CaMMV-infected plants, with 35 to 45% of specimens having detectable levels of the pathogen (Puig et al. 2021). Although *P. jackbeardsleyi* and *M. hirsutus* were not included in transmission studies in Trinidad, these species have been reported on CSSV-infected cacao in Cote d'Ivoire (N'Guessan et al. 2019) and may be vectors of CaMMV.

Long-distance movement of the virus is likely caused by the movement of infected budwood and pods from infected plants. After infection of a seedling through the grafting of infected budwood, symptoms become visible when new leaves are produced (Posnette 1944). However, because CaMMV is unevenly distributed in infected plants, not all budwood taken from infected plants will transmit the virus. It is estimated that only 50% of grafted plants were infected with CaMMV when budwood from infected plants was used in propagation (Posnette 1944). With pods from infected plants, CaMMV was detected in over 50% of the resulting seedlings when the pods had been highly symptomatic (Puig 2021a). A subsequent assay done with seeds from pods with few symptoms found that 41.7% of offspring carried the virus at 6 weeks post-germination (Puig et al. 2023). Most infected seedlings developed symptoms such as leaf mosaic and vein banding, but these symptoms were variable, repeatedly appearing and disappearing throughout the lifetime of the plants.

Alternative hosts contribute to disease spread by serving as inoculum reservoirs in areas where the virus is present and can initiate infections if mealybugs move from these infected hosts to newly established farms or nearby healthy orchards (Tinsley 1971). CSSV, a closely related virus, was found to have multiple alternate hosts serving as inoculum reservoirs in the cacao-growing hotspot of West Africa (Posnette 1981; Posnette et al. 1950; Tinsley and Wharton 1958). In the Americas, several relatives of *Theobroma cacao* are commonly found near commercial cacao production areas and could serve as inoculum reservoirs or sources of infection of CaMMV. The goal of this study was to use a recently developed PCR-based diagnostic test (Puig 2021b) to identify additional virus hosts in Puerto Rico, Florida, and Hawaii, three regions of the United States producing cacao or maintaining cacao germplasm.

Materials and Methods

Sampling and DNA extraction

Six sites in three geographic regions were chosen to sample for hosts of CaMMV (Table 1). In Puerto Rico, sampling was done at the USDA-ARS Tropical Agricultural Research Station in Mayaguez, as well as three sites on, or adjacent to, commercial cacao farms throughout the island. In Florida and Hawaii, samples were only taken from plants at or near USDA-ARS research stations. These were the Subtropical Horticultural Research Station in Miami and the Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center in Hilo, respectively. All three USDA-ARS research stations in this study house significant germplasm collections of *T. cacao* (Irish et al. 2010), and CaMMV has been confirmed to be present at all sites (Keith et al. 2024; Puig 2021b; Puig et al. 2021). Leaves were collected from potential hosts. Sixty-three plants were sampled from 27 species belonging to six families: Malvaceae ($n = 21$), Fabaceae ($n = 2$), Anacardiaceae ($n = 1$), Bixaceae ($n = 1$), Meliaceae ($n = 1$), and Moraceae ($n = 1$). To verify the presence of CaMMV on or near commercial farms, leaves from *T. cacao* plants were also sampled and tested.

Due to the uneven distribution of CaMMV in infected plants, two leaves were tested per plant to minimize false negatives. Preference was given to symptomatic or tender leaves when present. Leaves were shipped overnight to the USDA-ARS Foreign Disease Weed Science Research Unit in Maryland. DNA was extracted from 60 mg of fresh leaf tissue from the leaf petiole using the Qiagen DNeasy Plant Mini kit (Qiagen, Valencia, CA, U.S.A.) with one final elution of 50 µl.

PCR amplification and sequencing

The presence of the virus was determined using CaMMV-specific diagnostic primers Mia1396F and Mia1667R (Puig 2021b). PCRs were performed in 25-µl volumes with 12 µl of Sigma-Aldrich JumpStart REDTaq ReadyMix, 8 µl of molecular grade water, 1 µl of each 10 µM primer (Mia1396F and Mia1667R), and 3 µl of DNA template. Amplification conditions were a preliminary denaturing step at 94°C for 2 min, followed by 38 cycles at 94°C for 20 s, 57°C for 20 s, and 72°C for 50 s, and a final extension at 72°C for 8 min.

Amplicons were visualized on a 1% agarose gel, and the amplified product was purified with ExoSAP-It (Applied Biosystems, Waltham, MA, U.S.A.), then bidirectionally Sanger sequenced by Eurofins Genomics (Louisville, KY, U.S.A.). Forward and reverse sequences were edited and aligned using Geneious 11.1.2 (Biomatters, Auckland, New Zealand) and analyzed using BLASTn to confirm the identity of amplified fragments. A plant was considered positive if the amplicon yielded a sequence that matched sequences of CaMMV available in GenBank.

Phylogenetic analysis

The phylogenetic relationships among CaMMV strains were analyzed using partial sequences (178 bp) of the movement protein-coat protein genes from 35 isolates, including 10 gen-

erated in this study (4 from *T. cacao* and 6 from other hosts), 17 from the GenBank database, and 8 previously unpublished sequences from plants in Puerto Rico (Table 2). The publicly available sequences are from Trinidad (KX276640), Puerto Rico (MT253656, MT253658, MT262890, and MW052520), Brazil (MW052521 and MW052522), Florida (MZ325897, MZ409678, MZ409679, MZ409693, and MZ409699), Hawaii (OQ692891 and OQ692892), and Indonesia (OR396882, OR396883, and OR396884). The author's previously unpublished sequences were amplified from symptomatic plants at the USDA-ARS cacao germplasm collection in Puerto Rico from clones ICS16 ($n = 2$), ICS61, RIM52, PRC9 ($n = 2$), ICS45, and an Amelonado accession whose seeds are used as the preferred seedling rootstock (A. S. Puig, *unpublished data*). This dataset includes isolates from six distinct geographical areas. A representative subset of sequences generated from this study have been deposited in GenBank (Table 2).

Sequences were aligned using the MUSCLE algorithm in the Geneious Primer software (2023.0.1; Biomatters, Boston, MA, U.S.A.), and ends were filled in with Ns (Edgar 2004). A maximum likelihood phylogenetic tree was constructed with IQ-TREE v1.6.12 (Nguyen et al. 2015), following the selection of the best nucleotide substitution model using the Bayesian information criterion (the best model was TIM2e + G4) and ultra-fast bootstrap analysis using a maximum of 1,000 iterations. The resulting tree was visualized using the Interactive Tree Of Life (iTOL) v4 (Letunic and Bork 2024).

Results

Detection of CaMMV in plant hosts

A total of 51 plants representing 21 Malvaceae species were tested for the presence of CaMMV. Seven (13.7%) of these samples were determined to be positive based on them yielding sequences with high identity to CaMMV: *Ceiba aesculifolia*, *Durio* sp., *Hibiscus* sp., *Luehea seemannii*, *Pterospermum acerifolium*, *Theobroma grandiflorum*, and *Thespesia grandiflora* (Table 3). Virus-like symptoms were only observed on *Durio* sp., *Hibiscus* sp., and *Pavonia* sp. As expected, the detection of virus was variable across the samples and the locations. For example, a sample of *Thespesia grandiflora* collected from a commercial farm in Puerto Rico tested positive; however, CaMMV was not detected in a sample of this species collected from the USDA germplasm collection in Florida.

The non-Malvaceae included in this study are commonly found near cacao farms. Among the 12 samples from six non-Malvaceae species, CaMMV was detected in one of the four *Inga vera* (Fabaceae) samples tested and in the only sample of *Swietenia macrophylla* (Meliaceae). In a different *Inga vera* sample, a sequenced fragment was obtained with almost double the length expected (over 400 versus 267 bp). Analysis in BLASTn showed that the last third of the sequence was inverted and in the opposite orientation to the rest of the sequence. The plant was not counted as positively infected with CaMMV due to the presence of this unexpected inversion.

TABLE 1

List of plant samples collected from six sites near cacao infected with cacao mild mosaic virus

Region	Site	Species – family	Number of plants
Puerto Rico	USDA germplasm collection	*Adansonia digitata* (baobab) – Malvaceae	2
		Ceiba pentandra (kapok) – Malvaceae	1
		Cola acuminata (cola) – Malvaceae	2
		Herrania umbratica (monkey cacao) – Malvaceae	2
		Pachira aquatica (money tree) – Malvaceae	1
		Sterculia apetala (Panama tree) – Malvaceae	1
		Theobroma grandiflorum (cupuassu) – Malvaceae	2
	Commercial farm-1	*Bixa orellana* (achiote) – Bixaceae	1
		Inga laurina (guamá) – Fabaceae	1
		Inga vera (river koko) – Fabaceae	2
		Mangifera indica (mango) – Anacardiaceae	1
		Pavonia sp. (mallow) – Malvaceae	2
		Swietenia macrophylla (big-leaf mahogany) – Meliaceae	1
		Theobroma grandiflorum (cupuassu) – Malvaceae	2
	Commercial farm-2	*Inga vera* (river koko) – Fabaceae	1
		Thespesia grandiflora (maga) – Malvaceae	1
		Ceiba pentandra (kapok) – Malvaceae	1
	Commercial farm-3	*Bixa orellana* (achiote) – Bixaceae	3
		Inga vera (river koko) – Fabaceae	1
		Artocarpus heterophyllus (jackfruit) – Moraceae	1
		Taliparitі elatum (blue mahoe) – Malvaceae	1
		Thespesia grandiflora (maga) – Malvaceae	1
Hawaii	USDA germplasm collection	*Durio sp.* (durian) – Malvaceae	5
		Hibiscus sp. (hibiscus) – Malvaceae	4
Florida	USDA germplasm collection	*Adansonia digitata* (baobab) – Malvaceae	3
		Carpodiptera (*Berrya*) *cubensis* (alzaprima) – Malvaceae	1
		Ceiba aesculifolia (pochote) – Malvaceae	3
		Guazuma ulmifolia (West Indian elm) – Malvaceae	1
		Luehea seemannii (guácimo colorado) – Malvaceae	2
		Pachira aquatica (money tree) – Malvaceae	2
		Pseudobombax ellipticum (shaving brush tree) –Malvaceae	5
		Pterospermum acerifolium (bayur tree) – Malvaceae	2
		Sterculia africana (African star-chestnut) – Malvaceae	1
		Sterculia foetida (Java olive) – Malvaceae	1
		Sterculia villosa (elephant rope tree) – Malvaceae	1
		Thespesia grandiflora (maga) – Malvaceae	1

CaMMV was not detected in the 11 species that were represented by relatively low numbers of samples. For example, seven species (*Carpodiptera cubensis*, *Guazuma ulmifolia*, *Sterculia africana*, *Sterculia apetala*, *Sterculia foetida*, *Sterculia villosa*, and *Talipariti elatum*) were represented by a single plant in this study. Despite observing amplification in at least one sample each of *Adansonia digitata*, *Pseudobombax ellipticum*, and *Pavonia* sp., these were not classified as positive for CaMMV based on inconclusive sequencing results.

Leaves from *T. cacao* plants on/near commercial farms in Puerto Rico were also tested to verify the presence of CaMMV. The virus was detected in 71.4% (20 of 28) of these samples. A genetically representative subset of four sequences from these was included in the phylogenetic analysis (Table 2). In this study,

TABLE 2

Representative sequences of cacao mild mosaic virus included in the phylogenetic analysis

ID	Host[a]	Location[b]	Accession number
Puerto Rico	*Luehea seemannii*	FL-germplasm collection	PQ682506
Np39b	*Theobroma cacao*	PR-commercial farm	PQ682507
Np46a	*Theobroma cacao*	PR-commercial farm	PQ788386
Np49a	*Swietenia macrophylla*	PR-commercial farm	PQ788379
Np51a	*Theobroma cacao*	PR-commercial farm	PQ788387
Np52b	*Theobroma grandiflorum*	PR-commercial farm	PQ682508
Np57b	*Inga vera*	PR-commercial farm	PQ682509
Np65a	*Theobroma cacao*	PR-commercial farm	PQ682510
Np88b	*Durio* sp.	HI-germplasm collection	PQ682512
Amel	*Theobroma cacao*, Amelonado	PR-germplasm collection	PQ788380
ICS16-2	*Theobroma cacao*, ICS16	PR-germplasm collection	PQ788381
ICS16-6	*Theobroma cacao*, ICS16	PR-germplasm collection	PQ788382
ICS45-3	*Theobroma cacao*, ICS45	PR-germplasm collection	PQ788383
ICS61-5	*Theobroma cacao*, ICS61	PR-germplasm collection	PQ788384
PRC9-1	*Theobroma cacao*, Criollo	PR-germplasm collection	PQ788389
PRC9-4	*Theobroma cacao*, Criollo	PR-germplasm collection	PQ788390
RIM52-4	*Theobroma cacao*, RIM52	PR-germplasm collection	PQ788391

[a] Clone name or genetic background is listed after species when known.
[b] FL, Florida; HI, Hawaii; PR, Puerto Rico.

TABLE 3

Results of cacao mild mosaic virus diagnostic PCR test using primers Mia1396F/Mia1667R

Species	Family	Plants	Amp[a]	Seq[b]	Region[c]
Mangifera indica	Anacardiaceae	1	0	0	CF_PR
Bixa orellana	Bixaceae	4	0	0	CF_PR
Inga laurina	Fabaceae	1	0	0	CF_PR
Inga vera[d]	Fabaceae	4	1	1	CF_PR
Adansonia digitata	Malvaceae	5	1	0	FL, PR
Carpodiptera (*Berrya*) *cubensis*	Malvaceae	1	0	0	FL
Ceiba aesculifolia[d]	Malvaceae	3	1	1	FL
Ceiba pentandra	Malvaceae	2	0	0	CF_PR, PR
Cola acuminata	Malvaceae	2	0	0	PR
Durio sp.[d]	Malvaceae	5	2	1	HI
Guazuma ulmifolia	Malvaceae	1	0	0	FL
Herrania umbratica	Malvaceae	2	0	0	PR
Hibiscus sp.[d]	Malvaceae	4	2	1	HI
Luehea seemannii[d]	Malvaceae	2	1	1	FL
Pachira aquatica	Malvaceae	3	0	0	FL, PR
Pavonia sp.	Malvaceae	2	1	0	CF_PR
Pseudobombax ellipticum	Malvaceae	5	1	0	FL
Pterospermum acerifolium[d]	Malvaceae	2	1	1	FL
Sterculia apetala	Malvaceae	1	0	0	PR
Sterculia africana	Malvaceae	1	0	0	FL
Sterculia foetida	Malvaceae	1	0	0	FL
Sterculia villosa	Malvaceae	1	0	0	FL
Talipariti elatum	Malvaceae	1	0	0	CF_PR
Theobroma cacao[d]	Malvaceae	28	21	20	CF_PR
Theobroma grandiflorum[d]	Malvaceae	4	2	1	CF_PR, PR
Thespesia grandiflora[d]	Malvaceae	3	1	1	CF_PR, FL
Swietenia macrophylla[d]	Meliaceae	1	1	1	CF_PR
Artocarpus heterophyllus	Moraceae	1	0	0	CF_PR

[a] Amp, PCR-tested positive plants.
[b] Seq, sequencing-verified positive plants.
[c] FL, USDA germplasm collection, Florida; HI, USDA germplasm collection, Hawaii; PR, USDA germplasm collection, Puerto Rico; CF_PR, commercial farms, Puerto Rico.
[d] Plant species in which cacao mild mosaic virus was detected.

only two of the positive plants had CaMMV detected in both sampled leaves, supporting previous observations of the uneven distribution of the pathogen.

Relationships between CaMMV strains

Evolutionary relationships among virus isolates found on *T. cacao* and other hosts were inferred in IQ-TREE using the maximum likelihood method and TIM2e + G4 model for sequences of the coat protein-movement protein region. The tree with the highest log likelihood (–686.15) is shown in Figure 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The forward and reverse sequences obtained for *Hibiscus* sp., *Ceiba aesculifolia*, and *Thespesia grandiflora* were too short (89 to 110 bp) to overlap, so an aligned consensus sequence was not obtained, and these hosts were omitted from the phylogenetic analysis. However, these sequences shared 100% identity to CaMMV from durian, *Inga vera*, and *T. grandiflorum*.

The sequences from the Americas clustered into two primary groups, with each containing at least one sequence from a non-cacao host (Fig. 1). The largest group ($n = 23$) contained sequences from five non-cacao hosts, as well as published sequences from Trinidad, Indonesia, Brazil, Hawaii, Puerto Rico, and Florida. Sequences in this group shared 92.1 to 100% identity among themselves. The smaller group ($n = 12$) contained the sequence from *Swietenia macrophylla*, one published sequence from cacao in Hawaii, three published sequences from cacao in Florida, and seven previously unpublished sequences from cacao on commercial farms and a germplasm collection in Puerto Rico. Sequences in this clade also shared high nucleotide identity (93.3 to 100%) among themselves. The reference strain from Indonesia did not cluster with any other sequences. All publicly available sequences from Brazil, Indonesia, and Trinidad clustered into the larger clade.

Sequences of CaMMV from *Durio* sp., *I. vera*, and *T. grandiflorum* were identical to each other. They shared 98.9 and 98.6%

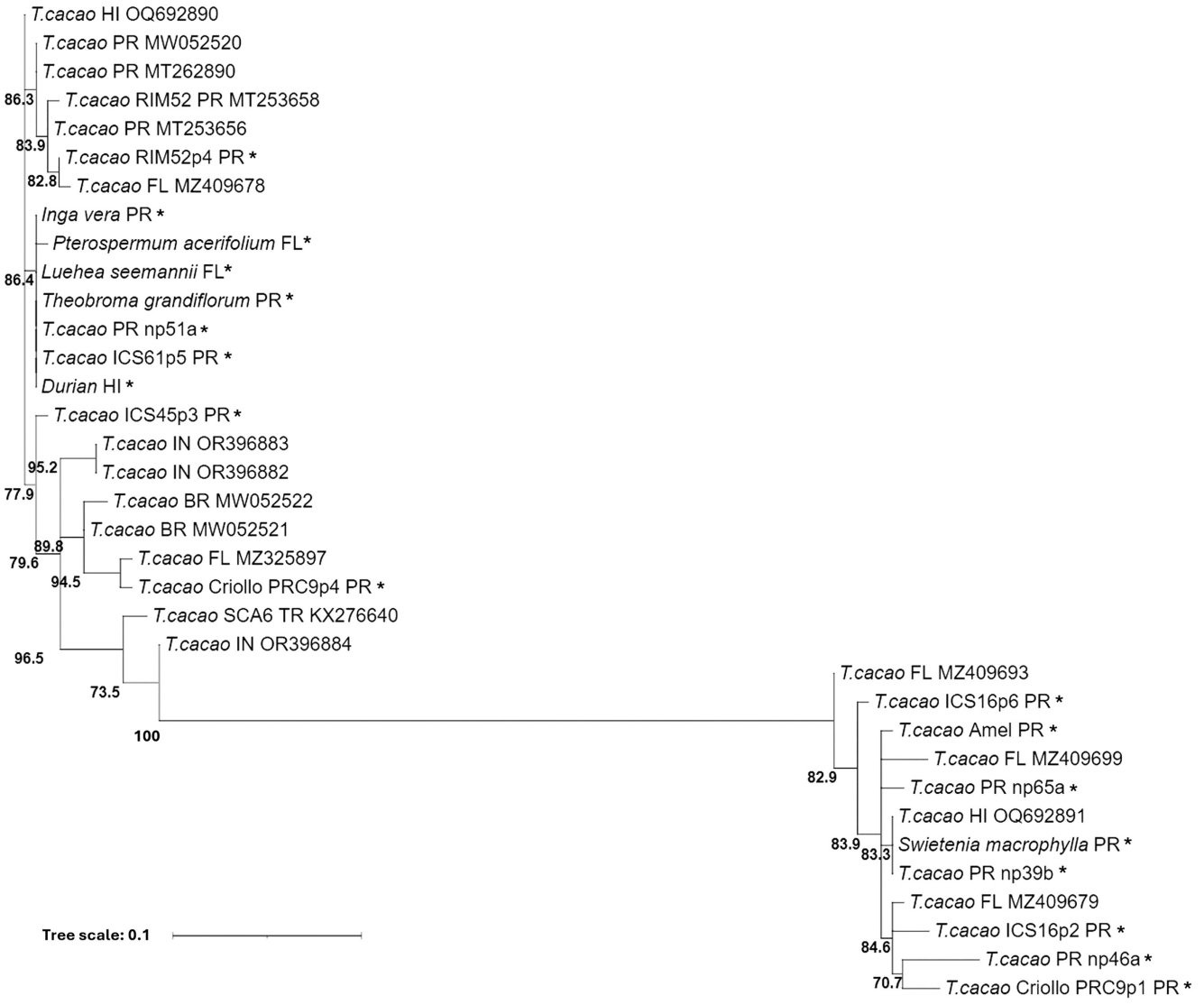

FIGURE 1

Phylogenetic tree constructed using sequences of the movement protein-coat protein (MP-CP) genome region using the maximum-likelihood method and neighbor-joining algorithm in IQ-TREE. Cacao mild mosaic virus sequences were obtained from plants in Puerto Rico (PR), Florida-USA (FL), Hawaii-USA (HI), Brazil (BR), Trinidad (TR), and Indonesia (IN). Geographic abbreviations are included in all sequence labels. Sequences obtained in this study are followed by an asterisk.

identity with sequences from *L. seemannii* and *P. acerifolium*, respectively. The CaMMV strain from *Swietenia macrophylla* shares 100% identity with a sequence from cacao from a different commercial farm in Puerto Rico and 99.7% identity with a published sequence from cacao in Hawaii (OR396882).

Discussion

This study identified nine potential alternative hosts for CaMMV, nearly one-third of the species tested in this study. Most of these, such as *T. grandiflorum*, *Durio* sp., *L. seemannii*, *P. acerifolium*, *Hibiscus* sp., *C. aesculifolia*, and *Thes. grandiflora*, are relatives of cacao and taxonomically classified in the Malvaceae family. However, two additional species, *I. vera* and *S. macrophylla* in the Fabaceae and Meliaceae, respectively, were also identified as hosts. In a few species, amplification of the appropriate 267-bp fragment was observed, but the bands were faint, and sequences were not obtained. Additional sampling is needed to determine whether this is caused by lower levels of the virus in these plants. Alternative hosts of CSSV were found to have a lower titer of the virus compared with cacao (Tinsley and Wharton 1958), which can make detection difficult because nonspecific amplification may outcompete virus-specific amplification in those situations (Nix et al. 2006).

An expanded host range can increase the prevalence of the virus by increasing the number of pathogen reservoirs in a given area and by maintaining high vector populations (McLeish et al. 2018). However, not all plants capable of becoming infected with virus can be sources of inoculum for subsequent infection. Studies on badnaviruses in West Africa showed low rates of transmission from alternative hosts to cacao, with more insect vectors needed for successful virus transmission (Wheeler and Suárez 1993). Transmission studies are needed to confirm that these hosts can act as sources of CaMMV that infect cacao. Additional studies are also needed including larger numbers of plants to determine the prevalence of CaMMV among different plant species.

In this study, few non-cacao hosts showed symptoms, which is consistent with the work by Baker and Dale (1947). Even in cacao, the presumed primary host of the virus, CaMMV infection is often asymptomatic, with foliar symptom expression affected by factors such as recency of infection and light levels (Posnette 1944; Puig 2021b; Ullah et al. 2021). The uneven distribution of CaMMV is supported by the finding that most positive plants only had one of two leaves testing positive. This emphasizes the importance of testing multiple leaves. Plants in this study were selected based on proximity to cacao plants known, or suspected, to be infected with CaMMV and are expected to have had similar levels of exposure to the virus.

The detection of CaMMV in durian has potential repercussions for cacao production in Southeast Asia, where 15% of the global cacao supply is produced. Although mild virus-like symptoms were observed on the durian leaves in this study (Fig. 2), a viral disease has been reported on durian in the Philippines based on leaf mosaic symptoms. This leaf mosaic was the second most prevalent foliar disease recorded on this crop in the Philippines during a disease survey (Leonida et al. 2023). Despite its prevalence, no follow-up work has been done to identify the causal organism.

Hibiscus species are popular ornamental shrubs cultivated worldwide for their colorful flowers. *Hibiscus* spp. are known to be hosts of a wide range of viruses, including those in the *Tobamovirus*, *Betacarmovirus*, *Carmovirus*, *Cilevirus*, *Begomovirus*, *Nepovirus*, *Tymovirus*, *Soymovirus*, *Rhabdovirus*, and *Ilarvirus* genera (Bhaskara Reddy et al. 2012; Givord et al. 1972; Lockhart 1987; Rajeshwari et al. 2005; Roy et al. 2024; Tang et al. 2008; Wang et al. 2023a). Of these, ornamental hibiscus in Hawaii have been found to be infected with members of the genera *Cilevirus*, *Tobamovirus*, *Betacarmovirus*, and *Soymovirus* (Melzer et al. 2013; Wang et al. 2023b). These viruses were reported to cause symptoms such as mosaic and chlorotic ringspot on infected plants. This is the first report of a badnavirus infecting *Hibiscus* spp.

Big-leaf mahogany (*Swietenia macrophylla*, family Meliaceae) is a valuable timber species in tropical regions. It was introduced to Puerto Rico in the early 1900s, where it was widely planted for reforestation and commercial timber (Francis 2003). It is grown commercially throughout Latin America, in regions where cacao is also cultivated. Although only one sample was included in this study, CaMMV was detected in both leaves tested

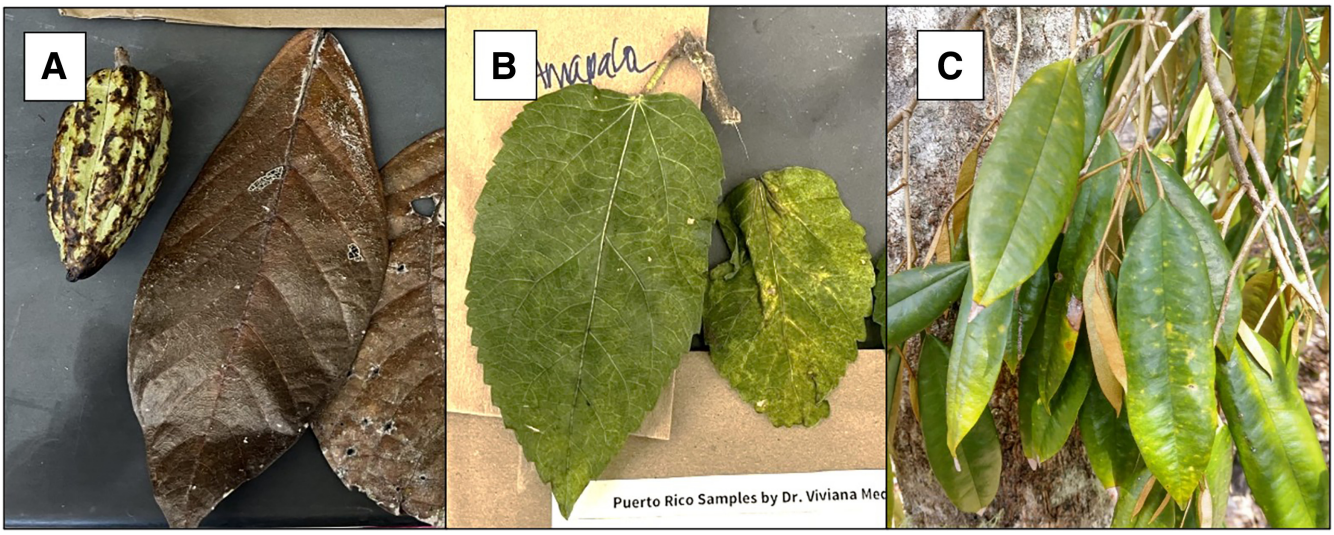

FIGURE 2
A, Samples from cacao (*Theobroma cacao*) with an abnormally small pod, as is sometimes seen in plants infected with Cacao mild mosaic virus. **B,** Leaves of mallow (*Pavonia* sp.) with mosaic symptoms characteristic of virus infection. **C,** Virus-like symptoms on durian (*Durio* sp.).

and yielded a high-quality sequence indicating moderate to high virus titer. *Inga vera* (family Fabaceae), the other non-Malvaceae species in which CaMMV was detected, is a common shade tree planted on cacao farms (Nygren et al. 2013). No symptoms were observed in this study, but a possible virus disease was previously reported on *Inga* spp. in El Salvador, based on leaf mottling symptoms (Stevenson and Wellman 1944). Although additional work is needed to determine the relative prevalence of CaMMV in these species and whether they can serve as sources of CaMMV inoculum for cacao, this information may be useful when selecting ornamental or shade trees for planting in cacao production areas.

Taken together, these results provide insight into the epidemiology of CaMMV, supporting more effective management strategies in affected locations. Due to the young age of many of these farms, these research results will have a significant impact by preventing the introduction of the disease and reducing virus transmission in areas where it is already established.

Conclusion

Of the nine plant species identified as possible hosts of CaMMV, *Swietenia macrophylla*, *Durio* sp., *Hibiscus* sp., *Thespesia grandiflora*, and *Inga vera* are of particular interest due to their economic importance or frequent cocultivation alongside *T. cacao*. However, additional studies are needed to determine whether these serve as alternative hosts to cacao. Although the mealybug vectors of CaMMV feed on a wide range of plants, in some plant species, viral titer may not reach the level needed for insect uptake and transmission (Kirkpatrick 1953; Tinsley and Wharton 1958). CaMMV strains in non-cacao hosts were highly similar to those in cacao, suggesting active pathogen movement rather than host-specific strains. As there is no treatment for infected plants, preventing transmission by controlling mealybug vectors and propagating from virus-free material are the most effective strategies for managing CaMMV.

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