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**TRANSMISSÃO DO cowpea mild mottle virus POR SEMENTES DE SOJA E
PRIMEIRO RELATO INFECTANDO *Euphorbia heterophylla*.**

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meus padrinhos, Maria de Lourdes e
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“Aprender é a única coisa de que a mente nunca se cansa, nunca tem medo e nunca se arrepende.”

Leonardo da Vinci

RESUMO

O cowpea mild mottle virus (CPMMV) é um dos principais vírus associados à cultura da soja no Brasil, transmitido pela mosca-branca *Bemisia tabaci* e responsável por prejuízos à produtividade. Este estudo teve como objetivo avaliar a importância epidemiológica da transmissão do CPMMV por sementes de soja e investigar o papel de plantas daninhas como hospedeiras alternativas do vírus no agroecossistema. Foram analisados grãos provenientes de diferentes genótipos de soja naturalmente infectados em áreas agrícolas da região de Paranapanema, estado de São Paulo. Embora a incidência do CPMMV nas plantas em campo tenha sido elevada, a transmissão via sementes ocorreu em baixa frequência, variando entre genótipos e atingindo valores entre 0,2 a 1,4%. Os resultados indicaram que a capacidade de transmissão do vírus por sementes é dependente do genótipo da soja, sugerindo que fatores genéticos do hospedeiro influenciam o estabelecimento do vírus no embrião e a transmissão verdadeira para a plântula. Paralelamente, foi identificado pela primeira vez o CPMMV infectando naturalmente *Euphorbia heterophylla*, uma planta daninha amplamente distribuída em áreas de cultivo de soja. Apesar da ausência de sintomas visuais, a planta apresentou elevada taxa de infecção, e análises moleculares confirmaram a identidade do vírus. Ensaio de transmissão por extrato vegetal demonstraram que o isolado proveniente de *E. heterophylla* é capaz de infectar soja, evidenciando seu potencial como hospedeira alternativa e reservatório viral. De forma integrada, os resultados demonstram que o CPMMV utiliza múltiplas estratégias para sua persistência e disseminação no ambiente agrícola. A transmissão por sementes, embora pouco frequente, pode atuar como fonte primária de inóculo, contribuindo para a introdução do vírus em novas áreas. Simultaneamente, a presença de plantas daninhas hospedeiras, associada à eficiência do vetor, favorece a manutenção do inóculo entre safras. Esses resultados reforçam a importância do uso de sementes certificadas, do manejo integrado de plantas daninhas e do controle da mosca-branca como estratégias essenciais para a redução dos impactos do CPMMV na cultura da soja.

Palavras-chave: carlavírus; mosca-branca; transmissão por semente; planta daninha.

ABSTRACT

Cowpea mild mottle virus (CPMMV) is one of the main viruses associated with soybean cultivation in Brazil, transmitted by the whitefly *Bemisia tabaci* and responsible for yield losses. This study aimed to evaluate the epidemiological importance of CPMMV transmission through soybean seeds and to investigate the role of weeds as alternative hosts of the virus in the agroecosystem. Grains from different soybean genotypes naturally infected in agricultural areas of the Paranapanema region, state of São Paulo, were analyzed. Although CPMMV incidence in field plants was high, seed transmission occurred at low frequency, varying among genotypes and reaching values between 0.2 and 1.4%. The results indicated that the virus's ability to be transmitted through seeds depends on the soybean genotype, suggesting that host genetic factors influence the establishment of the virus in the embryo and its true transmission to the seedling. In parallel, CPMMV was identified for the first time naturally infecting *Euphorbia heterophylla*, a weed widely distributed in soybean-growing areas. Despite the absence of visual symptoms, the plant showed a high infection rate, and molecular analyses confirmed the virus's identity. Transmission assays using plant extract demonstrated that the isolate from *E. heterophylla* is capable of infecting soybean, highlighting its potential as an alternative host and viral reservoir. Taken together, the results show that CPMMV employs multiple strategies for its persistence and dissemination in the agricultural environment. Seed transmission, although infrequent, may act as a primary source of inoculum, contributing to the introduction of the virus into new areas. At the same time, the presence of weed hosts, combined with the efficiency of the vector, favors inoculum maintenance between growing seasons. These findings reinforce the importance of using certified seeds, integrated weed management, and whitefly control as essential strategies to reduce the impacts of CPMMV on soybean cultivation.

Keywords: carlavírus; whitefly; seed transmission; weed plants.

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INTRODUÇÃO GERAL

A soja (*Glycine max*) e seus derivados são a commodity agrícola mais comercializada, representando quase 9% do valor total do comércio agrícola global. O Brasil se destaca como o líder mundial na produção e exportação do grão, sendo responsável por 40% da produção mundial, com mais de 171,5 milhões de toneladas, e 59% da exportação mundial, com mais de 98,8 milhões de toneladas de soja em grão exportada (USDA, 2025). Apesar de tal liderança, devido à longa duração do fenômeno climático, El Niño, nas principais regiões produtoras, a safra 2023/24 teve uma produção reduzida para 147,38 milhões de toneladas, um recuo de mais de 7,2 milhões de toneladas se comparada com a temporada anterior, quando foram colhidas 154,6 milhões de toneladas (CONAB, 2024).

Este evento climático também favoreceu o aumento da população de insetos-praga nas lavouras brasileiras, dentre eles, a mosca-branca (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)), considerada uma das principais pragas em sistemas agrícolas de regiões tropicais e subtropicais, devido ao seu alto nível de dano. *Bemisia tabaci* é um inseto fitófago que se alimenta da seiva das plantas, possuindo aparelho bucal do tipo picador-sugador. A ocorrência de sua infestação pode ocasionar prejuízos às plantas por meio de quatro mecanismos principais de dano (Bink-Moenen, 1990; Walker *et al.*, 2009; Perring *et al.*, 2018; Horowitz *et al.*, 2020).

Primeiro, tanto ninfas quanto adultos de *B. tabaci* realizam a sucção de seiva diretamente do floema presente em caules e folhas (Horowitz *et al.*, 2020; Da Silva *et al.*, 2019). Em condições de elevada densidade populacional, esse processo de alimentação pode resultar em sintomas como clorose, dessecamento foliar e até abscisão prematura (Schuster *et al.*, 1995). Infestações severas são capazes de provocar a morte de plântulas, além de induzir redução no crescimento, perda de vigor e diminuição da produtividade em plantas mais desenvolvidas (Schuster *et al.*, 1995; Gangwar *et al.*, 2018).

Segundo, durante o processo de alimentação, ninfas e adultos de *B. tabaci* podem introduzir secreções salivares nos tecidos vegetais, desencadeando alterações fisiológicas adversas (McCollum *et al.*, 2004). Entre os distúrbios associados à ação desse inseto destacam-se o prateamento observado em abóboras, a manifestação de estrias cloróticas em pimentão (*Capsicum annuum* L.) e o

amadurecimento irregular em frutos de tomate. (Maynard *et al.*, 1989; Schuster *et al.*, 1990, 2001; Yokomi *et al.*, 1990; Summers *et al.*, 1996; Dinsdale *et al.*, 2010)

Terceiro, ninfas e adultos de *B. tabaci* excretam honeydew (exsudato açucarado), que recobre as folhas com uma camada pegajosa e favorece o desenvolvimento de fumagina (Horowitz *et al.*, 2020; Naranjo *et al.*, 2002). A fumagina, por sua vez, ocasiona danos indiretos ao comprometer processos fisiológicos essenciais da planta, como a respiração e a fotossíntese (Oliveira *et al.*, 2001). Esse honeydew também atrai formigas, cuja presença interfere na atuação dos inimigos naturais de *B. tabaci* e de outras pragas (Abd-Raboou *et al.*, 2011).

Quarto, *B. tabaci* é considerado um “supervetor”, uma vez que é capaz de transmitir mais de centenas de vírus fitopatogênicos. Entre os patógenos disseminados por essa espécie destacam-se o Tomato yellow leaf curl virus (TYLCV), Tomato chlorosis virus (ToCV), Cucurbit yellow stunting disorder virus (CYSDV), Cucumber vein yellowing virus (CVYV), Squash vein yellowing virus (SqVYV), e Bean golden mosaic virus (BGMV) (Jones *et al.*, 2003; Hogenhout *et al.*, 2008; Gilbertson *et al.*, 2015; Polston *et al.*, 2014). Dentre esses, o TYLCV é um dos vírus mais devastadores, responsável por severas perdas em cultivos de tomate em escala global (Duffus, 1986; Picó *et al.*, 1996).

B. tabaci integra um complexo de espécies crípticas, no qual a linhagem Middle East Asia Minor 1 (MEAM1), conhecida como biótipo B ou *Bemisia argentifolii*, se destaca por ser a mais amplamente disseminada e responsável por atacar diversas culturas no Brasil (Brown *et al.*, 2023; de Moraes *et al.*, 2018; Fernandes *et al.*, 2022). Outro integrante desse complexo é a espécie críptica Mediterranean (MED), popularmente chamada de biótipo Q e atualmente reconhecida como *B. tabaci* *sensu stricto* (Brown *et al.*, 2023; Tay *et al.*, 2012). Essa espécie foi registrada pela primeira vez no Brasil em 2014 (Barbosa *et al.*, 2015) e, desde então, sua presença tem se expandido, com relatos recentes de ocorrência em plantações de soja (Da Silva *et al.*, 2020; Barreto da Silva *et al.*, 2024).

As moscas-brancas tornaram-se um desafio relevante durante a safra de soja em razão de seu hábito polífago, da capacidade de desenvolver resistência a inseticidas e do papel crucial que desempenham como vetores de vírus em plantas. Nas lavouras de soja, *Bemisia tabaci* atua como vetor do cowpea mild mottle virus (CPMMV), atualmente considerado um dos principais vírus que comprometem essa cultura no Brasil. O CPMMV tem sido amplamente identificado em campo e está

associado à doença denominada necrose da haste da soja, a qual representa uma séria ameaça à estabilidade da produtividade agrícola (Barreto da Silva *et al.*, 2020).

O cowpea mild mottle virus (CPMMV), pertencente à família *Betaflexiviridae* e ao gênero *Carlavirus* (Walker *et al.*, 2022). É pertencente à espécie *Carlavirus vignae* (ICTV, 2025). O CPMMV possui um genoma de RNA de fita simples de senso positivo, composto por aproximadamente 8.200 nucleotídeos e organizado em seis fases abertas de leitura (ORFs) (Menzel; Winter; Vetten, 2010; Zanardo *et al.*, 2017). A ORF1 codifica a replicase, que apresenta quatro domínios conservados: metiltransferase, peptidase C23, RNA helicase e RNA polimerase dependente de RNA (RdRp) (Zanardo *et al.*, 2017). As ORFs 2, 3 e 4 correspondem ao triplo gene bock proteins (TGB 1-3p), essenciais para o movimento viral. A ORF 5 é responsável pela síntese da capa proteica (CP), enquanto a ORF 6 codifica uma proteína rica em cisteína (CRP) com atividade de ligação ao ácido nucleico (NABP) (Menzel; Winter; Vetten, 2010).

O vírus infecta principalmente culturas agrícolas da família Fabaceae, como soja e feijão (*Phaseolus vulgaris*), juntamente com inúmeras espécies de plantas daninhas de diferentes famílias botânicas, como *Sida sp.* (Malvaceae); *Macroptilium spp.*, *Senna spp.*, *Desmodium glabrum*, *Rhynchosia minima* (Fabaceae); *Mirabilis jalapa* (Nyctaginaceae); *Cleome affinis* (Cleomaceae) e *Blainvillea rhomboidea* (Asteraceae) (Barreto da Silva *et al.*, 2024; Kitajima, 2020; Lamas *et al.*, 2017). No Brasil, foram relatadas plantas de soja infectadas por CPMMV na safra 2000/2001 no estado de Goiás e posteriormente identificadas em outros campos de soja nos estados da Bahia, Mato Grosso, Maranhão, Paraná (Almeida *et al.*, 2003, 2005; Almeida, 2008), e em 2008, em Minas Gerais e no estado do Tocantins (Almeida, 2008). Os sintomas causados por CPMMV em soja são altamente variáveis, indo de sintomas severos (nanismo, necrose de folhas e caules) a sintomas leves/moderados (folhas enrugadas/bolhosas, deformação foliar, mosaico amarelo e clareamento de nervuras) (Zanardo *et al.*, 2017; Marubayashi *et al.*, 2010).

A oscilação das condições climáticas também cria condições favoráveis para a presença constante de plantas daninhas. Essas plantas podem servir como hospedeiras de diversos vírus que atacam as culturas agrícolas e, ainda mais preocupante, permitem a ocorrência contínua de diferentes vetores (Kitajima, 2020). As plantas daninhas são amplamente reconhecidas como hospedeiras alternativas da mosca-branca; em especial, *Euphorbia heterophylla*, comumente encontrada em

áreas de soja (Sacilotto *et al.*, 2024), que tem destaque devido à dificuldade no manejo ocasionado pela resistência ao glifosato (Adegas *et al.*, 2020), a herbicidas inibidores da ALS (cloransulam, diclosulam, flumetsulam, imazethapyr, metsulfuron e nicosulfuron) e a Protox (acifluorfen, fomesafen, lactofen e saflufenacil) (Vargas *et al.*, 2016). Esses mecanismos de resistência dificultam o controle em campo, destacando a necessidade de manejo integrado. Somada a isso, a ausência de um sistema de quarentena sólido e eficaz contribui para a disseminação de pragas e patógenos variados, incluindo vírus (Kitajima, 2020).

A disseminação secundária ocorre quando insetos vetores se alimentam tanto das plantas cultivadas quanto das daninhas presentes no agroecossistema (Kumar *et al.*, 2021). Embora funcionem como reservatórios, muitas dessas plantas não apresentam sintomas visíveis, o que dificulta a identificação das fontes de inóculo e o manejo eficiente (Wisler e Norris, 2005). O Squash vein yellowing virus (SqVYV), transmitido pela *B. tabaci* MEAM1, é responsável por severos prejuízos à melancia (*Citrullus lanatus*) na Flórida (Webb *et al.*, 2012). Ensaios de transmissão identificaram cucurbitáceas espontâneas, como *Cucumis melo* var. *dudaim* e *Momordica charantia*, como hospedeiras naturais do vírus. Observou-se ainda que a mosca-branca apresenta preferência de oviposição em plantas infectadas, favorecendo a rápida disseminação do patógeno no sistema produtivo (Adkins *et al.*, 2008; Shrestha *et al.*, 2016). Gramíneas espontâneas também desempenham papel relevante como hospedeiras do Wheat streak mosaic virus (WSMV) e de seu vetor, o ácaro wheat curl mite (Ito *et al.*, 2012).

Casos como estes evidenciam que plantas daninhas contribuem significativamente para a manutenção e disseminação de patógenos vegetais, aumentando sua agressividade e complexidade de manejo. Assim, compreender os processos epidemiológicos envolvidos é essencial para o desenvolvimento de estratégias eficazes de controle (Darrasse *et al.*, 2010). Práticas culturais adequadas e manejo integrado de plantas daninhas e patógenos são fundamentais para reduzir fontes de inóculo.

O CPMMV é transmitido por *B. tabaci* de forma não persistente, em que o período de acesso à aquisição (PAA) ocorre de segundos a minutos, com uma retenção pelo vetor de minutos ou horas (ou dias em alguns casos) (Almeida *et al.*, 2008; Marubayashi *et al.*, 2010; Barreto da Silva *et al.*, 2024). A MED é particularmente preocupante devido à sua alta eficiência na transmissão do CPMMV, exigindo apenas

um curto período de aquisição de cerca de dois minutos (Barreto da Silva *et al.*, 2024), combinada com sua reduzida sensibilidade a inseticidas (Horowitz *et al.*, 2014), o que representa uma ameaça emergente significativa à produção de soja.

Além da transmissão por vetores, alguns vírus de plantas são capazes de infectar a semente produzida pela planta hospedeira, caracterizando a transmissão vertical do vírus. A transmissão de vírus por sementes consiste no processo pelo qual partículas virais são transferidas da planta-mãe infectada para a planta-filha durante o desenvolvimento da semente (Maule e Wang, 1996; Regassa, 2022). De modo geral, essa via de transmissão é considerada menos frequente quando comparada à transmissão por vetores, porém, quando ocorre, exerce papel estratégico na epidemiologia dos vírus vegetais, especialmente para vírus que não dispõem de vetores eficientes ou que apresentam baixa taxa de transmissão horizontal (Regassa, 2022; Shanmugam, 2024).

A infecção da semente pode ocorrer em diferentes fases do desenvolvimento reprodutivo da planta durante a formação do óvulo; no processo de fecundação, por meio da infecção de gametas; durante o enchimento da semente, via movimento célula a célula até o embrião; ou por contaminação externa, quando partículas virais permanecem aderidas ao tegumento da semente (Maule e Wang, 1996; Escalante *et al.*, 2024). Entretanto, apenas quando o vírus atinge e se estabelece no embrião, ocorre a chamada transmissão verdadeira, capaz de originar uma plântula sistemicamente infectada (Maule e Wang, 1996). Nos casos em que o vírus permanece restrito aos tecidos externos da semente, como o tegumento, a semente é considerada apenas contaminada, não resultando necessariamente em infecção da planta emergente (Escalante *et al.*, 2024).

O CPMMV tem sido amplamente identificado em lavouras de soja no Brasil, causando impactos relevantes na produtividade. Estudos destacam que sua transmissão por sementes pode favorecer a disseminação do vírus entre diferentes países (Da Silva *et al.*, 2020). Após observarmos diversos genótipos de soja, em estágio reprodutivo já na fase de enchimento de grãos, naturalmente infectados em áreas agrícolas estratégicas da região de Paranapanema, no estado de São Paulo, este estudo visa verificar se os grãos provenientes dessas lavouras infectadas são capazes de transmitir o CPMMV para novas plantas por meio do plantio de sementes contaminadas.

Embora o CPMMV possua uma ampla gama de hospedeiros, a identificação de novos hospedeiros e sua ocorrência trazem implicações relevantes para o manejo da doença. Quando não há culturas suscetíveis disponíveis, esses vírus permanecem viáveis em hospedeiros secundários ou mesmo no interior de vetores.

Nesse contexto, este estudo busca a intensificação de programas de monitoramento e identificação de plantas daninhas como hospedeiras alternativas, a fim de reduzir a pressão de inóculo e mitigar os impactos do CPMMV sobre a produtividade da cultura.

CAPÍTULO 1

FIRST REPORT OF cowpea mild mottle virus NATURALLY INFECTING *Euphorbia heterophylla*¹

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During the 2023/2024 soybean season in Brazil, producers and extension agents reported increased whitefly (*Bemisia tabaci*) infestations, especially in São Paulo State, prompting intensified surveillance for cowpea mild mottle virus (CPMMV, *Carlavirus vignae*), a whitefly-transmitted carlavirus infecting soybean, common bean, and various weeds (Barreto da Silva *et al.* 2024; Kitajima 2020; Lamas *et al.* 2017). Field sampling included soybean leaves (one hundred samples) and nearby weeds (six samples each species): *Euphorbia heterophylla*, *Macroptilium sp.*, *Commelina benghalensis*, *Portulaca oleracea*, *Raphanus raphanistrum*, *Senna occidentalis*, *Ipomoea purpurea*, *Ricinus communis*, *Digitaria insularis*, *Amaranthus hybridus*, and *Richardia brasiliensis*. Ten individual adult whiteflies from soybean were PCR-identified as MEAM-1 using Bem23 primers (De Barro *et al.* 2003). Total RNA was extracted (Bertheau *et al.*, 1998) from the plant samples and tested by RT-PCR with CPMMV CP primers (Barreto da Silva *et al.*, 2024). CPMMV was detected in soybean

¹ Capítulo redigido de acordo com as normas do periódico **Plant Disease**

(100/100), *Macroptilium* sp. (6/6), and *E. heterophylla* (5/6). Symptoms, including foliar mottling and deformation, were observed in soybean and *Macroptilium*, whereas *E. heterophylla* was asymptomatic. To confirm the identity of the CPMMV collected from *E. heterophylla*, the RT-PCR was realized using specific primers from RdRp, 1280 F and 1696 R (De Marchi *et al.*, 2017), and the RT-PCR product was Sanger sequenced bidirectionally and analyzed using Geneious Prime 2024.0 software. The CPMMV isolate from *E. heterophylla* (GenBank acc. no. PQ825744) exhibited 97.7% nucleotide identity with a Brazilian reference isolate from *Phaseolus vulgaris* (acc. no. MK202583). Genetic divergence was assessed by amplifying the RdRp (1,076 bp; CPMMV-RdRp-F/R) and CP (1,106 bp; CPMMV-CP-F/R) genes from both CPMMV-Eh (*E. heterophylla*) and CPMMV-soybean. Amplicons were sequenced and analyzed via BLASTn. CPMMV-Eh (PX132324) shared the highest identity at 96.3% in RdRp with acc. no. PV460962 from *Passiflora* sp. (Brasília), whereas CPMMV-soybean (PX132325) showed the highest identity at 98.5% with acc. no. MW657117 from *Glycine max* (Goiás). Pairwise RdRp identity between CPMMV-Eh and CPMMV-soybean was 80.8%. In the CP region, CPMMV-Eh (PQ834428) exhibited the highest identity (98.0%) with acc. no. KF554101 from *Glycine max* (Goiás), while CPMMV-soybean (PQ834429) showed the highest identity (94.3%) with acc. no. OP611426 from *Glycine max* (Buritama, SP). The pairwise CP identity between CPMMV Eh and CPMMV-soybean was 84.3%. These findings highlight genetic divergence between CPMMV-Eh and CPMMV-soybean. Sap transmission assays were conducted to evaluate the infectivity of CPMMV-Eh using phosphate-buffered saline (0.5 M, pH 7.2) and carborundum (600 mesh) on five soybean and five *E. heterophylla* plants. Plants were kept insect-free for 30 days after infection. No symptoms were observed in *E. heterophylla*, whereas soybean developed yellow mosaic. RT-PCR confirmed CPMMV-Eh infection in both hosts. This constitutes the first documented natural infection of *E. heterophylla* by CPMMV globally. Given *E. heterophylla*'s established resistance to glyphosate, acetolactate synthase (ALS) inhibitors, and protoporphyrinogen oxidase (PPO) inhibitors (Adegas *et al.* 2020; Vargas *et al.* 2016), its role as a persistent viral reservoir is of agronomic concern. Integration of *E. heterophylla* into virus surveillance and weed management protocols, particularly along field margins and during fallow periods, is imperative to mitigate inoculum pressure and curtail whitefly-mediated transmission to soybean crops.

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CAPÍTULO 2

FROM SEED TO FIELD: INVESTIGATING cowpea mild mottle virus (CPMMV) PERSISTENCE IN SOYBEAN SEEDS²

2.1 INTRODUCTION

Soybeans (*Glycine max* (L.) Merrill) and their derivatives represent the most widely traded agricultural commodity globally, constituting a key component of global food security as a source of protein, animal feed, and biofuel (Hartman *et al.*, 2009). As the leader, global producer, and exporter, Brazil accounts for approximately 40% of global soybean production and 59% of international exports (USDA, 2025). Despite this leadership position, Brazil's soybean agriculture remains highly vulnerable to climate phenomena. The prolonged impact of irregular rainfall and extended dry periods during the 2023/2024 growing season led to a reduction in the national soybean harvest to 147.38 million tons, down from 154.6 million tons in the previous season (CONAB, 2024).

These adverse climatic conditions also contributed to increased pest pressures in soybean fields, particularly favoring the proliferation of the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), a key pest in tropical and subtropical cropping systems. Whiteflies emerged as a major challenge during the growing season due to their polyphagous behavior, insecticide resistance, and role as plant viruses (Marubayashi *et al.*, 2010). In soybean fields, *B. tabaci* serves as the vector of cowpea mild mottle virus (CPMMV), one of the major viral pathogens currently affecting Brazilian soybean crops. CPMMV has been widely detected in the field and is responsible for soybean stem necrosis disease, which poses a serious threat to yield stability (Almeida, 2008; Barreto da Silva *et al.*, 2020).

CPMMV is a member of the family *Betaflexiviridae* and the genus *Carlavirus*, and possesses a positive-sense single-stranded RNA genome, and is classified under the species *Carlavirus vignae* (ICTV, 2025). The virus primarily infects plants within the family *Fabaceae*, along with certain weed species (Lamas *et al.*, 2017; Kitajima, 2020; Barreto da Silva *et al.*, 2024). Symptoms caused by CPMMV isolates in soybeans are highly variable, ranging from severe symptoms (bud blight, dwarfing,

² Capítulo redigido de acordo com as normas do periódico **Plant Pathology**

leaves and stem necrosis) to mild symptoms (crinkled/blistering leaves, leaves deformation, yellow mosaic and vein clearing) (Barreto da Silva *et al.*, 2020).

CPMMV is transmitted by *B. tabaci* in a non-persistent mode (Barreto da Silva *et al.*, 2024). This insect belongs to a cryptic species complex, within which Middle East Asia Minor 1 (MEAM1) species, also referred to as the B biotype or *Bemisia argentifolii*, being the most widespread species affecting major crops in Brazil (Moraes *et al.*, 2018; Fernandes *et al.*, 2022; Brown *et al.*, 2023). Another member of the complex, the Mediterranean (MED) species, commonly known as biotype Q and currently identified as *B. tabaci* (Tay *et al.*, 2012; Brown *et al.*, 2023), was first reported in Brazil in 2014 (Barbosa *et al.*, 2015) and is also considered a good vector of CPMMV (Barreto da Silva *et al.*, 2024). Its distribution has since expanded, with recent records in soybean fields (Barreto da Silva *et al.*, 2020, 2024). MED is particularly concerning due to its high transmission efficiency of CPMMV, requiring only a brief acquisition period of about two minutes (Barreto da Silva *et al.*, 2024), combined with its reduced sensitivity to insecticides (Horowitz & Ishaaya, 2014). Together, these traits represent a significant emerging threat to soybean production in Brazil.

Plant viruses can be naturally transmitted between plants through two main ways (Escalante *et al.*, 2024). The first is horizontal transmission, which may occur with the assistance of insects, human activity, or mechanical spread such as agricultural tools or equipment (Maule & Wang, 1996; Hull, 2014). The second is vertical transmission, which involves virus transmission through seed, pollen, or ovule (Matsushita *et al.*, 2018; Cobos *et al.*, 2019; Pagán, 2022). Moreover, viral infection during the early stages of embryo development may result in harmful morphological or even genetic alterations, ultimately causing yield reductions and economic losses (Escalante *et al.*, 2024).

Given the extensive detection of CPMMV and its significant impact on yield, recent studies indicate that seed transmission may serve as a pathway for CPMMV dissemination across regions and countries. To better understand the role of seed transmission in the epidemiology and spread of CPMMV in soybean fields, this study investigated the potential seed transmission of CPMMV in naturally infected soybean genotypes. Additionally, *Bemisia tabaci* specimens collected from the same locations were molecularly identified to determine the presence of cryptic species.

2.2 MATERIALS AND METHODS

2.2.1. CPMMV detection in soybean genotypes

Soybean leaves were collected during the 2023/2024 growing season from commercial soybean fields in the Paranapanema region, São Paulo, Brazil, to assess the incidence of CPMMV. Samples were taken from the following ten genotypes: Titanium TF I2X, DM 56I59 RSF IPRO, AS 3590 IPRO, NS 5933 IPRO, FIBRA IPRO, BMX Lança IPRO, Soytech 580 I2X, DM 54IX57 I2X, M 5939 I2X, and M 5710 I2X. For each genotype, leaves were collected from 100 randomly selected plants. Samples were placed in labeled plastic bags and transported to the laboratory under refrigerated conditions.

Total RNA was extracted using the method described by (Bertheau & Frechon, 1998). Reverse transcription-Polymerase Chain Reaction (RT-PCR) was performed using AMV reverse transcriptase (Promega, cat. #M5101) and GoTaq Green Master Mix (Promega, cat. #M7123), with the primer pair CPMMV1280-F and CPMMV1696-R (De Marchi *et al.*, 2017) (100 pmol each).

Reaction volume was 12.5 μ L containing 6.5 μ L of GoTaq Green Master Mix, 0.125 μ L of each primer (100M), 0.05 μ L of AMV reverse transcriptase, 3.7 μ L of nuclease-free water, and 2 μ L of RNA template.

Thermal cycling was performed on an Eppendorf® Mastercycler® Nexus. PCR products were separated on a 2% agarose gel (Sigma-Aldrich, cat. #A9539) stained with ethidium bromide (Sigma-Aldrich, cat. #E7637) and visualized under UV light. Selected amplified fragments were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, cat. #A9282) and sequenced in both directions. The resulting nucleotide sequences were compared with CPMMV isolates available in the NCBI GenBank nucleotide (nt) database using BLASTn to confirm viral identity.

In addition, soybean seeds from the same genotypes evaluated were harvested using a combine harvester, placed in labeled plastic bags, and transported to the laboratory under refrigerated conditions for subsequent analysis of seed transmission of CPMMV.

2.2.2 Soybean seed for CPMMV transmission assay

To evaluate the natural transmission of CPMMV, soybean seeds were collected from CPMMV-infected plants during the 2023/2024 growing season. For each of the ten genotypes analyzed, a total of 1200 seeds were sown individually in polystyrene

trays filled with commercial substrate. Genotypes were handled separately throughout the assay to prevent cross-contamination. Seedlings were maintained under controlled greenhouse conditions until the development of their first trifoliate leaf. At this stage, leaf tissues were collected for CPMMV detection, as described in the previous section. For initial screening, 100 pooled samples were prepared per genotype. Each pool consisted of 10 leaf discs circles (8.5 mm in diameter, corresponding to approximately 0.57 cm² each), with one disc collected from each of 10 individual plants, resulting in a total of 1,000 plants evaluated per genotype.

Pooled samples were subjected to total RNA extraction followed by RT-PCR using the same parameters described in the previous section.

Pools testing positive for CPMMV were subsequently analyzed at the individual plant level. Total RNA was extracted from each plant within the positive pools, and RT-PCR was performed on these individual samples to determine the exact number of CPMMV-infected plants attributable to seed transmission.

2.2.3. Molecular identification of cryptic species of *Bemisia tabaci* in soybean fields

Adult whiteflies were collected from soybean leaves in the same fields and during the same period as the CPMMV incidence evaluation/survey conducted in the 2023/2024 growing season. Insects were collected using a manual aspirator and immediately transferred into 1.5 mL microtubes containing 100% ethanol for preservation. Species confirmation using the mtCOI analysis; total DNA was extracted using the Chelex[®] 100 sodium form (Sigma-Aldrich, cat. #C7901) (Walsh *et al.*, 1991). PCR was performed using the primers Bem23F and Bem23R (De Barro *et al.*, 2003), for *B. tabaci* MEAM1 and MED identification. This primer pair targets a microsatellite locus, generating fragments of approximately 200 base pairs in MEAM1 and about 400 base pairs in MED (Skaljic *et al.*, 2010).

Reaction volume was 12.5 µL containing 6.5 µL of GoTaq Green Master Mix, 0.125 µL of each primer (100M), 3.75 µL of nuclease-free water, and 2 µL of DNA template.

Thermal cycling was performed on an Eppendorf[®] Mastercycler[®] Nexus. PCR products were separated on a 2% agarose gel (Sigma-Aldrich, cat. #A9539) stained with ethidium bromide (Sigma-Aldrich, cat. #E7637) and visualized under UV light.

2.2.4. Incidence of CPMMV

Incidence was determined using the percentage method, where the total number of samples was treated as 100. The proportion of positive plants was then used to calculate both the field infection rate and the seed transmission rate.

2.3 RESULTS

2.3.1. CPMMV detection in soybean genotypes

During the regular field visits, symptoms associated with CPMMV were observed across all genotypes evaluated in this study. Symptoms of severity varied among genotypes, with most of them showing strong and widespread symptoms, while others displayed only mild symptoms. In fields where genotypes were planted side by side, it was visually evident that the plants were more severely affected by CPMMV, particularly in areas with high whitefly populations (Figure 1).



Figure 1. (A) CPMMV symptoms in different soybean genotypes planted side by side in the same pivot: M 5939 I2X on the left and Titanium TF I2X on the right side. (B) Symptoms in M 5939 I2X. (C) Symptoms in Titanium TF I2X.

The highest CPMMV incidence was observed in genotype DM 56I59 RSF IPRO, with 100% of samples testing positive. This was followed by AS 3590 IPRO, NS 5933 IPRO, and DM 54IX57 I2X, FIBRA IPRO, with rates ranging from 97% to 99%. These results indicate the apparent absence of resistance traits in these genotypes, suggesting a high susceptibility to CPMMV infection. In contrast, Titanium TF I2X exhibited the lowest infection rate at 74%, indicating a potential genetic factor that may reduce viral establishment and/or vector efficiency. BMX Lança IPRO and M 5939 I2X demonstrated intermediate susceptibility, with infection rates of 88%, while Soytech 580 I2X and M 5710 I2X presented slightly lower rates of 95% and 90%, respectively (Table 1).

Table 1. Detection and incidence of cowpea mild mottle virus (CPMMV) in naturally infected soybean genotypes during the 2023–2024 growing season.

Genotypes	No. samples	Positive	Negative	Incidence of CPMMV (%)*
Titanium TF I2X	100	74	26	74
DM 56I59 RSF IPRO	100	100	0	100
AS 3590 IPRO	100	99	1	99
NS 5933 IPRO	100	99	1	99
FIBRA IPRO	100	97	3	97
BMX Lança IPRO	100	88	12	88
Soytech 580 I2X	100	95	5	95
DM 54IX57 I2X	100	97	3	97
M 5939 I2X	100	88	12	88

Note: *Incidence of CPMMV was calculated as no. positive/total samples x 100%.

2.3.2. Soybean Seeds for CPMMV Transmission Assay

Most of the soybean-infected seedlings present CPMMV symptoms in the first trifoliolate leaf, including mottling, yellow mosaic, blistering, and leaf deformation (Figure 2).

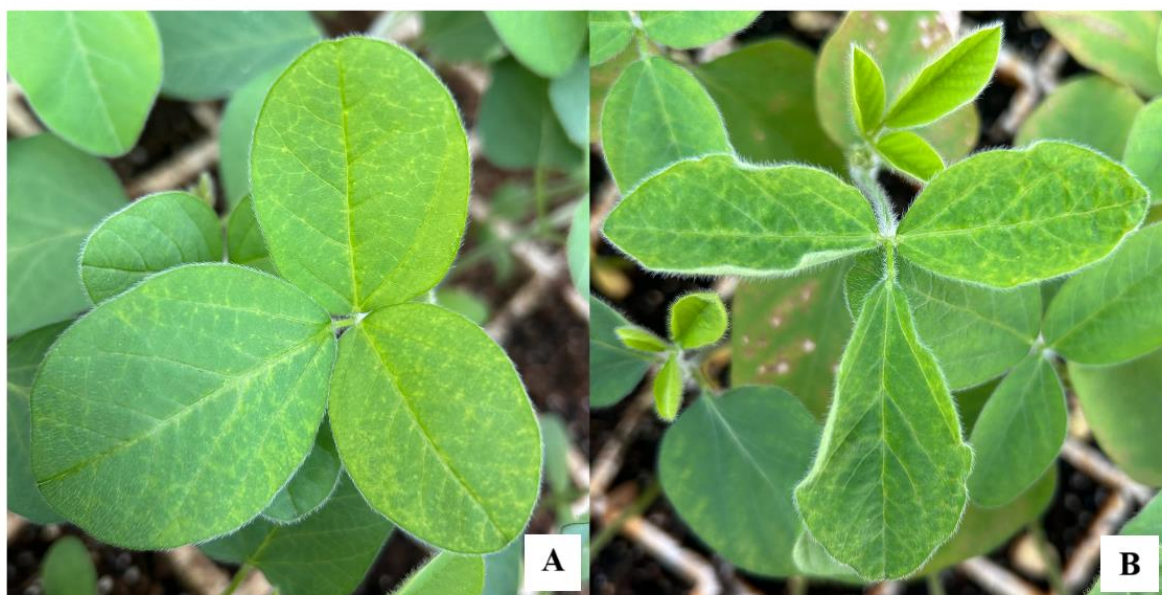


Figure 2. Symptoms of CPMMV infection from different soybean genotypes, A- M5939 Fibra IPRO; B- BMX Lança.

Among the tested genotypes, DM 56I59 RSF IPRO, AS 3590 IPRO, and FIBRA IPRO showed 0% seed transmission. Genotypes NS 5933 IPRO, Soytech 580 I2X, and DM 54IX57 I2X exhibited low infection rates (0.2%), indicating minimal natural seed transmission potential. BMX Lança IPRO had a slightly higher incidence than the previous group (0.4%). Titanium TF I2X (0.8%), M 5939 I2X (1.1%), and M 5710 I2X (1.4%) showed the highest CPMMV transmission rates, suggesting potential genotypic variability affecting virus retention in seeds naturally infected.

Table 2. Detection of CPMMV on soybean seedling samples

Variety	No. samples	Positive	Negative	Incidence of CPMMV (%)*
Titanium TF I2X	1000	8	992	0.8
DM 56I59 RSF IPRO	1000	0	1000	0.0
AS 3590 IPRO	1000	0	1000	0.0
NS 5933 IPRO	1000	2	998	0.2
FIBRA IPRO	1000	0	1000	0.0
BMX Lança IPRO	1000	4	996	0.4
Soytech 580 I2X	1000	2	998	0.2
DM 54IX57 I2X	1000	2	998	0.2
M 5939 I2X	1000	11	989	1.1
M 5710 I2X	1000	14	986	1.4

Note: *Incidence of CPMMV was calculated as no. positive/total samples x 100%.

2.3.3. Identification of cryptic species of *Bemisia tabaci* in soybean fields

Adult whiteflies were collected from soybean leaves in the same fields and during the same period as the CPMMV incidence assessment for the 2023/2024 growing season. Molecular identification confirmed the presence of the cryptic species MEAM1, with no detection of other *B. tabaci* species among the sampled genotypes.

2.4 DISCUSSION

Our findings indicate that CPMMV is widely distributed across soybean production areas in the Paranapanema region. The significant variation in the CPMMV infection rates among soybean genotypes suggests varying degrees/levels of susceptibility and potential presence of tolerance mechanisms.

Although the absolute values observed in this study remain low, a notable rise is epidemiologically relevant, as even minimal seedborne infection can contribute to the introduction and establishment of the virus in new areas. This finding underscores the importance of further research into genotypic resistance mechanisms and the development of seed treatment strategies to minimize/mitigate seedborne inoculum in soybean fields.

A direct comparison between foliar infection (Table 1) and seed transmission (Table 2) underscores the complexity of CPMMV epidemiology. Foliar infection was consistently high in several genotypes, reaching 100% in DM 56I59 RSF IPRO and 97–99% in AS 3590 IPRO, NS 5933 IPRO, and DM 54IX57 I2X. In contrast, seed transmission was generally low, with most cultivars showing transmission rates between 0% and 1.4%. Interestingly, DM 56I59 RSF IPRO, AS 3590 IPRO, and FIBRA IPRO, despite their high foliar susceptibility, exhibited no detectable seed transmission, suggesting that the ability of CPMMV to invade and persist in developing seeds is highly genotype-dependent and may be governed by specific physiological or structural barriers.

However, the varieties with the highest seedling infection rates, M 5710 I2X (1.4%) and M 5939 I2X (1.1%), suggest that although seed transmission is not the primary pathway, it remains an important factor in viral spread. Notably, these genotypes also had high virus incidence in field samples, reinforcing the need for further investigation into genotypic resistance factors.

Seed transmission constitutes a fundamental mechanism for the long-term persistence of plant viruses, particularly in situations where appropriate hosts or vectors are absent (Bhagwatkar *et al.*, 2025). Numerous seed-borne viruses have been reported to remain viable within seeds for extended periods, thereby ensuring their continuity across successive cropping cycles (Sastry, 2013). From an epidemiological standpoint, infected seeds represent a primary source of inoculum for vertically transmitted viruses, which may subsequently be disseminated by insect vectors (Dwyer *et al.*, 2007). In the context of climate change, this transmission pathway provides viruses with a significant adaptive advantage, facilitating their survival under adverse environmental conditions and enabling their expansion into new geographic regions (Jones, 2016).

In general, the seeds of all genotypes exhibited a normal external appearance, with no visible pigmentation or discoloration (data not shown), as is observed in seeds infected with soybean mosaic virus, which express severe yield losses on soybean (Kun *et al.*, 2010). Depending on the replication strategy of the plant virus, as well as the age and resistance level of the host, viruses can infect seeds at different stages of development, leading to progeny infection rates ranging from 0.10 to 92% (Escalante *et al.*, 2024). These results suggest a potential increase in seed transmission rates compared to previous studies, where seed transmission was only 0.375% (Barreto da Silva *et al.*, 2020).

Seed transmission is a key factor in the persistence of viruses across growing seasons (Johansen *et al.*, 1994). Thus, even when occurring at very low frequencies, the transmission of viruses through seed can significantly contribute to their spread, survival during unfavorable periods, and long-range dissemination, serving as an initial source of virus inoculum for vector, which may greatly influence in crop yield (Ali & Kobayashi, 2010).

Seed quality is crucial for successful germination, seedling vigor, plant establishment, yield potential, and resistance to pathogens in soybeans. Good quality seeds can ensure a uniform crop stand. In contrast, disease-infected seeds can reduce productivity and have an adverse economic impact associated with reduced market value and oil and protein levels (Bhagwatkar *et al.*, 2025). These findings reinforce the need for integrated strategies that combine vector control with the selection of genotypes showing low seed transmission. Our data highlights the necessity of seed

health testing, particularly for genotypes with measurable transmission rates, to prevent dissemination in the field.

2.5 CONCLUSION

CPMMV infection in soybean fields continues to be primarily mediated by *Bemisia tabaci*, which serves as the critical vector for the virus dissemination. However, the increase in seed transmission observed in this study, compared to previous research, highlights the need for greater attention to this pathway. This shift presents challenges for disease control and presents significant risks to seed production areas, reinforcing the necessity for enhanced management strategies that address both vector and seed-borne pathways.

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CONSIDERAÇÕES FINAIS

O cowpea mild mottle virus (CPMMV) apresenta múltiplas estratégias de persistência e disseminação em áreas de cultivo de soja no Brasil. Embora a transmissão do vírus ocorra predominantemente por meio da mosca-branca, a transmissão por sementes possui relevância epidemiológica, pois possibilita a manutenção do patógeno entre safras e sua introdução em novas áreas.

A transmissão por sementes mostrou-se dependente do genótipo da soja, indicando que características do hospedeiro influenciam a capacidade do vírus de alcançar o embrião e estabelecer infecção nas plântulas. Esse resultado reforça a importância do uso de sementes certificadas e da seleção de materiais genéticos menos suscetíveis à transmissão vertical.

Adicionalmente, a identificação de *Euphorbia heterophylla* como hospedeira natural do CPMMV evidencia o papel das plantas daninhas na manutenção do inóculo viral no agroecossistema. Mesmo sem manifestação de sintomas, essa espécie pode atuar como reservatório do vírus e contribuir para sua disseminação via vetor.

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