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CAROLINE DA CRUZ MARTINES

AVALIAÇÃO DA INCIDÊNCIA E TRANSMISSÃO VERTICAL DO passiflora virus Y EM SOJA E CARACTERIZAÇÃO BIOLÓGICA E MOLECULAR DO bidens mosaic virus EM PATCHOULI

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Dissertação apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Mestre em Proteção de Plantas.

Orientadora: Profa. Dra. Renate Krause Sakate

Coorientador: Dr. Gabriel Madoglio Favara

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Título:

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Aos meus amados pais,

Pedro e Rosana,

Dedico

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“Grandes coisas fez o SENHOR por nós, e, por isso, estamos alegres”.

BÍBLIA SAGRADA. Salmos. Capítulo 126, versículo 3.

ABSTRACT

This work was divided into two chapters. In Chapter 1, the objectives were to assess the occurrence of the potyvirus passiflora virus Y (PaVY) in soybean crops in the state of São Paulo and investigate aspects related to the seed transmission of this potyvirus. The results demonstrated a low incidence of PaVY in soybean fields in the major producing regions of São Paulo state. It was also observed that PaVY was seedborne but was not seed transmitted in soybean seeds. Therefore, vertical transmission of PaVY in soybean plants is unlikely to have epidemiological significance. In Chapter 2, the objective was to perform the biological and molecular characterization of bidens mosaic virus (BiMV) infecting patchouli (*Pogostemon cablin*) in Brazil. Potyvirus infections have been previous documented in patchouli plants from the states of São Paulo, Pará, and Sergipe in Brazil. Despite the possibility of BiMV infection in the previously identified plants, the identification of the potyvirus responsible for these infections could not be determined due to the absence of nucleotide sequence data in the previous findings. Thus, based on biological and molecular tests, we present here the first confirmation of BiMV infection in patchouli plants in Brazil. Further studies are needed to assess the incidence of this potyvirus in commercial crops and to determine the damage caused to plant development, as well as the quantity and quality of essential oil produced by patchouli plants.

Keywords: Potyvirus; PaVY; BiMV; *Glycine max*; *Pogostemon cablin*.

RESUMO

Este trabalho foi dividido em dois capítulos. No Capítulo 1, os objetivos foram avaliar a ocorrência do potyvirus passiflora virus Y (PaVY) em campos de soja no estado de São Paulo e investigar aspectos relacionados à transmissão do vírus através das sementes. Os resultados demonstraram uma baixa incidência do PaVY em campos de soja das principais regiões produtoras do estado de São Paulo durante as safras de 2021/2022 e 2022/2023. Também foi observado que o PaVY pode ser detectado nas sementes, mas não foi transmitido através delas para as mudas. Portanto, a transmissão vertical do PaVY em plantas de soja parece não possuir importância epidemiológica. No Capítulo 2, o objetivo foi realizar a caracterização biológica e molecular do potyvirus bidens mosaic virus (BiMV) infectando plantas de patchouli (*Pogostemon cablin*) no Brasil. Infecções de plantas de patchouli com potyvirus foram anteriormente relatadas nos estados de São Paulo, Pará e Sergipe. Apesar da possibilidade destas plantas estarem infectadas com o BiMV, o potyvirus presente nelas não foi identificado devido à ausência de sequências de nucleotídeos. Assim, com base em testes biológicos e moleculares, apresentamos aqui a primeira confirmação da infecção de plantas de patchouli com o BiMV no Brasil. Estudos adicionais são necessários para avaliar a incidência deste potyvirus em campos comerciais, os danos ocasionados no desenvolvimento das plantas, bem como na quantidade e qualidade do óleo essencial produzido pelas plantas de patchouli.

Palavras-chave: Potyvirus; PaVY; BiMV; *Glycine max*; *Pogostemon cablin.*

SUMMARY

GENERAL INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the major agricultural crops worldwide, playing a crucial role in human and animal nutrition, as well as serving as a versatile source of biomass and biodiesel (Bergmann *et al.*, 2013). Presently, Brazil stands as the leading global producer of soybean grains and is also recognized as the largest exporter of this commodity (USDA, 2023).

Soybean cultivation faces constant challenges due to the presence of various pathogens that can cause significant losses in yield and grain quality. Several diseases affect soybean production, and some of them are caused by viruses. In Brazil, soybean plants have been reported naturally infected with viruses belonging to the genera *Potyvirus, Orthotospovirus, Carlavirus, Alfamovirus, Begomovirus, Comovirus, Ilarvirus*, and *Sobemovirus* (Kitajima 2020).

A crucial step in the epidemiology of viral diseases is the transmission of the virus from infected plants to healthy ones. Plant viruses can be transmitted through aphids, whiteflies, thrips, beetles, nematodes, fungi, vegetative propagation organs, and seeds (Tomlinson, 1987). Seed transmission plays a particularly important role, enabling the spread and survival of the viruses in different environments and geographical regions. Seed transmission also allows for long-distance dissemination of viruses. Furthermore, it can serve as a source of primary inoculum and facilitate virus spread through vectors present in the area (Pagán, 2022). Seed transmission is a characteristic shared by the main viruses currently infecting soybean crops in Brazil (Costa, 1971; Porto & Hagedorn, 1975; Barreto da Silva *et al.*, 2020; Uzan, 2023).

In 2020, the potyvirus passiflora virus Y (PaVY) was identified infecting soybean in Brazil (Ribeiro-Junior *et al.*, 2022). PaVY was first described infecting *Passiflora foetida* plants in Indonesia in the early 2000s (Parry *et al.*, 2004). PaVY was also found infecting commercial passionfruit (*P. edulis*) plants in orchards in Australia. Infected plants exhibited symptoms of yellow/green mosaic, sometimes with ringspots and chlorotic spots (Parry et al., 2004). Subsequently, PaVY was identified naturally infecting *Macroptilium atropurpureum, Rhynchosia minima,* and *Vigna trilobata* plants in Australia, *M. atropurpureum* in Taiwan (Chiang *et al.*, 2012), and *Passiflora* sp. in China (Chen *et al.*, 2021).

The nearly complete nucleotide sequence of the Brazilian isolate of PaVY (PaVY-Br) is 9,679 nt long and shares 84.6% nucleotide and 96.5% amino acid

sequence identity with the PaVY isolate from China (Ribeiro-Junior, 2022). PaVY-Br induced chlorotic spots and systemic mosaic on soybean, as well as chlorotic local lesions on *P. edulis* and *Sesamum indicum*. The virus was successfully transmitted to soybean plants by *Myzus persicae* (Ribeiro-Junior, 2022). Due to its recent introduction in Brazil, little is known about the incidence of this potyvirus in soybean fields in the major producing regions of the country. It is also unknown whether PaVY can be transmitted through soybean seeds, which has significant epidemiological importance.

Pogostemon cablin Benth. is a plant species belonging to the Lamiaceae family, commonly known as patchouli. It is a highly valued medicinal aromatic plant due to its high demand for its essential oil. Patchouli is extensively used in the fragrance and pharmaceutical industries (van Beek & Joulain, 2018). Its essential oil possesses a unique woody scent and is an important component in many women's and men's fragrances, as well as various cosmetic products. It is considered one of the most important elements available to perfumers, serving as a fragrance fixative, and ranks among the top ten most important essential oils (van Beek & Joulain, 2018). In patchouli plants, viral infections have been found to have negative consequences, including impaired growth and reduced essential oil production (Sugimura *et al.*, 1995).

Bidens mosaic virus (BiMV) is a member of the *Potyviridae* family, and genus *Potyvirus*. Initially identified in 1961, found infecting *Bidens pilosa* in Brazil (Kitajima *et al.*, 1961). Since its discovery, BiMV has been documented naturally infecting plants of *Arracacia xanthorrhiza, Helianthus annuus, Lactuca sativa*, and *Pisum sativum*. Additionally, ornamental plants such as *Coreopsis lanceolata, Zinnia elegans, Centella asiatica*, and *Galinsoga parviflora* have also been reported as hosts of BiMV in Brazil (Kitajima, 2020; Camelo-Garcia *et al.*, 2021).

In 2022, six patchouli plants showing mosaic symptoms were found in a flower shop in Botucatu, São Paulo state, Brazil. The plants were taken to the laboratory and maintained in a greenhouse for identification of the causal agent. In this study, we conducted biological and molecular analyses to identify the virus present in the symptomatic patchouli plants.

The present study was structured into two chapters. In the first chapter, the objectives were to determine the occurrence of PaVY in soybean fields in the state of São Paulo and investigate its transmission through soybean seeds to subsequent

generations. The second chapter focused on the biological and molecular characterization of BiMV infecting patchouli plants in Brazil.

CHAPTER 1

UNLIKELY TRANSMISSION OF passiflora virus Y THROUGH SOYBEAN SEEDS

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Chapter elaborated following the guidelines of Plant Pathology[1]

Abstract

In 2020, the potyvirus passiflora virus Y (PaVY) was reported infecting soybean in Brazil. Considering its recent detection, little is known about the incidence of the virus in soybean-producing regions and if PaVY can be transmitted through seeds. The objectives of this study were to assess the occurrence of PaVY in soybean crops in São Paulo state and investigate aspects related to its seed transmission and host range. Soybean samples from fields located in 12 and 4 municipalities were collected during the soybean seasons of 2020/2021 and 2021/2022, respectively. PaVY was not detected in any of the 800 samples analyzed. Twenty five soybean plants cv. TMG 7063 IPRO were mechanically inoculated with this potyvirus. The harvested seeds from the PaVY-infected soybean plants were subsequently used in experiments to evaluate seed transmission of PaVY, detection of PaVY in seeds, and to assess the infectivity of the seeds. An average, 40% of the seeds produced by infected plants exhibited symptoms of coat mottling. PaVY were not detected in 1219 seedlings originated from 811 non-mottled and 408 mottled seeds produced by PaVY-infected soybean plants. The potyvirus were detected on 9.8% of mottled and 37.7% of non-mottled seeds. The virus infected *Canavalia ensiformis, Gossypium*

[1] Chapter elaborated following the guidelines of Plant Pathology.

hirsutum, *Nicotiana benthamiana*, *Phaseolus vulgaris*, *Raphanus sativus*, and *Vigna unguiculata*, expanding the host range of the PaVY-Br isolate. Overall, the results demonstrated a low incidence of PaVY in soybean fields in the major producing regions of the state of São Paulo and that transmission of PaVY through soybean seeds is unlikely.

Keywords: PaVY, potyvirus, host range, *Glycine max*

1.1. INTRODUCTION

Soybean [Glycine max (L.) Merr.] is among the most important crops worldwide. Since 2020, Brazil has been the largest soybean producer in the world (USDA, 2023). One of the main limitations to soybean production is the damage caused by phytopathogens. The occurrence of diseases, their severity, and the impact on soybean productivity and seed quality are influenced by various factors, such as the region and year of cultivation, the climatic conditions throughout the growth cycle, the agricultural practices adopted, the disease control measures employed, and the genetic diversity of both the pathogens and the soybean cultivars (Lin et al., 2022).

Regarding viral infections, 60 viruses have been identified infecting soybean plants worldwide (Sastry et al., 2019). The main viruses that infect soybean in Brazil are the potyvirus soybean mosaic virus (SMV), which causes soybean mosaic disease; the carlavirus cowpea mild mottle virus (CPMMV), which causes stem necrosis disease; the ilarvirus tobacco streak virus (TSV), the causal agent of bud blight disease; and the orthotospovirus groundnut ringspot virus (GRSV) (Almeida et al.; De Marchi et al., 2019; Porto & Hagedorn, 1975; Barreto da Silva et al., 2020). The viruses SMV, CPMMV, TSV and GRSV are transmitted through soybean seeds (Costa, 1971; Porto & Hagedorn, 1975; Barreto da Silva et al., 2020; Uzan, 2023). Seed transmission of viruses plays a significant role in the epidemiology of diseases, as it contributes to the virus survival, long-distance dissemination, and serves as the primary source of inoculum, enabling subsequent spread of the viruses by vectors (Johansen et al., 1994; Ali & Kobayashi, 2010).

Recently, the potyvirus passiflora virus Y (PaVY) was naturally identified infecting soybean in São Paulo State, Brazil (Ribeiro-Junior et al., 2022).

Experimentally, PaVY was transmitted to soybean plants by Myzus persicae and upon mechanical inoculation the PaVY-Br induced chlorotic spots and systemic mosaic on soybean and chlorotic local lesions on passion fruit (Passiflora edulis) and sesame (Sesamum indicum) (Ribeiro-Junior et al., 2022). Due to its recent detection, little is known about the incidence of PaVY in soybean fields in the country , the host range and if the virus can be transmitted through soybean seeds. Thus, the objectives of this study were to evaluate the occurrence of PaVY in soybean fields in the state of São Paulo and to determine if this potyvirus can be transmitted through soybean seeds.

1.2. Materials and Methods

Plant Material

Healthy plants of the soybean cultivar TMG7063 IPRO, were cultivated in 20-liter pots containing a mixture of soil, sand, and cow manure (1:1:1). These plants were maintained in a greenhouse, receiving daily irrigation and fertilization according to the crop's needs.

PaVY isolate and mechanical inoculation

The PaVY-Br isolate (GenBank MZ190341) used in this study was obtained in 2020 from a soybean infected plant located in the municipality of Taciba, São Paulo, Brazil (Ribeiro-Junior *et al.*, 2022). The isolate was periodically renewed through mechanical inoculation to young soybean plants.

PaVY-infected soybean leaves or soybean seeds, according to the experiment, were macerated in 0.02 M phosphate buffer, pH 7, at a ratio of 1:10 (w:v). The extract was mechanically inoculated by rubbing it with the finger onto leaves of the test plants at the 2-3 true leaf stages. Afterward, the plants were washed with water to remove the inoculum and abrasive.

Detection of PaVY by RT-PCR

Total RNA was extracted from soybean leaf tissues or seeds following the protocol described by Bertheau & Frechon (1998). Total RNA extracted from leaves of PaVY-infected soybean plants and from leaves or seeds of healthy soybean plants were used, respectively, as positive and negative controls in the molecular analysis.

The extracted RNA was used for PaVY detection using one-step reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was performed with specific primers, HC-PRO F (5' TCCAACAGGGGGCTATGAGA 3') and HC-PRO R (5' GAGCGTCACGCGTTTTCTTT 3'), designed in this study based on the complete nucleotide sequence of the genome of the PaVY-Br isolate (GenBank MZ190341) using the Primer-Blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). These primers amplify a fragment of 431 bp of the HC-Pro gene. The RT-PCR reactions consisted of 2 µl of total RNA, 6.25 µl of GoTaq Green Master Mix (Promega), 3.70 µl of nuclease-free water, 0.25 µl of each primer at a concentration of 10 µM, and 0,5 U of the reverse transcription enzyme AMV (Promega). The amplification conditions for RT-PCR included reverse transcriptase at 42°C for 50 min, initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The resulting RT-PCR product was analyzed by agarose gel electrophoresis, and the amplified fragments were visualized using a UV transilluminator. Some amplicons were purified using the Wizard SV purification kit and PCR Clean-up System (Promega) and were sent for nucleotide sequencing, in the sense direction, at the Institute of Biotechnology (IBTEC) of the University of the State of São Paulo, Brazil. The obtained nucleotide sequences were compared to related sequences deposited in GenBank using the BLASTn algorithm, available at https://www.ncbi.nlm.nih.gov/blast.

Obtaining PaVY-infected soybean plants for seed harvest

Twenty five soybean plants cv. TMG7063 IPRO were mechanically inoculated with PaVY at the V2 vegetative stage. The confirmation of PaVY infection in the inoculated plants was assessed by symptoms observation and RT-PCR performed 30 days post-inoculation (dpi). The plants were kept in a greenhouse for their development until the end of their growth cycle for seed harvesting. The harvested seeds from the PaVY-infected soybean plants were subsequently used in the experiments to evaluate seed transmission of PaVY, detection of PaVY in soybean seeds, and to assess the infectivity of PaVY-contaminated soybean seeds. Seeds harvested from healthy soybean plants were used as negative controls in the experiments.

Evaluation of seed transmission of PaVY

A total of 1,219 seeds, harvested from 14 PaVY-infected soybean plants cv. TMG7063 IPRO, were tested to evaluate the seed transmission of this potyvirus. During harvest, it was noted that some seeds exhibited a brown coat mottling indicating alterations in the coat pigmentation. These seeds were then categorized into two groups: mottled seeds and non-mottled seeds (Figure 1). Subsequently, all the mottled seeds and non-mottled seeds obtained from the infected plants were sowed in trays containing substrate and maintained in a greenhouse for development. To evaluate the seed transmission of PaVY, the progeny seedlings were monitored for symptoms and subjected to RNA extraction and RT-PCR performed 40 days after emergence. Leaf tissue samples were collected in a bulk manner, with each sample comprising six seedlings. Preliminary experiments were conducted to determine the sensitivity of PaVY detection in composite samples at a ratio of 1 infected plant to 5 healthy plants (1:5). The results showed that one PaVY-infected soybean plant could be efficiently detected in a composite sample of 5 healthy plants using RT-PCR.

An additional experiment was carried out to evaluate the seed transmission of PaVY among different soybean cultivars. In this experiment, the soybean cultivars 96R90, 96Y90, C2531E, BMX Potência RR, and 96R10 were used. The experiment was conducted as described earlier.

Detection of PaVY in soybean seeds by RT-PCR

Two hundred and forty-nine seeds, harvested from 5 PaVY-infected soybean plants cv. TMG7063 IPRO, were tested to evaluate the presence of this potyvirus in the seeds. After harvesting, the seeds from the infected plants were placed on Petri dishes with moist filter paper for a seven-day germination period. The seeds were divided into two groups: mottled seeds and non-mottled seeds. Total RNA was then extracted individually from the germinated seeds using the method described by Bertheau & Frechon (1998), and detection of PaVY was performed using RT-PCR.

Evaluation of the infectivity of soybean seeds produced by PaVY-infected plants

Three hundred and seventy-nine seeds, harvested from 5 PaVY-infected soybean plants cv. TMG7063 IPRO, were tested to evaluate the infectivity of the seeds. The seeds were divided into two groups: mottled seeds and non-mottled seeds. The seeds were placed on Petri dishes with moist filter paper for germination. After germination, groups of 6 to 12 seeds were transferred to a mortar containing 10 ml of phosphate buffer 0.02 M pH 7 and were macerated using a pestle. The obtained extract was used for inoculation of soybean plants cv. TMG7063 IPRO at the V2 vegetative stage. One soybean plant was inoculated for each group of 6 to 12 evaluated seeds. The inoculated plants were kept in a greenhouse for symptom observation, and PaVY detection was performed 30 dpi using RT-PCR.

PaVY partial host range

PaVY-infected soybean leaves were macerated in phosphate buffer 0.02 M pH 7. The obtained extract was used for mechanical inoculation of 4 plants of the following species: *Canavalia ensiformis, Capsicum annuum, Cichorium intybus, Cucurbita pepo, Datura stramonium, Glycine max, Gossypium hirsutum, Helianthus annuus, Lactuca sativa, Nicotiana benthamiana, N. clevelandii, N. glutinosa, N. tabacum, Passiflora edulis* cvs. FB-200 and IAC-7*, P. edulis* (purple)*, P. cincinnata, P. setacea, P. nítida, P. caerulea, Petunia hybrida, Phaseolus vulgaris, Raphanus sativus, Sida cordifolia, Solanum aethiopicum, S. lycopersicum, S. melongena* e *Vigna unguiculata.* Two plants of each species were inoculated with the buffer only as a negative control. The inoculated plants were kept in a greenhouse to monitor symptoms, and PaVY infection was assessed 30 dpi using RT-PCR.

Evaluation of the incidence of PaVY in soybean fields in São Paulo State

The incidence of PaVY-infected soybean plants was assessed during the 2021/2022 and 2022/2023 crop seasons in various production regions of São Paulo State, Brazil. Fifty symptomatic or asymptomatic leaf samples were collected from soybean plants in each field under evaluation. The samples were individually labeled, placed in plastic bags, and stored at -80 °C in an ultra-freezer until further molecular analysis. PaVY detection was conducted using RT-PCR. The collected samples were also analyzed to verify the presence of other viruses that commonly infect soybean and whose symptoms can be confused with those caused by PaVY. The detection of the carlavirus CPMMV was performed using the specific primers CPMMV 1280-F and

CPMMV 1696-R (De Marchi *et al.*, 2017), while the universal primers for orthotospovirus BR60 and BR65 (Eiras *et al.*, 2001) were used for the detection of GRSV.

1.3. Results

Seed transmission of PaVY

In order to establish the sensitivity of our assay, we tested mixed samples at ratio of 1:5 (PaVY-infected plant/healthy plants). The results showed that one PaVY-infected soybean plant could be efficiently detected in a composite sample of 5 healthy plants using RT-PCR.

A total of 1,219 seeds, harvested from 14 PaVY-infected soybean plants cv. TMG7063 IPRO, were tested to evaluate the seed transmission of this potyvirus. The infection of the soybean plants with PaVY was confirmed by symptoms observation (Figure 1) and RT-PCR. Some harvested seeds from the infected plants showed alterations in the pigmentation exhibiting coat mottling (Figure 1).

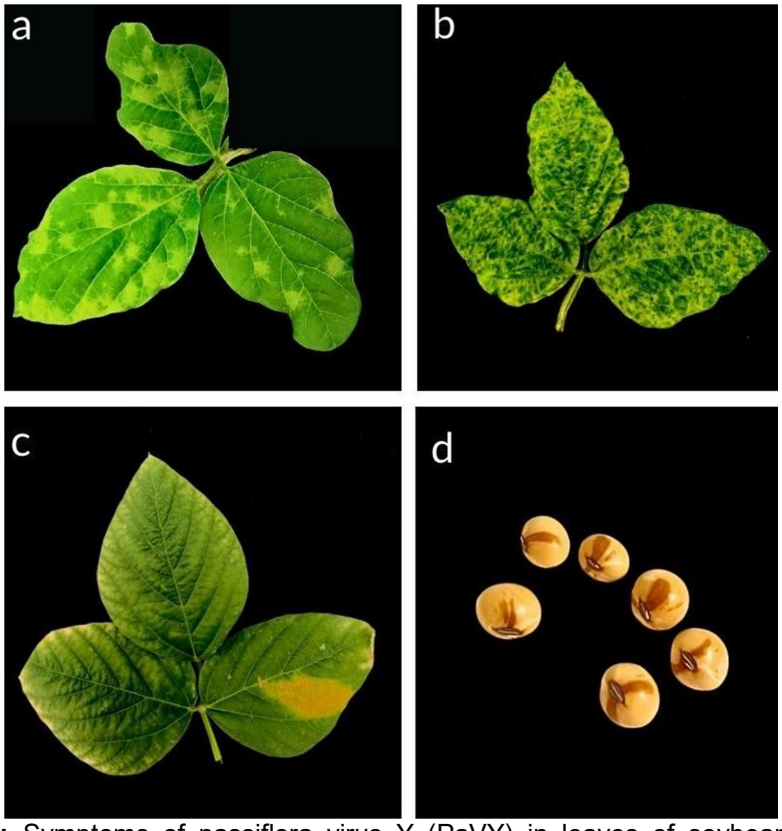

Figure 1: Symptoms of passiflora virus Y (PaVY) in leaves of soybeans infected plants (A, B, and C). Mottled seeds produced by PaVY-infected soybean plants cv. TMG7063 IPRO (D)

The number of mottled and non-mottled seeds produced by PaVY-infected soybean plants was showed in Table 1 (Table 1). Most of the PaVY-infected soybean plants produced both mottled seeds and non-mottled seeds. None of the seeds produced by healthy soybean plants (control) exhibited coat mottling. However, none of the 1,219 progeny seedlings derived from both mottled or non-mottled seeds exhibited symptoms of PaVY infection. Furthermore, the virus was not detected in the progeny seedlings by RT-PCR (Table1).

Table 1: Seed transmission of passiflora virus Y (PaVY) from mottled and non-mottled seeds to the progeny seedlings of soybean cv. TMG7063 IPRO.

Plants	no. of mottled seeds/no. of PaVY-infected progeny seedlings	no. of non-mottled seeds/ no. of PaVY-infected progeny seedlings
1	24/0	24/0
2	-	81/0
3	66/0	20/0
4	54/0	44/0
5	18/0	11/0
6	-	69/0
7	36/0	124/0
8	-	157/0
9	24/0	80/0
10	12/0	27/0
11	30/0	70/0
12	36/0	81/0
13	66/0	01/0
14	42/0	22/0
TOTAL	**408/0**	**811/0**

-: PaVY-infected soybean plants that did not produced mottled seeds.

Detection of PaVY by RT-PCR

Two hundred and forty-nine seeds, harvested from 5 PaVY-infected soybean plants cv. TMG7063 IPRO, were individually tested to evaluate the presence of this potyvirus in the seeds. The presence of PaVY was observed in 9.8% (14 out of 143) of mottled seeds and in 37.8% (40 out of 106) of non-mottled seeds (Table 2). The presence of PaVY varied among the seeds produced by each PaVY-infected soybean plant, with detection rates ranging from 0% to 57% (Table 2). The presence

of PaVY in the seeds was confirmed through nucleotide sequencing of three amplicons obtained from mottled seeds.

Table 2: Detection of passiflora virus Y (PaVY) in mottled and non-mottled seeds produced by infected soybean plants cv. TMG7063 IPRO

Plants	no. of mottled seeds/no. of seeds positive for PaVY	no. of non-mottled seeds/ no. of seeds positive for PaVY
1	43/3	-
2	32/4	70/40
3	42/5	-
4	-	36/0
5	26/2	-
Total	**143/14**	**106/40**

-: PaVY-infected soybean plants that did not produced mottled or non-mottled seeds

Evaluation of the infectivity of soybean seeds produced by PaVY-infected plants

Three hundred and seventy-nine seeds, harvested from 5 PaVY-infected soybean plants cv. TMG7063 IPRO, were tested to evaluate the infectivity of PaVY-contaminated soybean seeds. The seeds were divided into groups of 6 to 12 seeds and used as sources of inoculum for the transmission of PaVY to healthy soybean plants (Table 3). Among the evaluated seeds, 24 groups were composed of non-mottled seeds, while 14 groups were composed of mottled seeds. No infectivity was observed in PaVY-contaminated seeds. None of 38 soybean plants was infected after mechanical inoculation using extracts obtained from contaminated seeds. (Table 3).

PaVY partial host range

PaVY-Br isolate infected plants of six species: *C. ensiformis* (4/4), *G. hirsutum* (4/4), *N. benthamiana* (2/4), *P. vulgaris* (4/4), *R. sativus* (2/4), and *V. unguiculata* (4/4). Infected *C. ensiformis* plants exhibited symptoms of mosaic, blistering and leaf malformation at 20-25 dpi. *G. hirsutum* plants showed chlorotic spots and vein clearing on the inoculated leaves at 15-20 dpi. In these plants, PaVY showed a limitation in the systemic movement as the virus was detected by RT-PCR in the inoculated leaves but not in the new young leaves. *N. benthamiana* plants exhibited symptoms of vein clearing at 15 dpi. *R. sativus* plants showed leaf mosaic at 15-20

dpi. *P. vulgaris* and *V. unguiculata* plants initially exhibited chlorosis on the leaves, which later progressed to necrosis of both leaves and shoots, resulting in plant death at 10-15 dpi. No symptoms were observed in the other inoculated plant species, and PaVY was not detected by RT-PCR.

Table 3: Mechanical transmission of passiflora virus Y (PaVY) from contaminated seeds to healthy soybean plants

Plant source of the seeds	no. of seeds used for inoculation	Status of the seeds	no. of infected plants/ no. inoculated plants
1	11	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	12	Mottled	0/1
	10	Mottled	0/1
2	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	8	Non-mottled	0/1
	10	Mottled	0/1
3	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Mottled	0/1
	10	Mottled	0/1
	6	Mottled	0/1
4	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	10	Non-mottled	0/1
	8	Non-mottled	0/1
	10	Mottled	0/1
	10	Mottled	0/1
	6	Mottled	0/1
5	10	Mottled	0/1
	10	Mottled	0/1
	10	Mottled	0/1
	10	Mottled	0/1
	10	Mottled	0/1

Incidence of PaVY in soybean fields in São Paulo State

The occurrence of PaVY was investigated in soybean fields located in 12 municipalities during the 2021/2022 growing season, and in soybean fields located in four municipalities during the 2022/2023 growing season. None of the examined soybean plants were infected with PaVY in both seasons (Supplementary Table 2). The symptomatic soybean plants collected tested positive for CPMMV or GRSV (data not shown).

1.4. Discussion

Seed transmission has profound ecological implications for the perpetuation, persistence, and spread of viruses, and it also carries economic consequences for plant growers (Johansen et al., 1994). In this study, we evaluated the seed transmission of the potyvirus PaVY in soybean plants. In the evaluation of 1,219 seedlings derived from PaVY-infected soybean plants cv. TMG7063 IPRO, the presence of the potyvirus was not detected in any of the progeny seedlings (Table 1). Furthermore, an additional experiment was carried out to evaluate the seed transmission of PaVY among different soybean cultivars. The analysis demonstrated the absence of vertical transmission of PaVY from seeds to progeny seedlings of soybean plants belonging to the cultivars 96R90 (n=125), 96Y90 (n=205), C2531E (n=151), BMX Potência (n=57), and 96R10 (n=215) (Supplementary table 2). Overall, the results demonstrated that seed transmission of PaVY in soybean is unlikely.

During the harvest of seeds produced by PaVY-infected soybean plants, it was observed the presence of mottled seeds (Figure 1d). The occurrence of seed coat mottling in soybeans is marked by the presence of dark streaks or patterns that radiate from the hilum, resulting in bleeding at the hilum region. In some instances, the mottling is evenly distributed over the entire seed coat. The coloration of the mottling, varying from buff to brown or black, is governed by distinct genes regulating hilum coloration (Hobbs et al., 2003). In the present study, seeds produced by healthy plants did not show any changes in pigmentation. However, the presence of mottling in the seeds did not indicate the transmission of PaVY to progeny, as the potyvirus was not detected in seedlings derived from both non-mottled and mottled seeds (Table 1).

Yellow seeds are a common characteristic in most commercially grown soybeans cultivars due to the absence of pigmentation in the seed coat. It has been proposed that the inhibition of seed coat pigmentation in yellow soybeans is controlled by a process called homology-dependent silencing, which targets the chalcone synthase (CHS) genes responsible for pigmentation (Senda et al., 2004). The presence of mottling symptoms on the seed coat of soybean plants infected with specific viruses is associated with the viral silencing suppressor protein's capacity to inhibit post-transcriptional gene silencing (PTGS) of the CHS gene, resulting in disrupted pigmentation patterns (Senda et al., 2004).

Although PaVY was not transmitted from seeds to progeny seedlings, the presence of viral RNA was detected in some seeds produced by PaVY-infected soybean plants (Table 2), indicating that PaVY is seedborne. The term "seedborne" describes the potential of a virus to be present in seeds without necessarily being transmitted to the next generation (Fortes et al., 2023). Similar results were found for the sobemovirus rice yellow mottle virus (RYMV) and the begomovirus tomato leaf curl New Delhi virus (ToLCNDV). ToLCNDV and RYMV were detected in melon seeds (*Cucumis melo*) and rice seeds (*Oryza sativa*), respectively, but were not transmitted from the seeds to the offspring (Konate et al., 2001; Fortes et al., 2023).

In the present study, none of 38 soybean plants was infected after mechanical inoculation using extracts obtained from seeds produced by PaVY-infected soybean plants (Table 3). As commented by Johansen et al (1994), viral transmission through seeds due to surface contamination or the presence of viruses in the seed coat is a rare occurrence because only a few virus particles possess the necessary stability to withstand the conditions associated with seed dehydration, harvest, and storage. This mode of transmission appears to be relevant only for highly stable viruses, such as tobamoviruses. It has been demonstrated that particles of the tobamovirus tomato brown rugose fruit virus (ToBRFV) present on contaminated tomato seeds remains viable and can cause infection from the contaminated seeds to seedlings through mechanical inoculation (Salem et al., 2022).

The PaVY-Br isolate used in this study was mechanically transmitted to plants of *C. ensiformis*, *G. hirsutum*, *N. benthamiana*, *P. vulgaris*, *R. sativus*, and *V. unguiculata*, expanding the experimental host range of this viral isolate. Plants of *P. edulis* cv. FB 200 and IAC-7, *P. edulis* purple, *P. setacea*, *P. nitida*, *P. cincinnata* and *P. caerulea* were not infected with PaVY-Br. PaVY has been reported naturally

infecting *Passiflora* plants in Indonesia, Australia, and China (Parry et al., 2004; Chen et al., 2021).

There is significant biological variability among PaVY isolates. During the evaluation of the host range of 8 PaVY isolates collected from different host plants, it was observed that two isolates obtained from *P. foetida* consistently infected *P. edulis* and *P. caerulea*, but showed only occasional infectivity in other leguminous plants. On the other hand, another PaVY isolate from *P. foetida* exhibited a similar pattern to the five isolates identified in leguminous plants, readily infecting legume plants but unable to infect *P. edulis* (Coutts et al., 2011). In a previous study conducted by our research group, it was found that the PaVY-Br isolate induced local chlorotic lesions in passion fruit, unlike the results obtained in the present study (Ribeiro-Junior et al., 2022). These divergent results may be associated with the use of different *P. edulis* cultivars, which may possess distinct levels of resistance to PaVY.

PaVY was not detected in the surveys conducted in the main soybean-producing regions of São Paulo state during the 2021/2022 and 2022/2023 soybean growing seasons (Supplementary table 1). The presence of PaVY was not detected, even in the assessments conducted in the same area of the municipality of Taciba where this potyvirus was first reported in 2020, in Brazil (Ribeiro-Junior et al., 2022). Furthermore, in August 2022, we conducted an assessment in four passion fruit orchards located in the municipality of Taciba, and the presence of PaVY was not detected (data not shown).

In conclusion, our results demonstrated a low incidence of PaVY in soybean fields in the major producing regions of the state of São Paulo. It was also observed that PaVY is seedborne but not seed transmitted in soybean seeds. Therefore, vertical transmission of PaVY in soybean plants is unlikely to have epidemiological significance. Further studies with other PaVY isolates should be conducted to confirm the non-transmission of this potyvirus trough soybean seeds.

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Supplementary table 1: Occurrence of passiflora virus Y (PaVY) in soybean fields located in different municipalities of the state of São Paulo during the crop seasons of 2021/2022 and 2022/2023.

Soybean crop season 2021/2022		
Collection site	**Coordinates**	**no. of PaVY-infected plants /no. of analyzed plants**
Araçatuba	21°10'33"S 50°31'33"W	0/50
Buritama	21°04'08"S 50°12'08"W	0/50
Casa Branca	21°55'48"S 47°04'08"W	0/50
Guaíra	20°04'03"S 48°'04'"04"W	0/50
Jaú	22°14'42"S 48°'33'"20"W	0/50
Mogi Mirim	22°28'36"S 47°03'36"W	0/50
Narandiba	22°24'26"S 51°31'28"W	0/50
Óleo	22°56'33"S 49°26'14"W	0/50
Paranapanema	23°28'26"S 48°45'16"W	0/50
Salto Grande	22°50'13"S 50°01'16"W	0/50
Taciba	22°24'38"S 51°19'30"W	0/50
Vargem Grande	21°50'07"S 46°54'40"W	0/50
Soybean crop season 2022/2023		
Collection site	**Coordinates**	**no. of PaVY-infected plants /no. of analyzed plants**
Araçatuba	21°10'33"S 50°31'33"W	0/50
Buritama	21°04'08"S 50°12'08"W	0/50
Narandiba	22°24'26"S 51°31'28"W	0/50
Taciba	22°24'38"S 51°19'30"W	0/50

Supplementary table 2: Seed transmission of passiflora virus Y (PaVY) from mottled and non-mottled seeds to the progeny seedlings of different soybean cultivars

Cultivar	no. of plants used to harvest seeds	no. of mottled seeds/ no. of PaVY-infected progeny seedlings	no. of non-mottled seeds/ no. of PaVY-infected progeny seedlings
96R90	3	54/0	71/0
96Y90	5	169/0	36/0
C2531E	5	-	151/0
BMX Potência RR	4	-	57/0
96R10	5	-	215/0
Total		**230/0**	**530/0**

CHAPTER 2

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF bidens mosaic virus INFECTING PATCHOULI (*Pogostemon cablin*) IN BRAZIL

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Chapter elaborated following the guidelines of Journal of Phytopathology[2]

Abstract

Six patchouli (*Pogostemon cablin* Benth.) plants showing symptoms of viral infection were found in Brazil. Transmission electron microscopy revealed flexuous particles and characteristic pinwheel-shaped cytoplasmic inclusions of potyvirus in sap and cells of symptomatic leaves. Potyvirus infection was confirmed through RT-PCR, using universal primers followed by nucleotide sequencing. The obtained sequences, comprising part of the CP gene and the 3' non-translated region, showed 97.71%-97.87% identity with an isolate of bidens mosaic virus (BiMV). The nucleotide sequences of the CP, HC-Pro and CI genes of BiMV isolates from patchouli plants were obtained through RT-PCR with specific primers followed by nucleotide sequencing. The obtained sequences showed 95.78%-97.1% identities with BiMV isolates from Brazil. One BiMV isolate from a patchouli infected plant was sap-transmitted to different plant species. *Chenopodium amaranticolor* exhibited local lesions, *Bidens pilosa* developed leaf mosaic, and *Nicotiana benthamiana* exhibited

[2] Chapter elaborated following the guidelines of Journal of Phytopathology

chlorosis and stunting. The BiMV isolate was transmitted from infected patchouli plant to *B. pilosa* using *Myzus persicae* aphids. This is the first confirmation of patchouli infected with BiMV in Brazil. Further studies are necessary to assess the incidence of this potyvirus in commercial patchouli crops, as well as the damage caused to plant development and the quantity and quality of essential oils produced.

Keywords: potyviruses, BiMV, viral infection, essential oil

SHORT COMMUNICATION

Patchouli (*Pogostemon cablin* Benth.) is a native plant to Southeast Asia that belongs to the family Lamiaceae. Patchouli is a highly valued aromatic plant that is widely cultivated in India, China, Indonesia, Mauritius, Malaysia, Thailand, West Africa, the Philippines, and Vietnam (Pandey et al., 2021). Patchouli holds economic importance due to the essential oil present in its leaves, which is widely used in the production of cosmetics and also in therapeutic treatments (Kumaraswamy & Anuradha, 2010; Swamy & Sinniah, 2015). Patchouli essential oil also possesses antibacterial, antifungal, and insecticidal properties (Hu et al., 2017). The largest producers of patchouli essential oil in the world are Indonesia, China, Malaysia, and Brazil (Pandey et al., 2021).

Patchouli is an herbaceous plant with fibrous roots and stems ranging in diameter from 10 to 20 mm. The plant can grow to a height of 1 m and develop branching that extends up to a radius of 60 cm. Patchouli leaves have a length between 5 and 10 cm and a width of 2 to 7 cm.. Additionally, patchouli leaves are smooth and have a velvety texture. The essential oil is stored in the trichome glands of the leaves (Swamy & Sinniah, 2016).

Infections caused by viruses can lead to a decrease in plant development and oil content in patchouli plants (Sugimura et al., 1995). In the world, patchouli plants have been reported naturally infected by viruses belonging to the genera *Cytorhabdovirus*, *Fabavirus*, *Potyvirus*, *Potexvirus*, and *Necrovirus* (Sastry et al., 2019; Kitajima, 2020; Kaufmann et al., 2022).

Bidens mosaic virus (BiMV) is a member of the species *Bidens mosaic virus*, genus *Potyvirus*, family *Potyviridae*. It was first reported in 1961, causing mosaic in

plants of *Bidens pilosa* in Brazil (Kitajima et al., 1961). Subsequently, BiMV has been reported infecting naturally plants of *Arracacia xanthorrhiza*, *Coreopsis lanceolata*, *Galinsoga parviflora*, *Helianthus annuus*, *Lactuca sativa*, *Pisum sativum*, *Zinnia elegans*, and *Centella asiatica* in Brazil (Kitajima, 2020; Camelo-Garcia et al., 2021). To date, there are no reports of BiMV presence in other countries worldwide.

In 2022, six patchouli plants showing mosaic symptoms (Figure 1) were found in a flower shop in Botucatu County, São Paulo state, Brazil. The plants were taken to the laboratory and maintained in a greenhouse for identification of the causal agent.

To observe possible virus particles, sap from leaves of three symptomatic patchouli plants was negatively stained with uranyl acetate and examined using a transmission electron microscope (JEOL JEM 1011). To visualize the cytopathic effect on cells of patchouli plants, small pieces of symptomatic leaves were fixed, post-fixed, dehydrated, infiltrated, embedded, and thin sectioned for examination with a transmission electron microscope following the procedures described by Kitajima and Nome (1999).

Total RNA from leaves of the six patchouli symptomatic plants was individually extracted following the protocol described by Bertheau et al. (1998). The obtained RNA was used for virus detection by one-step reverse transcription-polymerase chain reaction (RT-PCR) with the potyvirus universal primers WCIEN/PV1, which amplifies a fragment of 800 bp comprising part of the coat protein (CP) gene and the 3' non-translated region (Maciel et al., 2011). The obtained amplicons were sent for nucleotide sequencing, in the sense direction, at the Institute of Biotechnology (IBTEC) of the University of the State of São Paulo, Brazil. The obtained nucleotide sequences were compared to related sequences deposited in GenBank using the BLASTn algorithm, available at https://www.ncbi.nlm.nih.gov/blast.

To obtain the complete nucleotide sequence of the CP gene of the BiMV isolates, total RNA extracted from leaves of the six symptomatic patchouli plants was used in RT-PCR performed with the BiMV-specific primer pair 8331/9046, which amplifies a fragment of 715 bp from the CP gene of this potyvirus (Suzuki et al., 2009). The obtained amplicons were sent for nucleotide sequencing, in the sense and antisense directions, at IBTEC.

For the purpose of obtaining genetic information of other regions of the genome of the BiMV isolates, two pair of primers was designed based on the only

complete nucleotide sequence of an isolate of BiMV available (GenBank KF649336). The primer pairs were designed using the Primer-Blast program, available at https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi. The primer pair BiMV-CI F (TTCTCATCCGGGGAGCTGTA) and BiMV-CI R (AAGCAAAGCAGGCTAGAGCA) amplifies a fragment of 933 bp from the cylindrical inclusion (CI) gene. The primer pair BiMV-HC-Pro F (TGTTAAGCGATTGCTCGGGT) and BiMV-HC-Pro R (GCATCGTGAACGTCTGGGTA) amplifies a fragment of 924 bp from the helper component protease (HC-Pro) gene. The reactions with both primer pairs were performed with 2 µl of total RNA, 6.25 µl of GoTaq Green Master Mix (Promega), 3.70 µl of nuclease-free water, 0.25 µl of each primer at a concentration of 10 µM, and 1 U of the reverse transcription enzyme AMV (Promega). The amplification conditions were 42°C for 50 min for reverse transcription, followed by 94°C for 3 min for initial denaturation, 35 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s, and a final extension at 72°C for 10 min. The RT-PCR product was subjected to agarose gel electrophoresis, and the amplicons were visualized under a UV transilluminator. The amplicons obtained were sent for nucleotide sequencing, in the sense direction, at IBTEC.

A pairwise comparison of the entire nucleotide sequences of CP gene, and partial nucleotide sequences of the CI and HC-Pro genes of the BiMV isolates was performed using Muscle 5.1 alignment within Geneious Prime. A Bayesian phylogenetic tree was constructed using the complete nucleotide sequences of the CP gene of BiMV isolates from patchouli plants and other potyviruses reported in Brazil. Nucleotide sequences of the carlavirus cowpea mild mottle virus were also included in the dataset. The sequences were aligned using the Muscle option in MEGA version X (Kumar et al., 2018), and phylogenetic analysis was performed using Mr. Bayes 3.2.2 (Ronquist and Huelsenbeck, 2003). The program MrModeltest v.2.3 (Nylander, 2004) was used to select the nucleotide substitution model GTR+G using the Akaike information criterion (AIC). Two independent runs were conducted simultaneously using 1 million generations and excluding 25% of the resulting trees as burn-in. Phylogenetic trees were visualized using FigTree v1.4.4 software (tree.bio.ed.ac.uk/software/figtree/).

One BiMV isolate from a patchouli infected plant was used in the biological assays. Leaf tissues from the symptomatic patchouli plant were homogenised in phosphate buffer 0.02 M, pH 7. The obtained extract was used for sap inoculation on

four plants of the following species: *Bidens pilosa, Canavalia ensiformis, Capsicum annuum, Chenopodium amaranticolor, Cichorium intybus, Crotalaria juncea, Cucumis melo, Cucurbita moschata, Cucurbita pepo, Datura stramonium, Glycine max, Gossypium hirsutum, Helianthus annuus, Lactuca sativa, Nicotiana benthamiana, Nicotiana clevelandii, Nicotiana glutinosa, Nicotiana tabacum, Passiflora edulis, Petunia hybrida, Phaseolus vulgaris, Pisum sativum, Raphanus sativus, Sida cordifolia, Solanum aethiopicum, Solanum lycopersicum,* and *Vigna unguiculata.* Two plants of each species were mock-inoculated as a negative control. The inoculated plants were kept in a greenhouse to monitor the symptoms, and infection with BiMV was evaluated 30 days after inoculation by RT-PCR using specific primers.

Transmission experiments were carried out using apterous adults of *Myzus persicae*, reared on healthy plants of *Brassica oleracea*. The aphids were collected from the colony and subjected to a fasting period of 30 min. They were then transferred to patchouli leaves infected with BiMV for an acquisition access period (AAP) of 10 min. Following this, the aphids (10 per plant) were transferred to eight healthy plants of *B. pilosa* and kept for an inoculation access period (IAP) of 24 h. After inoculation, the aphids were manually eliminated. The inoculated plants were maintained in a greenhouse to monitor symptoms, and confirmation of BiMV infection was performed 30 days after inoculation using RT-PCR.

Flexuous and elongated particles were observed using transmission electron microscopy in the sap of the three symptomatic patchouli plants analyzed. Potyvirus-like particles and potyvirus-like cytoplasmic inclusion bodies were also observed in epidermal and mesophyll cells of ultra-thin sections from infected tissues (Figure 2) (Inoue-Nagata et al., 2022).

RT-PCR performed with the universal primers WCIEN/PV1, followed by nucleotide sequencing confirmed the infection of the six symptomatic patchouli plants with potyvirus. The obtained sequences showed 97.71% to 97.87% identity with the corresponding sequence of an isolate of BiMV (GenBank AY960151) identified in plants of *B. pilosa* in Brazil (Inoue-Nagata et al., 2006). The infection of the six patchouli plants with this potyvirus was further confirmed by RT-PCR using the BiMV-specific primer pairs (Suzuki et al., 2009), followed by nucleotide sequencing. The BiMV isolates from the patchouli infected plants were named BiMV-PC1, BiMV-PC2, BiMV-PC3, BiMV-PC4, BiMV-PC5, BiMV-PC6. The sequences of the BiMV isolates from infected patchouli plants obtained by Sanger sequencing using the primers

WCIEN, 8331, and 9046 were assembled and generated consensus nucleotide sequences ranging from 1221 to 1285 nt, comprising part of the nuclear inclusion b (NIB) gene, the entire CP gene, and part of the 3' non-translated region of the BiMV isolates. The sequences have been deposited in GenBank with the accession numbers OR085478, OR085479, OR085480, OR085481, OR085482, and OR085483.

The primer pairs BiMV-CI F/ BiMV-CI R and BiMV-HC-Pro F/ BiMV-HC-Pro R efficiently detected the BiMV isolates in total RNA extracted from leaves of symptomatic patchouli plants. The partial nucleotide sequences of the CI gene (GenBank OR085484, OR085485, OR085486, OR085487, OR085488, and OR085489) and HC-Pro gene (GenBank OR085490, OR085491, OR085492, OR085493, OR085494, and OR085495) of the BiMV isolates shared, respectively, 96.90% to 97.06% and 95.78% to 96.04% identity with the sequence of a BiMV isolate (GenBanK KF649336) identified in infected plants of *B. pilosa* in Brazil (Sanches et al., 2014).

Regarding to the complete nucleotide sequence of the CP gene, the BiMV isolates from infected patchouli plants shared 99.8% to 100% identity among themselves (Table 1). The complete nucleotide sequence of the CP gene from the BiMV isolates found in patchouli plants was compared to the three available complete nucleotide sequences of CP gene of BiMV isolates in GenBank. These sequences correspond to BiMV isolates identified in infected plants of *B. pilosa* (GenBank AY960151, KF649336) and *P. sativum* (GenBank AY960150) in Brazil (Inoue-Nagata et al., 2006; Sanches et al., 2014). The complete nucleotide sequences of the CP gene among the BiMV isolates showed identity ranging from 96.8% to 97.1% (Table 1). The phylogenetic analysis revealed that all the isolates of BiMV clustered together in a group and were distantly related to the BiMV isolates identified in infected plants of *B. pilosa* and *P. sativum* in Brazil (Figure 3).

The BiMV-PC1 was used in the biological assays. The partial host range of BiMV-PC1 was evaluated by monitoring symptoms after sap inoculation in plants of different species, and RT-PCR with specific primers. BiMV-PC1 was detected only in plants of three species: *B. pilosa*, *N. benthamiana*, and *C. amaranticolor*. Infected plants of *B. pilosa* exhibited symptoms of leaf mosaic between 10 and 15 days post-inoculation (dpi). *N. benthamiana* plants showed chlorosis and stunting 10 dpi, while

C. amaranticolor reacted with local lesions 15 dpi. No symptoms were observed in the others inoculated plant species and the potyvirus was not detected by RT-PCR.

Studies evaluating the partial host range of BiMV have revealed high biological diversity among isolates of this potyvirus. An isolate of BiMV identified in *L. sativa* plants experimentally infected *Chenopodium quinoa*, *C. amaranticolor*, *P. sativum,* and *L. sativa*, while it did not infected *H. annuus* and *B. pilosa* (Suzuki et al., 2009). Conversely, the BiMV isolate identified in *B. pilosa* plants experimentally infected *H. annuus*, *L. sativa*, *N. clevelandii*, *Nicotiana occidentalis*, *Nicotiana rustica, N. tabacum*, *P. sativum*, *Z. elegans*, and *C. pepo* (Sanches et al., 2014). Another BiMV isolate identified in *C. asiatica* did not infect *B. pilosa*, however, it caused severe mosaic symptoms in *N. benthamiana* and local lesions in *C. amaranticolor*, and *C. quinoa* (Camelo-Garcia et al., 2021).

The BiMV-PC1 isolate was transmitted from infected patchouli plant to *B. pilosa* plants by *M. persicae* aphids, with a transmission rate of 38% (3 infected plants out of 8 inoculated). The infected *B. pilosa* plants exhibited symptoms of leaf mosaic 17 days post-inoculation (dpi).

Patchouli plants are cultivated through vegetative propagation, primarily through the utilization of stem cuttings. This method of propagation is widely practiced due to the limited availability of seeds (Pandey et al., 2021). In this way, the transmission of BiMV during the vegetative propagation of infected plants may represent the main way of dissemination of this potyvirus in patchouli crops. Furthermore, the establishment of a new planting using cuttings from infected plants poses a significant risk, as these plants can serve as sources of primary inoculum and enable secondary spread of BiMV to other plants in the area by aphid vectors. Obtaining virus-free mother plants through meristem culture, as demonstrated for other potyviruses-infecting patchouli plants (Sugimura et al., 1995), followed by molecular or serological tests to confirm plant health, is a important step in managing BiMV in patchouli crops.

In Brazil, patchouli plants have been naturally identified infected with the potexvirus patchouli virus X (PatVX), the alphanecrovirus tobacco necrosis virus (TNV), the tobravirus pepper ringspot virus (PepRSV), and a cytorhabdovirus and nucleorhabdovirus not completely characterized (Kitajima, 2020). Recently, patchouli plants showing symptoms of vein chlorosis and chlorosis spots were found to be

infected with a cytorhabdovirus tentatively named patchouli chlorosis-associated cytorhabdovirus (PCaCV) (Kaufmann et al., 2022).

Potyvirus infections have been documented in patchouli plants from the states of São Paulo, Pará, and Sergipe in Brazil. Despite the possibility of BiMV infection in the previously identified plants (Kitajima, 2020), the identification of the potyvirus species responsible for these infections could not be determined due to the absence of nucleotide sequences data in the previous findings. Thus, based on biological and molecular tests, we present here the first confirmation of BiMV infection in patchouli plants in Brazil. Further studies are needed to assess the incidence of this potyvirus in commercial crops and to determine the damage caused to plant development, as well as the quantity and quality of essential oil produced by patchouli plants.

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Table 1: Pairwise comparison of the complete nucleotide sequences of the coat protein gene of bidens mosaic virus (BiMV) isolates.

Isolates	Identity (%) nt								
	1	2	3	4	5	6	7	8	9
1-BiMV-PC1 OR085478		99.9	99.8	99.9	99.9	99.8	97.0	96.9	96.8
2-BiMV-PC2 OR085479	99.9		99.9	100	100	99.9	97.1	97.0	96.9
3-BiMV-PC3 OR085480	99.8	99.9		99.9	99.9	99.8	97.0	96.9	96.8
4-BiMV-PC4 OR085481	99.9	100	99.9		100	99.9	97.1	97.0	96.9
5-BiMV-PC5 OR085482	99.9	100	99.9	100		99.9	97.1	97.0	96.9
6-BiMV-PC6 OR085483	99.8	99.9	99.8	99.9	99.9		97.0	96.9	96.8
7-BiMV-KF649336	97.0	97.1	97.0	97.1	97.1	97.0		96.9	96.8
8-BiMV-AY960150	96.9	97.0	96.9	97.0	97.0	96.9	96.9		98.4
9-BiMV- AY960151	96.8	96.9	96.8	96.9	96.9	96.8	96.8	98.4	

Figure 1: Symptoms of mosaic on leaves of patchouli plants infected with bidens mosaic virus (BiMV).

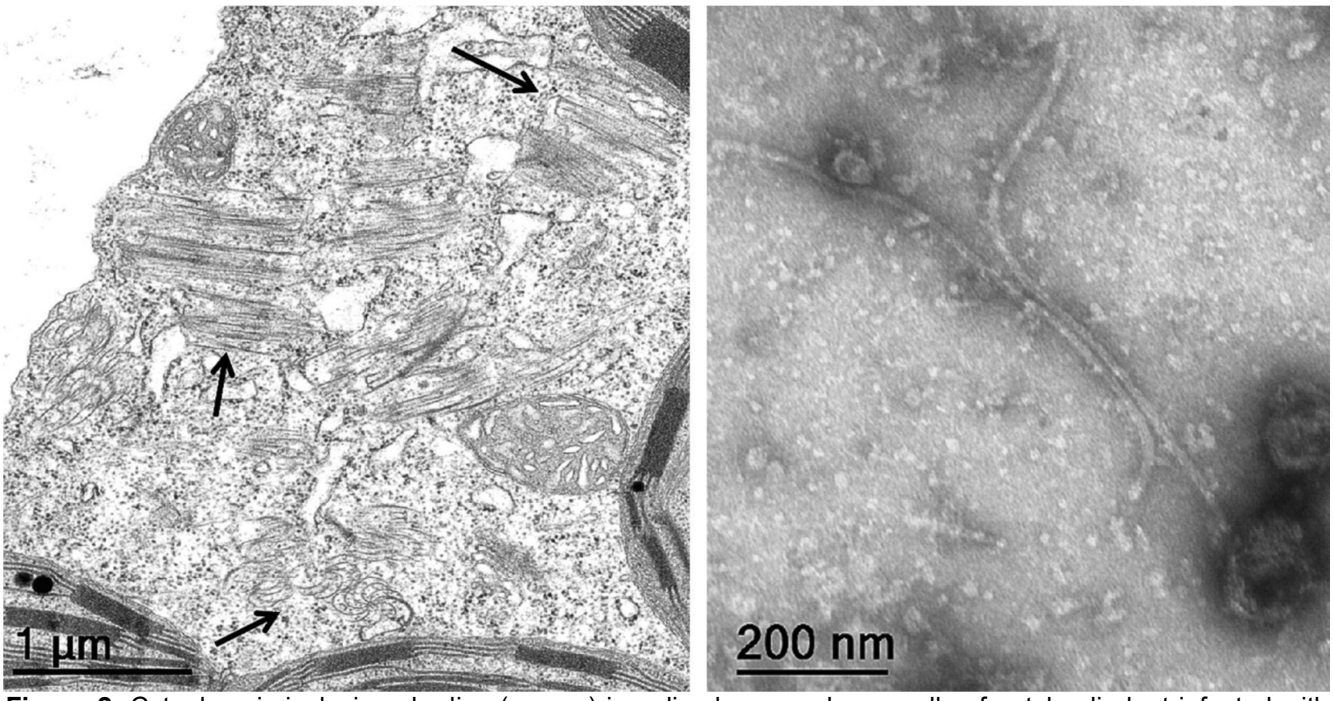

Figure 2: Cytoplasmic inclusions bodies (arrows) in palisade parenchyma cells of patchouli plant infected with bidens mosaic virus (BiMV) (left panel). Elongated and flexuous particles observed in sap negatively stained from BiMV-infected patchouli plant (right panel).

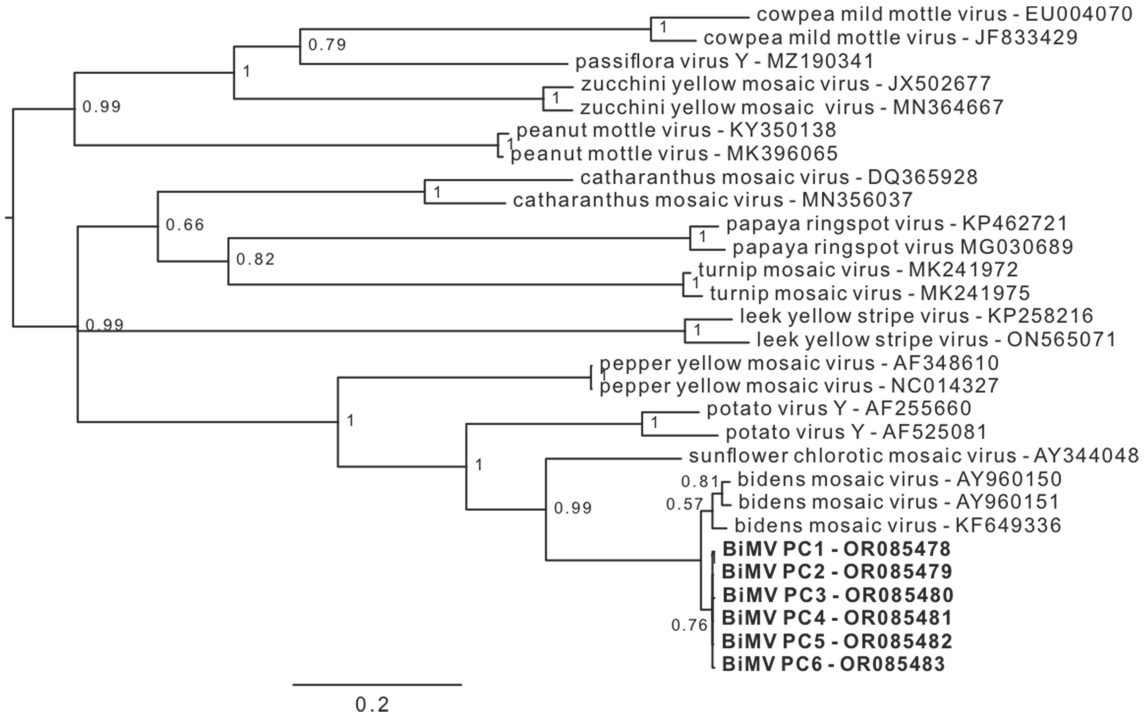

Figure 3: Midpoint-rooted Bayesian phylogenetic tree based the complete nucleotide sequences of the coat protein gene of bidens mosaic virus isolates from patchouli plants and other potyviruses reported in Brazil. The BiMV isolates from patchouli plants are highlighted in bold. Nucleotide sequences of the carlavirus cowpea mild mottle virus were included as outgroup. The corresponding GenBank accession number of each virus sequence is given in the figure Bar= number of substitution per site.

FINAL CONSIDERATIONS

Currently, the potyvirus passiflora virus Y (PaVY) occurs at a low incidence in soybean fields in the major producing regions of the state of São Paulo. This potyvirus is seedborne but not seed transmitted in soybean seeds. Therefore, vertical transmission of PaVY in soybean plants is unlikely to have epidemiological significance.

The potyvirus bidens mosaic virus (BiMV) was identified naturally infecting plants of patchouli (*Pogostemon cablin*) in Brazil. This is the first confirmation of BiMV infection in patchouli plants in the country. Further studies are needed to assess the incidence of this potyvirus in commercial crops and to determine the damage caused to plant development, as well as the quantity and quality of essential oil produced by patchouli plants.

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