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Molecular and biological characterization of Cowpea Mild Mottle Virus (CPMMV) isolates infecting soybean in Bali and its host range

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Abstract. *Wirya GNAS, Listihani L, Selangga DGW, Phabiola TA, Temaja IGRM, Jayasanti NNS, Ariyanta IPB. 2026. Molecular and biological characterization of Cowpea Mild Mottle Virus (CPMMV) isolates infecting soybean in Bali and its host range. Biodiversitas 27 (4): d270441. https://doi.org/10.13057/biodiv/d270441*. Cowpea Mild Mottle Virus (CPMMV) is one of the main pathogens infecting soybean (*Glycine max*), characterized by mosaic symptoms on young leaves. The host range of CPMMV in Indonesia remains unclear, and there have been no reports of it in Bali soybean plants. This research aims to analyze the CPMMV spread in soybean plants in Bali and to test the CPMMV host range on several plants from Fabaceae, Solanaceae, Amaranthaceae, and Chenopodiaceae. This study was carried out by surveying and sampling symptomatic soybean plants from nine regencies in Bali. Samples obtained from this study were 270 symptomatic soybeans. The samples were then used for CPMMV detection via RT-PCR, CP gene cloning, sequencing analysis, and host range test on 11 test species. The CPMMV infection in soybean resulted in mosaic symptoms, leaf malformations, and stunting. The percentage of symptomatic soybean samples testing positive for CPMMV in nine regencies in Bali ranged from 23.08% to 92.37%. DNA cloning was successfully conducted, as indicated by the ±958 bp insert gene in the plasmid recombinant. The CPMMV isolate from Indonesia has the closest homology with Taiwan and Chinese isolates (93.38-95.71%). Of the 11 test plants, *Chenopodium giganteum* and *Gomphrena globosa* showed local symptoms, while the other nine showed systemic symptoms. After reverse inoculation on soybean, all test plants exhibiting systemic symptoms were suitable as sources of CPMMV inoculum. Therefore, the Bali CPMMV isolate may serve as a host and an inoculum source for soybeans. Thus, these results are necessary for intercropping practices involving soybean cultivation.

Keywords: Bali, Betaflexiviridae, CPMMV, host range, RT-PCR, soybean

INTRODUCTION

Soybean (*Glycine max* L.) is among Indonesia's main food commodities and a superior strategic commodity after rice and corn. In 2019, national soybean production declined by 34.74% from 2018, reaching 650,000 tons (Ministry of Agriculture 2021). Many factors influence soybean productivity. The presence of pests and diseases, which reached 76.97%, is one of the challenges associated with soybean cultivation (Central Bureau of Statistics 2024). A viral infection is one of the plant-disturbing pathogens that attack soybeans. Several types of viruses have been reported as the cause of soybean productivity decrease in various countries, among them are Alfalfa Mosaic Virus (AMV), Bean Common Mosaic Virus (BCMV), Bean Yellow Mosaic Virus (BYMV), Blackeye Cowpea Mosaic Virus (BlCMV), Cucumber Mosaic Virus (CMV), Pea Enation Mosaic Virus (PEMV), Peanut Mottle Virus (PeMoV), Soybean Mosaic Virus (SMV), Tobacco Mosaic Virus (TMV), Tobacco Ringspot Virus (TRSV), Tobacco Streak Virus (TSV), Tomato Ringspot Virus (ToRSV), and Tomato Spotted Wilt Virus (TSWV) (Elmore et al. 2022; Deligoz et al. 2025). In Indonesia, several viruses have been reported in soybean plants. They are Soybean Mosaic Virus (SMV), Cucumber Mosaic Virus soybean strain (CMV-S), Pepper Yellow Leaf Curl Virus (PYLCV), and Cowpea Mild Mottle Virus (CPMMV) (Sutrawati et al. 2021).

CPMMV is a member of the genus *Carlavirus* from the Betaflexiviridae family (Kareem et al. 2023). CPMMV is assumed to be seed-borne and spread by an insect vector, but can also spread mechanically. CPMMV is transmitted by whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), in contrast to other Carlavirus members that are spread by aphids (da Silva et al. 2020). Transmission from one plant to another via whitefly takes only a few seconds. In soybeans, whiteflies are recognized as a major pest. Therefore, this insect vector has significant potential to rapidly transmit CPMMV from one infected plant to other healthy plants.

The losses from CPMMV infection have been reported in several countries. CPMMV has been reported to cause serious problems to cowpea in Sub-Saharan Africa (Horn and Shimelis 2020). Zongo et al. (2018) reported CPMMV infection in bambara peanuts (*Vigna subterranea* L.), which reduced the width index of CPMMV-infected plant leaves

by 70% compared to non-CPMMV-infected plants; CPMMV infection also reduced pod and bean numbers and bean weight. The CPMMV infection in Indonesian soybean resulted in a decrease in soybean crop residue dry weight ranging from 15.5-5.4%, a decrease in soybean weight ranging from 11.5-51.6%, and a decrease in bean quality, in the form of abnormal beans, ranging from 7.6-54.35% (Sutrawati et al. 2020).

Researchers from various countries have reported a range of properties among CPMMV isolates, including sequence variations, host-range differences, and diverse CPMMV infection symptoms across hosts. This virus is reported to infect *Solanum melongena* L. (Solanaceae) in Jordan (Mansour et al. 2008). However, the CPMMV isolate is not the same as the CPMMV isolate that infects Fabaceae (Mansour et al. 2008). Brito et al. (2012) reported that CPMMV infects long beans with a disease incidence of 15-40% in Venezuela. Natural CPMMV infection in long beans and green beans is also reported in Taiwan (Chang et al. 2013). This means CPMMV infection continues to spread across hosts and countries.

In Indonesia, CPMMV is one of the viruses causing endemic disease in Java and Sumatra. Also in Bali, the CPMMV infection has not been reported before. Furthermore, no host range has been reported for CPMMV, nor has natural infection in plants other than soybean (Sutrawati et al. 2021). Intercropping with other vegetables, such as Solanaceae, is a common practice in secondary crop cultivation. It is currently unclear whether the CPMMV in Indonesian soybeans can infect Solanaceae, and whether Solanaceae could serve as a source of CPMMV inoculum for soybean plants. Thus, this study aims to determine the field symptomatology, disease incidence, and molecular characteristics of the CPMMV Bali isolate in soybean using CP gene homology and phylogeny, and to identify its host range. This information is valuable in determining appropriate CPMMV control strategies.

MATERIALS AND METHODS

Survey and sampling

The survey and sampling were conducted in nine districts of Bali, Indonesia: Badung, Gianyar, Bangli, Karangasem, Jembrana, Tabanan, Buleleng, Klungkung, and Denpasar City, from May to September 2025. Thirty soybean samples with mosaic symptoms were collected from each district. The total number of detected samples was 270. The soybean variety used in this research was based on the soybean cultivated during sampling. The soybean varieties in each district were listed in Table 1.

Detection and identification of CPMMV by RT-PCR

Total RNA extraction was performed using RNeasy Plant Mini Kit (Qiagen, Germany, catalog number: 74904) according to Qiagen protocol. The extracted RNA was synthesized into cDNA by the Reverse Transcription (RT) method. The RT reactant has 10 µL total volume, comprised of: 2 µL total RNA, 1 µL RTbuffer10x, 0.35 µL 50 mM DTT (dithiothreitol), 2 µL 10 mM dNTP (deoxyribonucleotide triphosphate), 0.35 µL MMuLV Rev (200U/µL), 0.35 µL RNase inhibitor (40U/µL), 0.75 µL oligo (dT) (10 µM), and 3.2 µL nuclease-free water. The reactant was used for one RT reaction. RT reaction was performed in an automated thermal cycler (GeneAmp PCR System 9700; PE Applied Biosystems, USA) with the following program: 65°C for 5 min, 42°C for 60 min, and 72°C for 15 min. The cDNA from the RT reaction would serve as a template for the PCR reaction.

The cDNA was amplified using specific primer pairs for the CP gene of CPMMV. The forward primer: 5'-ATTAAGGATCCGAGTTGATTTAAATAAGT-3' and the reverse primer: 5'-ATTAAGAATTCCTTGTGATTGAAATTGCG-3' with the expected amplification product being 958 bp in length. The composition of the PCR reaction comprises 12.5 µL Dream Taq PCR master mix (Thermo Scientific), 1 µL primer forward 10 µM, 1 µL primer reverse 10 µM, 1 µL cDNA, and 9.51 µL H_2O. The cDNA amplification stage was 94°C for 5 min, followed by 35 cycles comprised of denaturation at 94°C for 1 min, primer attachment (annealing) 45°C for 1 min, and DNA synthesis (extension) 72°C for 2 min, and finally extension at 72°C for 10 min, and the cycle ended at 4°C. Amplicon checking was performed by electrophoresis on 2% agarose in 0.5X TBE buffer for 28 min at 100 V. CPMMV isolates showing clear and thick ±958 bp DNA bands were then used for the CP gene cloning stage.

DNA cloning

One CPMMV isolate per regency that showed severe disease symptoms, or 9 isolates in total, were cloned. The CP-CPMMV fragment was ligated into the plasmid vector pTZ57R/T. Ligation reaction (volume 10 µL) comprised of 3 µL H_2O, 3 µL PCR product, 2 µL buffer T4 DNA ligase 2x, and 1 µL (3 U/µL) T4 DNA ligase, and 1 µL vector plasmid pTZ57R/T 55ng/µL. The mixture was incubated at 4°C for 16 hours. The transformation was conducted by mixing 2 µL of the ligation product with 50 µL of DH5α competent cells in a 1.5 mL Eppendorf tube (stored on ice) for 20 min. The stage was followed by heat shock at 45°C for 60 s in the water bath so that the plasmid can be inserted into the bacterial cell and then cooled on ice for 2 min into an Eppendorf tube, 500 µL of C-Medium (liquid) was added, and the mixture was incubated and shaken at 200 rpm at 37°C for 60 min, and then centrifuged at 4000 rpm for 3 min at 4°C. The resulting supernatant was discarded, while the pellet and C-medium were left to 100 µL and homogenized by tapping them using fingertips. The suspension was then spread onto LB agar containing 100 µg/mL ampicillin and incubated overnight (16 hours) at 37°C.

Table 1. Symptoms and percentage of positive samples from symptomatic plants of CPMMV in soybeans in Bali based on RT-PCR

Location (Village, District)	Variety	Symptoms	Percentage of soybean samples positive for CPMMV
Dawan, Klungkung	Detam2	Mosaic, yellowing	87.61%
Mendoyo, Jembrana	Wilis	Mosaic, leaf malformation, dwarf	92.37%
Tanguwisia, Buleleng	Wilis	Mosaic, yellowing	83.90%
Buduk, Badung	Devon2	Weak mosaic	45.12%
Ketewel, Gianyar	Dena1	Mosaic	74.53%
Tembuku, Bangli	Anjasmoro	Weak mosaic	34.25%
Baturiti, Tabanan	Detam 3	Mosaic	79.63%
Penatih, Denpasar City	Anjasmoro	Weak mosaic	27.41%
Sidemen, Karangasem	Devon2	Weak mosaic	23.08%

Five to seven colonies from each petri dish were taken from the single colony growing in the LB media containing ampicillin to be confirmed by PCR using the specific primer pairs for CPMMV CP gene. A transformant clone was taken by the microtip and put inside a PCR tube filled with PCR components. The PCR reaction was performed with a total volume of 25 µL, comprised of 8.5 µL H_2O, 12.5 µL premix PCR (Green Taq, Promega), 1 µL forward primer CP CPMMV and 1 µL reverse primer CP CPMMV. Amplification reaction was conducted by Perkin Elmer 480 Thermocycler with reaction stages being 94°C for 5 min, followed by 35 cycles comprised of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 2 min, followed by 72°C for 10 min and ended at 4°C. Amplification in an agarose gel was performed as previously described. One colony was taken from colonies found positive to contain recombinant plasmid and inoculated into 3 mL LB broth. It was then incubated for 16 hours or overnight at 37°C in the incubator. Plasmid purification was performed by GeneJET Plasmid Miniprep Kit (Thermo Scientific).

CPMMV cp gene nucleotide analysis

The plasmid obtained in this study was sequenced using M13 primers by commercial service (First BASE Laboratories, Malaysia), arranged through PT Genetika Science, Jakarta. The M13 primer was designed from the plasmid nucleotide. So, the entire CP gene can be sequenced. The CP gene length is 958 bp. The sequences were then aligned with other CPMMV nucleotide sequences in GenBank. The analysis for the CPMMV CP gene genetic variety was done by Bio Edit version 7.05 (http://mbio.ncsu.edu/BioEdit/bioedit.html). The CP-CPMMV homology analysis uses information from CPMMV isolate sequences from various countries in GenBank. Homology analysis was conducted by the ClustalW software (www.ebi.ac.uk). The CPMMV phylogenetic tree was constructed using the ClustalX and BioEdit programs (version 7.05) and the MEGA 11 program with the neighbor-joining algorithm and 1000 bootstrap replications (Tamura et al. 2021). This analysis is based on nucleotide sequences. The nucleotide substitution model used in this study is K2P, with gaps treated as complete deletions. Sequences that were outside the ingroup but closely related to the study group were chosen as the outgroup (Jamil et al. 2019). In this study, isolate sequences belonging to the same genus as CPMMV and located close to Indonesia were selected as outgroup sequences.

CPMMV inoculum purification and propagation

The CPMMV isolate used in this research is ASW 13, originating from Klungkung Regency. The CPMMV-infected soybean leaf is ground in phosphate buffer 0.1 M pH 7.0 (1:5 b/v) to obtain diseased plant extract or sap. The sap was brushed onto the primary leaves of soybean plants that had been treated with 600-mesh carborundum. The leaf surface was then washed with running water to remove residual carborundum. Inoculated plants were kept in insect-proof cages. CPMMV single infection was confirmed by RT-PCR using CPMMV-specific primer, which shows the characteristic symptoms of systemic mosaic and leaf deformation.

CPMMV biological test with host range

The CPMMV host range test was performed on 11 plant species from the family Fabaceae (*Vigna unguiculata* subsp. *sesquipedalis*, *Vigna radiata*, *Phaseolus vulgaris*, *Arachis hypogaea*, and *Glycine max*, Solanaceae (*Nicotiana tabacum*, *Nicotiana benthamiana*, *Solanum lycopersicum*, and *Solanum melongena*), Chenopodiaceae (*Chenopodium giganteum*, and Amaranthaceae (*Gomphrena globosa*). The test was performed in a greenhouse with 11 test plants and 20 repetitions per plant. This host range test was inoculated with the ASW 13 isolate because it causes severe disease symptoms. CPMMV inoculation was performed mechanically as previously explained. Control plants are plants inoculated only with a phosphate buffer. Symptom type and incubation period were observed. CPMMV was confirmed by the RT-PCR method in plants exhibiting symptoms. CPMMV reverse inoculation to soybean was done to confirm the CPMMV infection in the test plant. Test plants showing systemic symptoms and positive for CPMMV are used as an inoculum source to infect healthy soybean plants through mechanical transmission, just as previously mentioned. The disease symptoms and duration period were observed. 30 days after inoculation, the plant showing CPMMV infection-like symptoms would be confirmed by RT-PCR.

RESULTS AND DISCUSSION

Symptoms of soybean plants infected with CPMMV

CPMMV infections generally cause similar symptoms, including mosaic-like patterns in young leaves. A mosaic pattern was observed in all samples from the nine regencies (Table 1). Leaf malformation, stunting, and mosaic symptoms were found in Jembrana soybean leaves (Figure 1.D and Figure 1.E). Furthermore, mosaic and yellowing symptoms were observed in Klungkung and Buleleng (Figure 1.B and Figure 1.C), whereas other regions only showed mosaic symptoms (Figure 1.A). The viral infection of CPMMV in soybean leaves from all regencies was confirmed by RT-PCR using specific primer CP CPMMV. RT-PCR with specific primers CPF/CPR detected a DNA band specific for CPMMV (data not shown). Based on the RT-PCR results, the percentage of symptomatic soybean testing positive for CPMMV in nine regencies is between 23.08-92.37% (Table 1). The lowest CPMMV infection rate is in Sidemen, Karangasem, while the highest is in Mendoyo, Jembrana. This result shows that virus infection symptoms in soybeans in the field vary a lot. This is the first report of natural CPMMV infection in soybean in Bali, and it was detected in nine regencies in Bali, which are Badung, Gianyar, Bangli, Karangasem, Jembrana, Tabanan, Buleleng, Denpasar City, and Klungkung. Previously, CPMMV was reported to cause damage in soybeans in Bantul, Musi Banyuasin, Cirebon, Kendari and Cianjur (Sutrawati et al. 2020). Soybean leaf samples from Badung, Bangli, Denpasar City, and Karangasem show weak mosaic symptoms, and the detection with RT-PCR shows a positive result for CPMMV.

Homology and phylogenetic analysis

The cDNA fragment for the CP CMMV gene was ligated into the vector plasmid pTZ57R/T and subsequently transformed into DH5α competent cells. This is demonstrated by the growth of the DH5α colony after being transformed by the recombinant vector pTZ57R/T-CP CPMMV in LB media containing ampicillin. The success of recombinant transformation in DH5α cells is further confirmed by PCR of the bacterial colony. The bacterial colony growing in LB media (data not shown) was taken for colony PCR. The colony PCR with primer CP CPMMV yielded a DNA band of ±958 bp, corresponding to the target gene length (Figure 2). This indicates that the bacterial colony is positive for carrying the recombinant with CPMMV inserted. The purification result showed the plasmid to be recombinant, with a size of ±3846 bp, consisting of a plasmid vector of 2886 bp and an insert gene CP CPMMV of ±958 bp. The observed size of plasmid recombinant variations aligns with the movement of supercoiled plasmid DNA.

The recombinant plasmid containing the CPMMV CP gene was sequenced. The sequences were then analyzed by comparing the study's sequences with GenBank reference sequences. Homology and phylogenetic analysis were conducted. Based on homology analysis of the CP gene sequence from nine CPMMV isolates against CPMMV sequences from various countries registered in GenBank, the CPMMV isolate from several regions of Bali shows similarities with isolates from other countries. The isolates from the nine regions in Bali show homology with other isolates ranging from 98.76% to 99.98% (Table 2). CPMMV isolates in Bali have the highest nucleotide homology with an isolate from Bantul, Yogyakarta, ranging from 98.71-98.82%. The nucleotide sequence similarities between isolates in Indonesia with Taiwan and China isolates range from 93.38-95.71%, higher than similarities with isolates from India, Brazil, and the US; while the lowest homology is with the Puerto Rico isolate. Based on homology analysis, CPMMV isolates from Indonesia have nucleotide homology of more than 72%. According to CPMMV demarcation criteria, an isolate is categorized as a single species if nucleotide homology exceeds 72% or amino acid homology exceeds 80% (King et al. 2011). Thus, this results confirming that nine isolates from Bali are one species with the CPMMV in Asia, America, and Africa. The phylogenetic analysis shows that the CPMMV isolates form three groups, which are group I (isolates from Indonesia and Taiwan), group II (isolates from China and India), and group III (isolates from Brazil, USA, and Puerto Rico) (Figure 3).

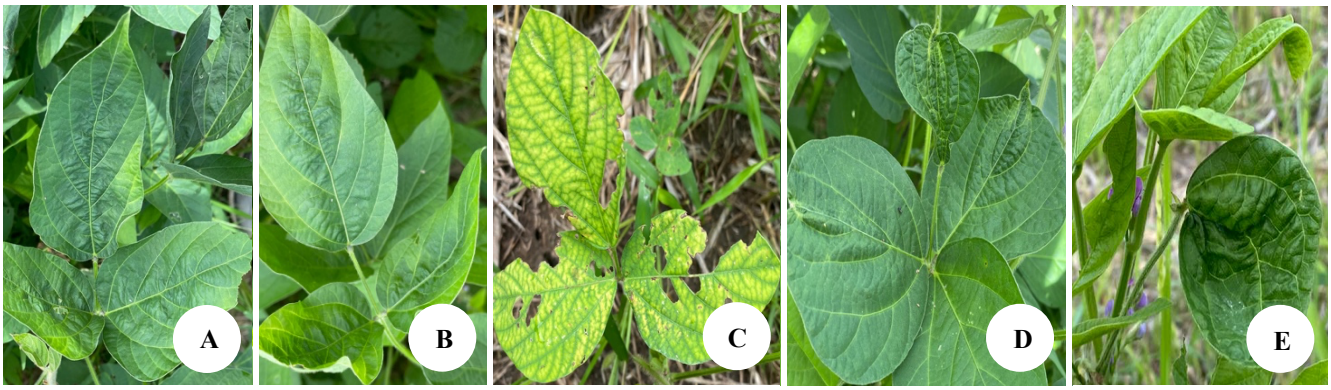

Figure 1. Symptoms of soybean plants infected with CPMMV found in nine regencies in Bali. A. Mottled, B. Mottled with yellow, C. Yellow, D. Mottled with leaf malformation, E. Dwarf. Mottled with leaf malformation were observed in Jembrana, mottled with yellow and yellow leaf were observed in Klungkung and Buleleng, while mottled observed in other regencies

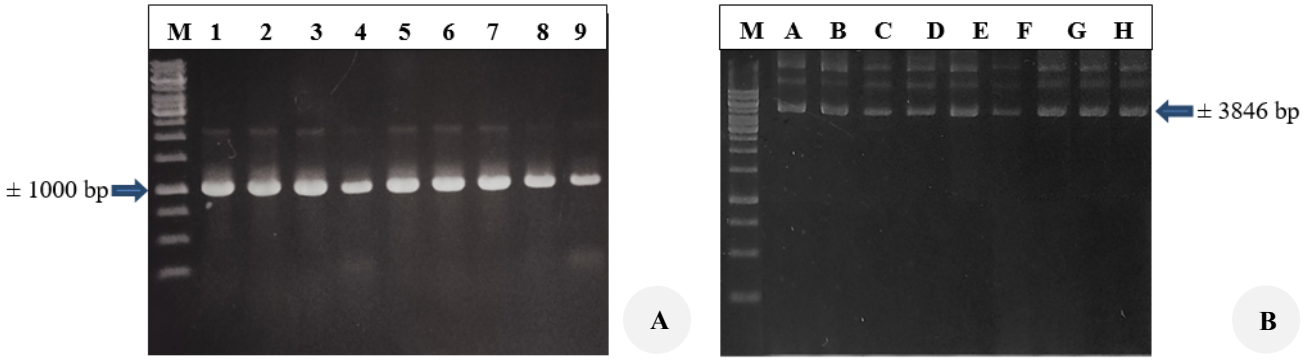

Figure 2. Visualization of CPMMV CP gene DNA amplification. A. Lanes 1-9 from colony PCR, separation of CPMMV CP gene DNA bands and PTZ57R/T vector after amplification using M13F (-20)/M13R (-26) pUC primers B. Lanes A-I, M. 1 kb DNA marker (Thermo Scientific)

Table 2. Homology nucleotide of CPMMV Bali isolate on soybean plants based on different locations against isolates from other countries available on GenBank

Isolate source	Isolate	Homology of nucleotide (%)									Accession number
		1	2	3	4	5	6	7	8	9	
IDN	Jembrana_Bali		99.98	99.94	99.90	98.89	99.87	99.85	98.85	98.83	PZ172663
IDN	Buleleng_Bali	99.98		99.82	98.84	98.88	98.86	98.83	98.78	98.76	PZ172664
IDN	Tabanan_Bali	99.94	99.82		98.81	99.63	99.40	98.83	98.82	98.77	PZ172665
IDN	Klungkung_Bali	99.90	98.84	98.81		99.84	99.53	98.87	98.84	98.87	PZ172666
IDN	Karangasem_Bali	98.89	98.88	99.63	99.84		98.93	98.85	98.85	98.83	PZ172667
IDN	Bangli_Bali	99.87	98.86	99.40	99.53	98.93		98.84	98.84	98.83	PZ172668
IDN	Badung_Bali	99.85	98.83	98.83	98.87	98.85	98.84		99.97	98.91	PZ172669
IDN	Gianyar_Bali	98.85	98.78	98.82	98.84	98.85	98.84	99.97		99.75	PZ172670
IDN	Denpasar City_Bali	98.83	98.76	98.77	98.87	98.83	98.83	98.91	99.75		PZ172671
IDN	Bantul_Yogyakarta	98.71	98.73	98.74	98.76	98.77	98.76	98.82	98.72	98.82	LC382366
IDN	Cirebon_West Java	98.69	98.64	98.71	98.73	98.76	98.74	98.79	98.69	98.69	LC382367
IDN	Cianjur_West Java	98.62	98.59	98.70	98.71	98.72	98.73	98.80	98.62	98.62	LC382370
IDN	Musi Banyuasin_South Sumatera	98.52	98.50	98.63	98.65	98.62	98.70	98.73	98.52	98.56	LC382369
TAI	Taiwan	94.20	94.19	95.71	94.84	94.63	94.95	94.95	94.20	94.27	JX020701
CHN	China	94.04	94.08	95.14	94.73	94.61	94.95	94.94	94.04	94.19	PP790740
CHN	China	93.82	93.81	94.65	94.64	93.57	93.93	94.82	93.82	93.80	PP790739
CHN	China	93.81	93.76	93.89	94.40	93.39	93.74	94.81	93.81	93.79	MW354945
CHN	China	93.65	93.62	93.75	94.48	93.38	93.83	94.63	93.65	93.49	OR667247
IND	India	93.03	93.08	93.17	93.47	93.28	93.82	94.08	93.03	93.26	OQ791180
IND	India	90.10	90.82	91.32	93.49	92.47	91.39	90.19	90.10	90.85	KJ534277
BRA	Brazil	88.82	88.87	89.63	89.38	89.42	88.94	88.95	88.82	88.82	KF554105
BRA	Brazil	88.81	88.86	89.37	89.32	89.29	88.94	88.95	88.81	88.84	MW656833
USA	Florida	88.81	88.85	88.93	88.73	89.29	88.94	88.94	88.81	88.84	KC774019
USA	Florida	88.66	88.73	88.84	88.84	89.04	88.82	88.89	88.66	88.66	KC774020
PRI	Puerto Rico	88.64	88.68	88.75	88.84	8894	88.75	88.86	88.64	88.61	GU191840
CHN	CarMV_China*	43.79	44.03	44.26	43.47	45.69	43.94	46.85	43.76	44.55	AF173879

Note: 1. Isolate from Jembrana, 2. Buleleng, 3. Tabanan, 4. Klungkung, 5. Karangasem, 6. Bangli, 7. Badung, 8. Gianyar, 9. Denpasar City. IDN: Indonesia, *CarMV from China is used as an out group

Host range test of CPMMV infection

In order to obtain the host range of CPMMV infection, host range tests were performed for Solanaceae, Fabaceae, Chenopodiaceae and Amaranthaceae families. The CPMMV infection in test plants from Fabaceae and Solanaceae shows systemic mosaic, leaf vein chlorosis, and leaf malformation; while in Chenopodiaceae and Amaranthaceae it causes local chlorotic spots. The incubation period in Fabaceae generally lasted 6-9 days post-inoculation (dpi), with early symptoms including leaf chlorosis and mosaic on the trifoliate leaf (Table 3). *V. unguiculata* differs by having a longer incubation period of 19 days.

After inoculation, clearer symptoms appearing are systemic mosaic, small leaf size, and leaf malformation. The leaf inoculated by CPMMV is a fully opened single leaf, while the infection symptom appeared in young leaves growing after the single leaf. This means that CPMMV infection in Fabaceae is systemic. Infection symptoms in *P. vulgaris* other than leaf mosaic include chlorosis in the leaf vein, making the leaf vein appear white (Table 3). CPMMV inoculation showed necrotic spots in *C. giganteum* and *G.*

globosa, and did not show systemic symptoms. Mechanical CPMMV inoculation in *N. tabacum* and *N. benthamiana* did not show local symptoms and caused systemic infection in the form of mosaic and leaf vein chlorosis. CPMMV causes systemic mosaic and leaf malformation in *S. lycopersicum* and *S. melongena*. This test shows that the CPMMV isolate isolated from soybean can infect Fabaceae and Solanaceae and causes systemic symptoms, as well as can infect Chenopodiaceae and Amaranthaceae and causes local symptoms on the inoculated leaf without causing systemic symptoms.

The host range test were confirmed by RT-PCR. Detection by RT-PCR shows that all plants with systemic mosaic and chlorosis are positive for CPMMV-specific primers, except *A. hypogaea* (Table 3). The chlorosis symptom in *A. hypogaea* is caused by injuries during inoculation; consequently, the RT-PCR result was negative. This shows that the symptoms in the test plants were caused by CPMMV infection. The Fabaceae and Solanaceae showing systemic symptoms in this test were then used as inoculum source to inoculate healthy soybeans. This test is conducted to determine the potential for cross-infection from the test plants to soybeans. The reverse inoculation from indicator plants showing systemic symptoms and positive for CPMMV successfully transmitted CPMMV into healthy soybean (Table 4). The CPMMV transmission to soybean has a 10-80% disease incidence with a 4-7-day incubation period. Thus, the seven plant species can become CPMMV host as well as CPMMV inoculum source for soybean.

According to the results above, the CPMMV were detected in soybean samples from nine regencies. The symptoms of viral infection depend on the variety of soybean planted, the cultivation method, and the virus strain in the region. Generally, CPMMV infection symptoms are often mixed with those of other viral infections, including striped leaf blade chlorosis, leaf distortion, vein banding, and plant stunting. Additionally, a single CPMMV infection showed the characteristic symptoms of CPMMV, which are mosaic, chlorosis, vein clearing, and leaf distortion (Andayanie et al. 2020; Wei et al. 2021; Luan et al. 2024). Leaves with mosaic and striped symptoms have dark green areas (green islands) and bright green or yellow (chlorotic) areas on the leaf tissue. The bright green or chlorosis area containing a lot of virus directly borders the dark green area free of virus (green islands). The mosaic pattern arises because the dark green and chlorotic areas result from virus-infected and uninfected cells (Listihani et al. 2019, 2020, 2022a, 2022b; Selangga and Listihani 2022; Selangga et al. 2022). Chlorosis symptoms in leaves infected by the virus is related to chlorophyll damage. When some viral sheath proteins enter the chloroplast, they bind to the thylakoid membrane and disrupt Photosystem II (PS II). A disturbance in PS II will induce an increase in Reactive Oxygen Species (ROS) production in the chloroplast, which causes damage to chlorophyll and other pigments (Li et al. 2025). Leaf vein blanching symptoms in the form of chlorosis around the leaf vein are not caused by chlorophyll damage but more due to the change in the cell when the infected cell expands (becoming transparent) more than the surrounding uninfected cells (Zheng et al. 2025).

This research shows that the CPMMV variety not only shows in the virulence and the type of symptoms shown, but also in the variety at the molecular level. Based on the nucleotide homology analysis, the CPMMV isolates obtained from Indonesia in this research are related to isolates from Asia (China, Taiwan). The close relationship with Asian isolates suggests shared ancestry and/or historical exchange of CPMMV between Indonesia and other Asian countries. However, the CPMMV from the various regions appears to show genetic variation in its nucleotide sequences. Genetic variety in a virus may occur in two ways: mutation and recombination.

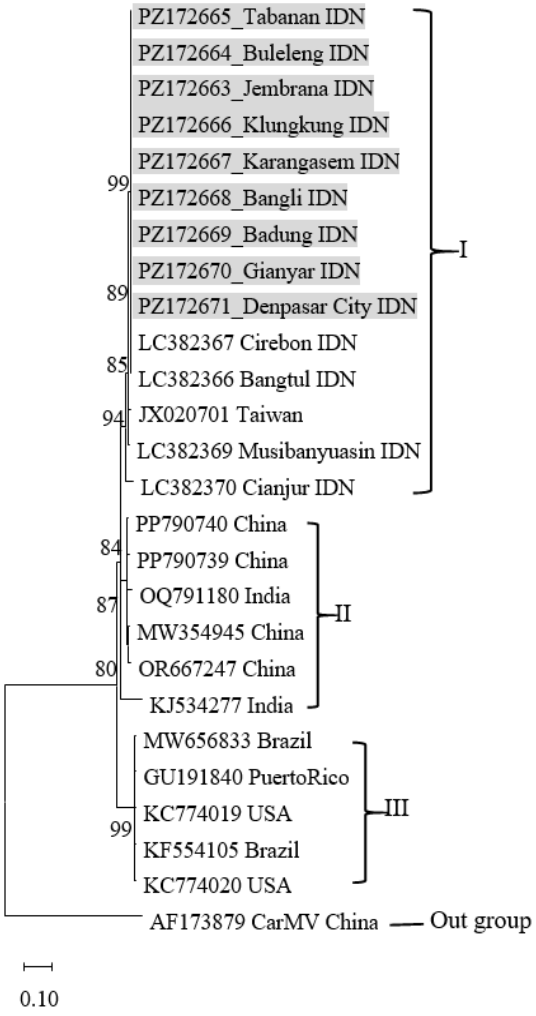

Figure 3. Phylogenetic tree based on the CP gene from CPMMV Bali isolates. Isolates marked with gray highlight are Bali isolates. Bootstraps values greater than 70% based on 1000 replicates are shown on tree branches

Table 3. Symptoms, number of symptomatic plants, incubation period, and number of plants infected with CPMMV isolate ASW 13 on several indicator plants

Indicator plants	Symptoms	Number of symptomatic plants (total number of plants)	Incubation period (IPR)	Number of samples infected with CPMMV*
Vigna unguiculata	Systemic mosaic, leaf malformation	19 (20)	19	17
Vigna radiata	Leaf vein chlorosis	6 (20)	8	6
Arachis hypogaea	Leaf vein chlorosis	6 (20)	9	0
Phaseolus vulgaris	Systemic mosaic, leaf vein chlorosis	11 (20)	6	11
Glycine max	Systemic mosaic, leaf malformation	20 (20)	6	20
Chenopodium giganteum	Local lesions	8 (20)	10	8
Gomphrena globosa	Local lesions	9 (20)	8	9
Nicotiana tabacum	Systemic mosaic	16 (20)	7	16
Nicotiana benthamiana	Leaf vein chlorosis	14 (20)	15	14
Solanum melongena	Systemic mosaic	15 (20)	8	15
Solanum lycopersicum	Systemic mosaic, leaf malformation	18 (20)	7	18

Note: *Confirmation of CPMMV infection was carried out using the RT-PCR method

Table 4. Symptoms, number of symptomatic plants, incubation period, and number of infected plants in CPMMV reverse inoculation from indicator plants to soybean plants

Indicator plants as a source of inoculum	Symptoms in soybeans	Number of symptomatic plants	Incubation period (IPR)	Number of samples infected with CPMMV*
Vigna unguiculata	Systemic mosaic, leaf malformation	7 (20)	4	7
Vigna radiata	Leaf vein chlorosis	2 (20)	7	2
Phaseolus vulgaris	Systemic mosaic	9 (20)	6	9
Glycine max	Systemic mosaic, leaf malformation	16 (20)	6	16
Nicotiana tabacum	Systemic mosaic	8 (20)	6	8
Solanum melongena	Systemic mosaic	11 (20)	6	11
Solanum lycopersicum	Systemic mosaic	6 (20)	6	6

Note: *Confirmation of CPMMV infection was carried out using the RT-PCR method

The high mutation rate in RNA viruses indicates a type of evolutionary strategy (Butković and González 2022; Selangga et al. 2023). According to LaTourrette and Garcia-Ruiz (2022), virus evolution occurs to adapt to environments, such as the host plant, virus strain, and insect vector. The different environmental conditions between regions in Indonesia and the climate differences between Indonesia and other countries may also contribute to different environmental constraints, causing virus genetic changes. Virus mutation may also occur because viruses are pathogenic and can readily undergo genetic recombination during replication in host cells. Recombination between the genomes of RNA virus species is one of the causes of the biological variety in plant viruses (Wang et al. 2022). Moreover, recombination may occur when two virus strains infect the same plant. This results in a new virus strain (a recombinant) that may exhibit different characteristics from the two strains (Sánchez-Tovar et al. 2025).

Several studies across various countries reported that CPMMV is seed-borne in soybeans. The widespread occurrence of CPMMV in various regions in Indonesia is assumed to be related to the distribution of soybean seeds. Most farmers use seeds from the previous harvest or buy from other soybean farmers in other regions from their previous planting season. This way of fulfilling the soybean seeds is known as the inter-region and inter-season seeds traffic system. Thus, there was no information on the quality and health of the seeds used, increasing the risk of virus spread during seed distribution between soybean-producing regions. The distribution of CPMMV via seed transmission was reported by Bhagwatkar et al. (2025). This study reported that the seedling transmission efficiency of CPMMV in soybeans are 75% in F1 and 100% in F2, indicating that CPMMV is transmissible in soybeans. Additionally, antioxidant enzymes such as CAT, POX, SOD, and PAL were increased as a defense response triggered by CPMMV. Seed quality, such as seed weight, germination percentage, fresh weight and seedling vigor indices I and II, decreased in soybean infected with CPMMV via vertical seed transmission. These results showed that seed transmission plays a key role in CPMMV transmission.

CPMMV isolate ASW 13 used in the host range test can cause systemic mosaic symptoms in Fabaceae and Solanaceae plants. Mansour et al. (2008) reported that CPMMV isolated from tomatoes in Israel differs from two isolates from Fabaceae based on host-range test, serological properties, and virion aggregates. Chang et al. (2013) tested the host range of CPMMV, and the result shows that the CPMMV Taiwan isolate can infect Fabaceae, but is unable to infect Cucurbitaceae and Solanaceae. The Chang study shows that the CPMMV isolate ASW 13 in this research differs from the CPMMV isolate reported by Yadav et al. (2013), as the CPMMV isolate from soybean in this research can infect both Solanaceae and Fabaceae. Different

CPMMV infection symptoms of the CPMMV isolate ASW 13 occur in other tested hosts. The CPMMV isolate ASW 13 infection in long bean (*V. unguiculata*) shows systemic mosaic, leaf vein blanching, wrinkling leaf lamina, and leaf malformation; while Yadav et al. (2013) in India reported that CPMMV in long bean shows chlorotic lesion symptoms developing into necrotic spot with weak mosaic and curling leaves. The green bean (*P. vulgaris*) response to the CPMMV isolate ASW 13 shows the same symptoms as reported by Yadav et al. (2013), including mosaic, leaf vein chlorosis, and curling leaves. The CPMMV isolate India infection in *N. tabacum* does not show symptoms, whereas the CPMMV isolate ASW 13 shows mosaic, leaf vein blanching and leaf malformation. The different responses from test plants against viral infection can be used as the basis to differentiate a virus strain from the others. Host response differences against the CPMMV isolate ASW 13 compared with other CPMMV isolates previously tested in other countries by Yadav et al. (2013) are assumed to be related to different virus strains, test plant varieties, and different environmental conditions. Symptom expression in the host plant is the result of a specific interaction between the virus and the host and is influenced by environmental factors.

During the CPMMV ASW 13 test, long bean symptoms did not begin with chlorosis and local necrosis in the inoculated leaf, but systemic mosaic. The CPMMV isolate from India in long beans caused chlorotic lesions that progressed to necrosis on the inoculated leaf, then the symptoms developed into weak mosaic and curling leaves. Necrotic symptoms in leaf inoculated by virus indicated virus localization in the area of the lesion, and the further development into systemic mosaic indicates that the virus has spread beyond the lesion's border (Listihani et al. 2019).

The CPMMV reverse inoculation test in indicator plants (Fabaceae and Solanaceae plants) back to soybeans can cause symptoms on the inoculated plants, and is proven to infect CPMMV based on the molecular CPMMV detection method. It shows the potential of Fabaceae and Solanaceae plants as inoculum sources for CPMMV in soybeans. A CPMMV isolate from soybean can also serve as inoculum for other Fabaceae and Solanaceae plants. CPMMV isolate ASW 13 has a different host range compared to previously reported CPMMV isolates: the isolates from Taiwan and India. The host range of CPMMV ASW 13 is quite wide and may become a source of infection for soybean. Thus, it is suggested that soybeans should not be planted with Fabaceae or Solanaceae, as they may transmit CPMMV to each other.

According to the results, this study provides information on CPMMV infection in symptomatic soybean leaf samples from nine regencies. CPMMV infections were determined only from soybean leaf samples (30 samples per regency) and carried out by RT-PCR using CPF/CPR primers. Regarding the possibility of mixed viral infection, further examination is necessary to differentiate CPMMV infection from other viruses. Additionally, the host-range test suggest that CPMMV could infect Fabaceae and Solanaceae plants. Thus, field host-range test is needed to evaluate its ability to transmit CPMMV and to provide recommendations for intercropping techniques. Furthermore, the study can also assess whitefly and seed transmission to determine the mechanisms and their effects on soybean productivity.

In conclusion, CPMMV has spread and infected soybean plants in nine regencies in Bali, with CPMMV-positive sample rates ranging from 23.08% to 92.37%. Based on the disease incidence, the Anjasmoro and Devon2 varieties have the lowest incidence of CPMMV infection. The homology analysis showed that CPMMV isolates from Bali have the highest homology, ranging from 98.71% to 98.92%, with CPMMV from Bantul and Yogyakarta. Also have the closest relationship with the CPMMV isolates from Taiwan and China, with 93.38-95.71% similarity. Asw 13 isolate obtained from this research can infect hosts in Fabaceae and Solanaceae and cause systemic infection, while causing local lesions in Chenopodiaceae and Amaranthaceae. Further field studies on the infection of natural CPMMV in Fabaceae and Solanaceae are necessary, followed by in-depth studies on whitefly and seed transmission to fully identify CPMMV in soybean.

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