



Analysis of 3'-Terminal Region and Mechanical Transmission of Yambean Mosaic Virus Isolates Infecting *Clitoria ternatea* in Indonesia

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Abstract: Among potyviruses infecting legumes, yambean mosaic virus (YBMV, *Potyvirus pachyrhizus*) is still less studied. A recent finding in Thailand expanded the virus host range to include butterfly pea (*Clitoria ternatea*). Leaves of butterfly pea plants in several spots in the Special Region of Yogyakarta, Indonesia, also exhibited systemic mosaic, chlorosis, and malformation, typical of a viral disease. Four symptomatic leaf samples were collected from Bantul Regency, and another one in Sleman Regency, along with non-symptomatic samples. Samples were tested using by RT-PCR with universal potyvirus primers and specific primers for bean common mosaic virus (BCMV, *Potyvirus phaseovulgaris*) and cucumber mosaic virus (CMV, *Cucumovirus CMV*), respectively. Results of RT-PCR and BLAST analyses of the obtained DNA sequences indicated that the five symptomatic samples were infected by with YBMV. Meanwhile, two other widespread legume viruses, BCMV and CMV, were not detected. Subsequently, the two non-symptomatic samples tested negative for the three viruses. The five new Indonesian isolates (acc. nos. PX398571-PX398575) were grouped closer to a butterfly pea isolate from Thailand (MJU-L1, PQ238432) than to another Indonesian isolate from yam bean (*Pachyrhizus erosus*) (Bogor, AB289438) in the constructed phylogenetic trees. The isolate from Sleman (SMN-00) was mechanically transmitted to yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*), but not to cucumber (*Cucumis sativus*) and chili pepper (*Capsicum annuum*). This study reported the first cases of YBMV in butterfly pea in Indonesia, while also enriching our understanding of the genetic diversity of the virus.

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1. Introduction

Butterfly pea (*Clitoria ternatea*) belongs to the Fabaceae family. The plant is not only an aesthetic ornamental plant but also an important component in the food, beverage, pharmaceutical, nutraceutical, cosmetic, and animal feed industries in many countries, including Indonesia (Oguis et al., 2019; Adisakwattana et al., 2020; Puspitojati, 2024; Faradilla et al., 2025; Rahayu et al., 2025). Therefore, a recent finding in neighboring Thailand, showing the presence of yambean mosaic virus (YBMV, *Potyvirus pachyrhizus*) in butterfly pea (Jenjai and Samarakoon, 2025), is of particular concern. Additionally, butterfly pea has long been known to be susceptible to infection by clitoria yellow mottle virus (CliYMV, *Tobamovirus clitoriae*) (Wei et al., 2012) and papaya ringspot virus (PRSV, *Potyvirus papayanuli*) (Sultana et al., 2019).

YBMV was described as a distinct virus within the *Potyvirus* genus following the analysis of the complete genome of an isolate from yambean (*Pachyrhizus erosus*) in Peru (Fuentes et al., 2012). The virus infection in yam bean had been reported in Indonesia prior to that, although at the time it was identified as a variant of bean common mosaic virus (BCMV, *Potyvirus phaseovulgaris*) (Damayanti et al., 2008). BCMV itself, together with cucumber mosaic virus (CMV, *Cucumovirus CMV*), remains widespread among fabaceous crops to this day (Melinda et al., 2015; Rahim et al., 2020; Ibrahim et al., 2023; Adak et al., 2026). Besides mechanical transmission, different aphid species help to disseminate YBMV, BCMV, and CMV from plant to plant (Sastry et al., 2019; Khaled-Gasmi et al., 2022; Inoue-Nagata et al., 2022).

Butterfly pea plants with viral symptoms were occasionally spotted at different locations in Indonesia. The potential losses resulting from viral disease on this commodity could directly impact the sustainability of agricultural businesses and the supply chain of the herbal-based industry. Therefore, a deeper understanding of the causal virus is crucial to protect the productivity and economic value of the butterfly pea in the country (Çelik et al., 2023).

2. Materials and Methods

2.1. Samples collection

Viral symptomatic as well as non-symptomatic leaf samples of butterfly pea were purposively collected from different locations within the Special Region of Yogyakarta, Indonesia. The leaf samples were then brought to the Plant Pathology Laboratory, at Universitas Gadjah Mada, and stored at -20° C in a freezer until further tests.

2.2. Virus identification using RT-PCR and sequencing

Total RNAs were extracted from butterfly pea leaves using 'Total RNA Mini Kit (Plant)' (Geneaid Biotech Ltd., Taiwan) following the manufacturer's standard procedures. The isolated RNA was applied in cDNA synthesis using ReverTra Ace kit (Toyobo, Japan), each was in a 10 µL reaction volume consisting of 1 µL dNTP, 0.5 µL (10 pmol µL⁻¹) Oligo(dT) primer, 0.5 µL RNase inhibitor, 0.5 µL ReverTraAce®, 2 µL RNA template, 2 µL 5× RT Buffer, and 3.5 µL nuclease-free water. The program for the reverse transcription was set at 42 °C (20 min), followed by 99 °C (5 min).

The obtained cDNAs were tested in PCRs using a universal primer pair U341-Forward (5'-CCGGAATTCATGRTITGGTGYATIGAIAAYGG-3')/Poty 1-Reverse (5'-GGATCCCGGGTTTTTTTTTTTTTTTTTV-3') to amplify 600 - 800 bp of the 3'-tail of potyviruses (Langeveld et al., 1991). Presence of other common viruses in the samples were also examined using primer pairs D1BCMV-Forward (5'-AAATGTGGTACAATGCTGTGAAGG-3')/RU-Reverse (5'-TCAGTATTCTCGCTGGTTGTTGC-3') amplifying 469 bp of partial coat protein (CP) gene of BCMV (Chiquito-Almanza et al., 2017) and CMV-RNA3-Forward (5'-GTAGACATCTGTGACGCGA-3')/CMV-RNA3-Reverse (5'-GCGCGAAACAAGCTTCTTATC-3') targeting 540 bp of partial RNA3 segment of CMV (de Blas et al., 1994).

Each PCR reaction was performed in a volume of 40 µL containing 4 µL cDNA template, 20 µL MyTaq HS Red Mix (Bioline, Germany), 2 µL (10 pmol µL⁻¹) of each reverse and forward primer, and 12 µL nuclease-free water. The PCR program included an initial denaturation step at 95° C (3 min), 35

cycles of denaturation at 95° C (1 min), annealing according to each primer pair, and elongation at 72° C (1 min), ending with a final elongation step at 72° C (10 min).

PCR products were separated on 1% agarose gel stained with Florosafe DNA Staining (1st BASE, Malaysia) using electrophoresis for 60 min at 50 V. The band was observed under a UV transilluminator (Optima Inc., Japan). Products with a single targeted band were delivered to the Integrated Laboratory for Research and Testing at Universitas Gadjah Mada for bidirectional Sanger sequencing. The obtained sequences were analyzed using the nucleotide BLAST online tool against the NCBI GenBank database (<https://blast.ncbi.nlm.nih.gov>) for species identification. Nucleotide sequences of the isolates obtained in this study were then deposited in NCBI GenBank with unique accession numbers.

2.3. Recombination event

Sequences of isolates obtained in this study and corresponding sequences of the same virus species available in NCBI GenBank were aligned and trimmed using the ClustalW algorithm in MEGA11 (Tamura et al., 2021). Corresponding sequences of the most closely related virus were also aligned as an outgroup. Potential sequence anomalies among the aligned sequences were scanned using the Recombination Detection Program version 5 (RDP v.5.30), which integrates several optional algorithms: RDP, GENECONV, MaxChi, 3Seq, Bootscan, Chimaera, and Siscan (Martin et al., 2021). The recombination event, a critical evolutionary mechanism of viruses, should be validated by at least 5 algorithms with a Bonferroni-corrected P-value < 0.05 to indicate statistical significance (Martin et al., 2021).

2.4. Phylogenetic and percentage identity analyses

Tamura-3 (Tamura, 1992) was determined to be the best parameter model for constructing phylogenetic trees based on the prepared nucleotide alignments using the Maximum Likelihood (ML) method in MEGA11. The statistical significance of the tree branching was estimated with 1000 bootstrap replicates. Percentage identities of the tested isolates at nucleotide (nt) and amino acid (aa) levels were calculated using the Sequence Demarcation Tool software (SDT v1.2) (Muhire et al., 2014).

2.5. Mechanical transmission

Leaves of a butterfly pea plant showing severe symptoms and tested positive for virus infection by RT-PCR were ground in a mortar and pestle to prepare the inoculum. Five ml of potassium phosphate buffer (0.01 M, pH 7.0) was added for each gram of leaf tissue. The homogenate sap was then filtered through a cheesecloth. Leaves of the three tested plant species: yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis* cv. Kanton, Fabaceae), cucumber (*Cucumis sativus* cv. Baresta, Cucurbitaceae), and chili pepper (*Capsicum annuum* cv. Pilar, Solanaceae) were dusted with carborundum (400 mesh). Four plants were used as replicates for each species. Mechanical inoculation was performed by gently rubbing the sap against the dusted leaves. The inoculated plants were rinsed with distilled water ten minutes later, and then maintained in a greenhouse at 25-27 °C. Control plants were mock-inoculated with double distilled water. Symptom expression on the inoculated plants was examined during a period of four to five weeks. The same RT-PCR described above was used to confirm infection in the inoculated plants (Zahra et al., 2024).

3. Results

3.1. Samples collection

Leaf samples from four butterfly pea plants (BTL-7, BTL-9, BTL-20, BTL-24) showing viral-like symptoms were collected in Bantul Regency, Special Region of Yogyakarta, in 2024. These plants were grown as ornamentals in a park. Another butterfly pea plant (SMN-00) with similar symptoms of a viral disease was found in a park in Sleman Regency, Special Region of Yogyakarta, in 2025. One non-symptomatic sample was also collected from each location. In total, seven samples were collected for testing in this study.

3.2. Virus identification using RT-PCR and sequencing

All five symptomatic samples tested positive for potyvirus infection according to RT-PCR using U341/Poty 1 primers. However, they were negative for BCMV and CMV infection using specific primers for the each virus. The two non-symptomatic samples were negative for potyviruses, BCMV, and CMV. BLAST analysis on the partial genome sequences confirmed that all five isolates belong to YBMV. The 3'-terminal region sequences were deposited in NCBI GenBank under accession nos. PX398571 - PX398575. Mosaic and vein banding with different severities were observed on the leaves of YBMV-infected plants (Figure 1).

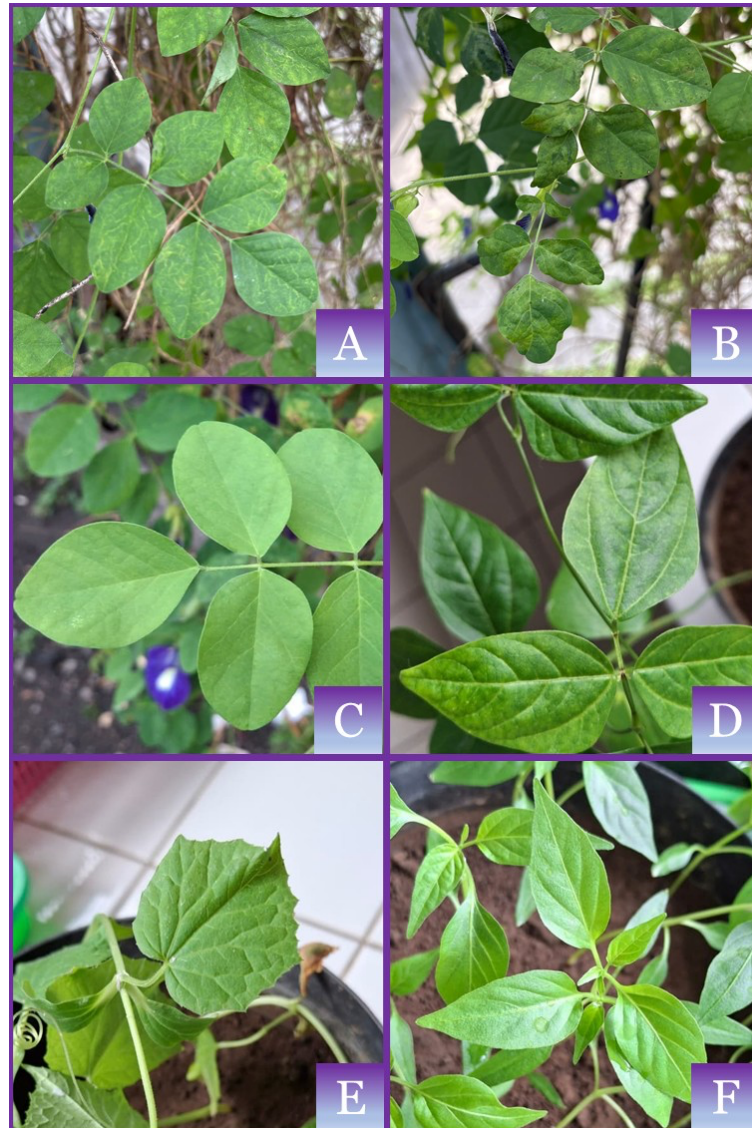


Figure 1. Different symptoms on butterfly pea plants infected by YBMV, and plants mechanically inoculated by YBMV. A. Mild chlorosis on butterfly pea leaves infected by BTL-7 isolate, B. Mosaic and wrinkling on butterfly pea leaves infected by SMN-00 isolate, C. Non-symptomatic butterfly pea plant from Sleman Regency tested negative for YBMV, D. Mild mosaic on leaves of yardlong bean, four weeks post mechanical inoculation using SMN-00 isolate, E. No symptom on leaves of cucumber, four weeks post mechanical inoculation using SMN-00 isolate, F. No symptom on leaves of chili pepper, four weeks post mechanical inoculation using SMN-00 isolate.

3.3. Recombination event

BCMV isolates, which had been shown to have the highest nt identities by BLAST analysis, were aligned with YBMV alignment as out-groups. RDP5 scan on the aligned 14 YBMV and 17 BCMV

isolates detected only a single recombination event on QLD isolate (from Australia, OQ377538) with beginning breakpoint at position 486 and ending breakpoint at 681. The recombinant strain used the SMN-00 isolate (from Indonesia, PX398575) as its major parent and the BL-C isolate (from India, MH744999) as its minor parent. The recombination was validated by RDP (3.816×10^{-3}), GENECONV (1.396×10^{-2}), BootScan (9.436×10^{-3}), MaxChi (2.408×10^{-5}), Chimaera (7.083×10^{-7}), and SiScan (7.209×10^{-11}) algorithms.

3.4. Phylogenetic and percentage identity analyses

According to both phylogenetic trees based on partial CP + 3' UTR (692 bp) and partial CP (435 bp), the five Indonesian YBMV isolates were shown to be closely related to each other, and shared a basal node with MJU-L1 which was isolated in Thailand also from butterfly pea as well as HGR-2, DSMV PV-0784, and BR-6 isolates. Another Indonesian isolate (Bogor) from an infected yam bean plant was clustered in a separate group, together with SR from yam bean in Peru (Figure 2). As expected, the recombinant QLD isolate formed a monophyletic YBMV branch that was genetically closest to BCMV (Figure 2).

SDT analysis on the nt sequences of partial CP + 3' UTR (692 bp) estimated that the five butterfly pea isolates from Indonesia shared 97-100% nt identity among them, and 83-97% with other YBMV isolates. In a partial CP sequence comparison (435 bp), all the compared YBMV isolates maintained 87-100% nt and 96-100% aa identities (Figure 3).

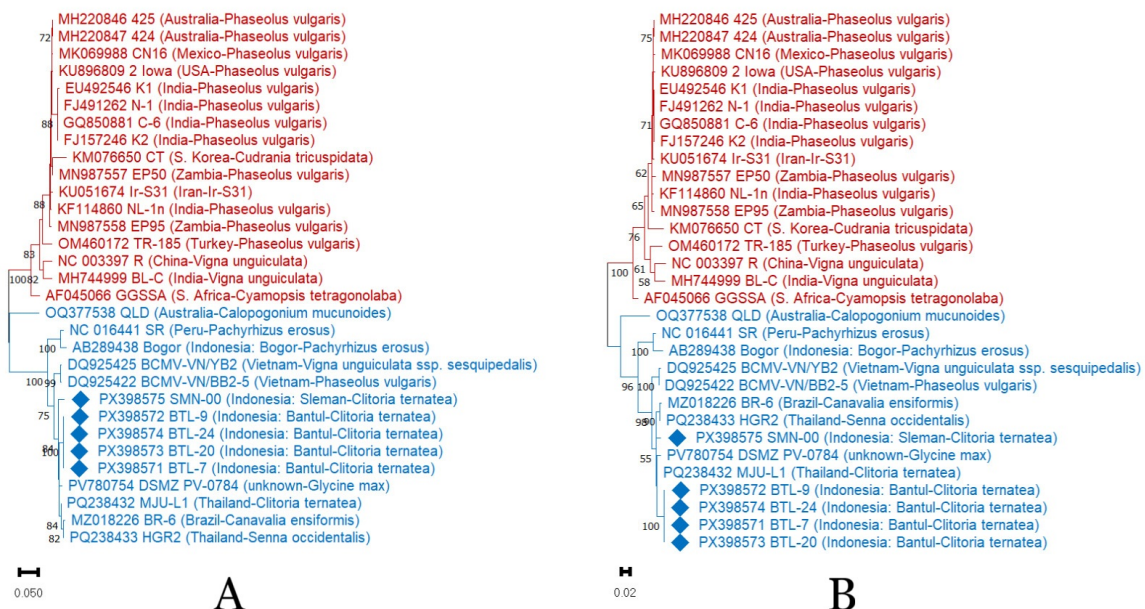
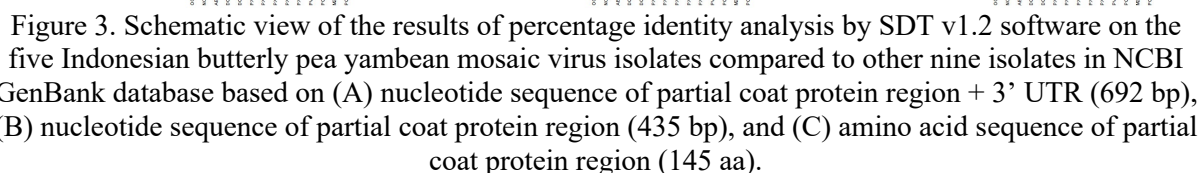


Figure 2. Phylogenetic trees based on nucleotide sequences of (A) partial coat protein region + 3' UTR (692 bp) and (B) partial coat protein region (435 bp) of yambean mosaic virus (YBMV) genome, highlighted in blue. The maximum-likelihood trees were constructed by MEGA11 software using Tamura-3 parameter model with 1000 bootstrap replicates, only values at least 50% were shown. Indonesian butterfly pea isolates reported here were pointed with rhombuses. Seventeen isolates of bean common mosaic virus (BCMV), highlighted in red, were used as out-group.



SMN-00 isolate from Sleman Regency was selected to be used as a source of inoculum for the mechanical inoculation experiment. Four weeks post-inoculation, young leaves of yardlong bean showed mild mosaic, whereas leaves of cucumber and chili pepper did not develop noticeable abnormalities compared to the controls (Figure 1). Furthermore, RT-PCR confirmed YBMV infection only in yardlong bean.

YBMV belongs to the *Potyviridae* family, within the *Potyvirus* genus, the largest genus of plant RNA viruses with more than 200 identified species (Inoue-Nagata et al., 2022). The virion is a flexible, uncoated, rod-shaped filament particle approximately 700 nm long, with a relatively consistent diameter of approximately 11-20 nm (Cascardo et al., 2021). YBMV has a monopartite +ssRNA genome that encodes a single large open reading frame (ORF), which is translated into a single polyprotein with an approximate molecular weight of 350 kDa (Cascardo et al., 2021). The coat protein alone has a molecular weight of ≈ 26.41 kDa (Acharya et al., 2022). The reported full genome length for an isolate from Peru is 9,635 nt (excluding the poly(A) tail) (Fuentes et al., 2012).

As research progressed, YBMV was found to have a wide global distribution and is not limited to the yam bean (Acharya et al., 2022). Several reports indicate that the virus also infects other legumes, for example, YBMV was found in sword bean (*Canavalia ensiformis*) in Brazil (de Sá Andrade Medeiros et al., 2019) and in kalopo (*Calopogonium mucunoides*) in Australia (Filardo et al., 2023). *Senna hirsuta* in Nigeria has also been reported as a host for YBMV (Ekpiken et al., 2023). Latest reports in Thailand (Jenjai and Samarakoon, 2025) and this study in Indonesia expanded the host range of YBMV to include butterfly pea.

The symptoms caused by the virus in yam bean plants are very distinctive. The most prominent symptoms are severe mosaic, leaf malformation, green vein stripes, and leaf distortion (de Sá Andrade Medeiros et al., 2019). In other legumes, symptoms can vary depending on the virus strain and host variety, including yellow-green mosaic, wrinkled leaves, stunted growth, and, in severe cases, necrosis and plant death (Sastry et al., 2019). In line with this, mild to severe mosaic, chlorosis, vein banding, and wrinkling were clearly evident on infected butterfly pea leaves in Thailand (Jenjai and Samarakoon, 2025) and Indonesia.

Phylogenetic analysis on the obtained 3'-tail region of YBMV isolates showed that the five Indonesian butterfly pea isolates are closer to a butterfly pea isolate from Thailand (MJU-L1) than to another Indonesian isolate from yam bean (Bogor) (Figure 2). This highlighted diversity in Indonesia as well as possible genetic distinction based on plant hosts. However, the butterfly pea phylogroup also contains isolates from *Senna occidentalis* in Thailand and sword bean in Brazil. Although there is considerable nucleotide variation among the observed partial CP region, notably most mutations are silent, as shown by the high amino acid identity among the compared isolates (Figure 3). Similar to our

findings, the recombinant QLD isolate was reported to share a relatively low 76% nt and 85% aa identities to the closest YBMV isolate, SR (NC_016441) (Filardo et al., 2023).

The primary mode of YBMV transmission is through insect vectors, particularly aphids, and it is non-persistent. Specific aphid vectors that have been identified in the transmission of YBMV include *Aphis craccivora*, *Aphis glycines*, and *Aphis gossypii* (Sastry et al., 2019). Research reports also showed that YBMV found in yam beans can be transmitted through seeds, thereby potentially accelerating the virus's spread in endemic areas and into new areas (Fuentes et al., 2012). The current study confirmed that the SMN-00 isolate from butterfly pea can be mechanically transmitted, causing clear symptoms in yardlong bean. Natural infection of YBMV in yardlong bean has been previously documented in Vietnam (Ha et al., 2008). The host range of the virus likely does not include Cucurbitaceae, as it did not infect cucumber according to the mechanical inoculation experiment. Therefore, the distribution of the still less studied YBMV on commonly cultivated legumes needs to be surveyed to understand its actual economic impact.

Conclusion

YBMV was detected infecting five symptomatic butterfly pea plants collected in Bantul and Sleman Regencies, Special Region of Yogyakarta, Indonesia, which tested negative for BCMV and CMV. This study confirmed the first identification of YBMV in butterfly pea in Indonesia, and validated a similar finding in Thailand. Analysis on the 3'-terminal region of Indonesian and global isolates improved our understanding of the diversity of YBMV, a still less studied legume-infecting plant virus.

Ethical Statement

Ethical approval is not required for this study because it did not involve human or animal subject.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Artificial Intelligence Declaration

The authors declare that no generative artificial intelligence tools were used at any stage of the preparation of this manuscript, including the writing, editing, or refinement of the text, or the creation of any images, figures, graphics, tables, or related titles.

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Author Contributions

Study conception and design: AIS, FNA; data collection: FRCW, RW, MNV; analysis and interpretation of results: AIS, FNA, FR-Z; writing-draft manuscript preparation: ATK, SR, RP; writing-review and editing: AIS, FR-Z. All authors reviewed and approved the final version of the manuscript.

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