Jurnal Perlindungan Tanaman Indonesia, Vol. 29, No. 1, 2025: 62−69
DOI: 10.22146/jpti.101371
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Short Communication

Molecular Characterization of Mungbean yellow mosaic India virus and Tomato leaf curl New Delhi virus in Sukoharjo and Magelang Regencies, Indonesia

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Received November 7, 2024; revised March 6, 2025; accepted June 3, 2025

ABSTRACT

Diseases caused by begomoviruses in black-eyed pea (*Vigna unguiculata* subsp. *unguiculata*) and cucumber (*Cucumis sativus*) remain insufficiently explored in Indonesia. One symptomatic black-eyed pea and two cucumber samples were collected from Sukoharjo and Magelang Regencies, respectively, and molecularly tested using Krusty/Homer and SPG1/SPG2 primers for universal detection of begomoviruses. NCBI BLAST analysis on the obtained nucleotide sequences confirmed mungbean yellow mosaic India virus (MYMIV, *Begomovirus vignaradiataindiaense*) infection in black-eyed pea sample and tomato leaf curl New Delhi virus (ToLCNDV, *Begomovirus solanumdelhiense*) infection in both cucumber samples. Sequences of partial AV1, and partial AC1 and AC2 genes of the three isolates were registered with accession nos. PQ539469-71 and PQ539476-78, respectively, in NCBI GenBank. No recombination signals were detected in the sequences of the new isolates using Recombination Detection Program (RDP v.5.30). In the phylogenetic trees built by MEGA 11 with Tamura-Nei parameter model, MYMIV H-2 as well as ToLCNDV N-8 and V-97 isolates shared basal nodes with Indonesian isolates indicating their close genetic relationship with other isolates also found in the country. While expanding our information regarding genetic diversity of begomoviruses, this study also reported the first cases of MYMIV in black-eyed pea in Indonesia and ToLCNDV in cucumber in Magelang, to the best of our knowledge.

Keywords: Cucurbitaceae; Fabaceae; molecular variation; plant virus; polymerase chain reaction

INTRODUCTION

Begomovirus, a prominent genus in the Geminiviridae family, consists of plant viruses responsible for widespread crop losses, especially in tropical and subtropical climates (Rojas *et al.*, 2018). These viruses are transmitted by the whitefly vector, *Bemisia tabaci*, an insect that enables the rapid spread of begomoviruses across various regions and crops (Fiallo-Olivé & Navas-Castillo, 2023). A notable feature of begomoviruses is their genetic diversity, which has led to the emergence of novel strains capable of infecting a various host plants (Nigam, 2021). In Indonesia, particularly in agricultural regions such as Central Java, begomoviruses have become a growing threat to high-value legume and vegetable crops, necessitating studies

on their detection, diversity, and impact (Mizutani *et al.*, 2011; Nurulita *et al.*, 2015; Santosa *et al.*, 2024).

Detection methods are critical to effectively manage *Begomovirus* outbreaks, enabling early intervention and limiting its spread. Molecular techniques utilizing universal primers, such as SPG1/SPG2 (SPG) and Krusty/Homer (KH), have been developed to detect begomoviruses in infected plants. The SPG primer set has been shown to amplify conserved partial AC1 and AC2 regions of the *Begomovirus* genome, making it an effective tool for initial detection across diverse isolates (Li *et al.*, 2004). This approach has been successfully implemented both internationally and in Indonesia to screen for begomoviruses presence in a wide range of crop hosts, such as mungbean yellow mosaic Indian virus (MYMIV, *Begomovirus vignaradiataindiaense*) (Nurulita *et al.*, 2015; Sutrawati *et al.*, 2020) and tomato leaf curl New Delhi virus (ToLCNDV, *Begomovirus solanumdelhiense*) (Listihani *et al.*, 2019). Additionally, the KH primer pair is used to complement SPG by enabling detection of multiple begomovirus strains within single infections, a common occurrence in fields with high disease prevalence (Duffy & Holmes, 2007).

This study aims to elucidate molecular characterization of *Begomovirus* isolates from infected black-eyed pea (*Vigna unguiculata* subsp. *unguiculata*) and cucumber (*Cucumis sativus*) from Sukoharjo and Magelang Regencies, Central Java Province, Indonesia. By using the universal primers SPG and KH, we attempt to provide molecular data of these begomoviruses from the still understudied black-eyed pea. Additionally, this results from this will contribute to the understanding of *Begomovirus* epidemiology in Southeast Asia and development of effective diagnostic and management strategies to mitigate their agricultural and economic impacts.

MATERIALS AND METHODS

Sample Collection

Black-eyed pea fields in Sukoharjo Regency and cucumber fields in Magelang Regency were surveilled in July 2024. Both regencies are in Central Java Province, Indonesia. Samples were purposively collected from fields with plants exhibiting severe *Begomovirus* symptoms and high *B. tabaci* populations. Samples were kept at -4 °C until further testing in Phytopathology Laboratory, Universitas Gadjah Mada.

DNA Extraction, PCR, and Sequencing

DNA extraction was conducted for each collected sample using the Genomic DNA Mini Kit for Plant, strictly following the manufacturer's protocol (Geneaid Biotech Ltd., Taiwan). To detect begomoviruses, two PCR reactions were performed per sample using universal primer pairs (Defitra *et al.*, 2025). The first primer pair, Krusty (F 5'-CCNMRDGGHTGTGARGGNCC-3')/Homer (R 5'-SVDGCRTGVGTRCANGCCAT-3'), targeted a ± 580 bp segment within the AV1 gene, enabling partial amplification of this genomic region (Revill *et al.*, 2003). The second primer pair, SPG1 (F 5'-CCC CKGTGCGWRAATCCAT-3')/SPG2 (R 5'-ATC CVAAYWTYCAGGGAGCT-3'), amplified ± 900 bp segment covering partial AC1 and AC2 genes (Li *et al.*, 2004).

Each PCR mixture was prepared in a reaction volume of 40 µL, consisting of 2 µl (10 pmol/µL) of each primer, 20 µL of MyTaq HS Red Mix (Bioline, Germany), 4 µL of extracted DNA template, and 12 µL of PCR-grade water. PCR conditions were optimized as follows: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for the KH primers or 59 °C for the SPG primers for 1 minute, and extension at 72 °C for 1 minute. A final extension was conducted at 72 °C for 10 minutes.

Electrophoresis was conducted on 1% agarose gels containing Florosafe DNA Stain (1st BASE, Malaysia) at 50 V for 50 minutes to confirm amplification success, with bands visualized under a UV transilluminator (Optima Inc., Japan). Amplified DNA fragments of expected sizes were sent to Integrated Research and Testing Laboratory (Universitas Gadjah Mada) for bidirectional sequencing using Sanger technology. Sequencing was aimed to obtain partial sequences of the AV1, AC1, and AC2 genes of each viral isolate. Sequence identity was established by comparing the new sequences to those in the NCBI GenBank database using nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov).

Novel sequences were deposited in GenBank to obtain unique accession numbers for each isolate.

Recombination Analysis

Complete genome sequences of *Begomovirus* isolates available in GenBank were aligned to the sequences of newly obtained isolates, which were then trimmed to uniform lengths using the ClustalW algorithm in MEGA11 software (Tamura *et al.*, 2021). To examine potential recombination events among isolates in this study with available isolates in NCBI GenBank, analysis was carried out using Recombination Detection Program (RDP v.5.30) (Martin *et al.*, 2021). Recombination events were considered significant only when detected by at least five of the following algorithms: Bootscan, MaxChi, Chimaera, 3Seq, Siscan, GENECONV, and RDP. Additionally, a Bonferroni-corrected P-value threshold of < 0.05 was applied to validate the events (Martin *et al.*, 2021).

Phylogenetic and Percentage Identity Analyses

Phylogenetic analysis of recombinant-free isolates was conducted separately for partial AV1, and partial AC1 and AC2 genes, utilizing the Maximum Likelihood (ML) method with the Tamura-Nei parameter model (Tamura & Nei, 1993) as implemented in MEGA11. Statistical support for each branch was evaluated through 1000 bootstrap. Percentage nucleotide (nt) identity among the compared isolates were estimated using Sequence Demarcation Tools (SDT v.1.2) (Muhire *et al.*, 2014).

RESULTS AND DISCUSSION

One leaf sample of black-eyed pea plant showing chlorosis and stunting growth was sampled from Sukoharjo Regency. Two cucumber leaf samples with mosaic symptom were collected from Magelang Regency (Figure 1). These were also observed in other reports on *Begomovirus* infection in *Fabaceae* and *Cucurbitaceae* (Santosa & Somowiyarjo, 2023; Santosa *et al.*, 2024).

All samples were infected by *Begomovirus* based on PCR results that produced the targeted band with specific sizes to KH and SPG primer pairs, respectively. Result of BLAST analysis on the obtained nucleotide sequences of the partial AV1, and partial AC1 and AC2 genes consistently identified

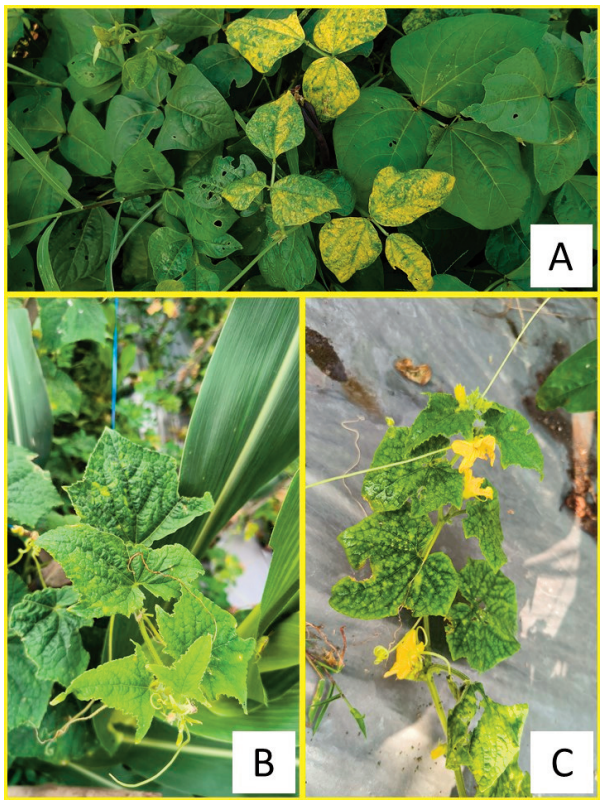

Figure 1. Symptoms of begomovirus infections observed on samples. (A) Black-eyed pea from Sukoharjo Regency showing strong chlorosis (yellowing), particularly on young leaves, was infected by mungbean yellow mosaic virus H-2 isolate; (B) Cucumber from Magelang Regency showing leaf mottle was infected by tomato leaf curl New Delhi virus N-8 isolate; (C) Cucumber from Magelang Regency showing leaf malformation and slight chlorosis was infected by tomato leaf curl New Delhi virus V-97 isolate

that the black-eyed pea isolate was mungbean yellow mosaic India virus (MYMIV) and both the cucumber isolates were tomato leaf curl New Delhi virus (ToLCNDV). Sequences of partial AV1, and partial AC1 and AC2 genes of the three isolates were submitted to NCBI GenBank to be given accession numbers, PQ539469-71 and PQ539476-78, respectively.

The analysis conducted using RDP 5 software revealed no significant recombination in the genomes of all isolates in this study with those retrieved from GenBank within the observed regions. Two phylogenetic trees were developed based on the sequence of partial AV1 gene (± 500 bp) and partial AC1 and AC2 genes (± 890 bp), respectively. Consistent with BLAST analysis, both trees grouped H-2 together

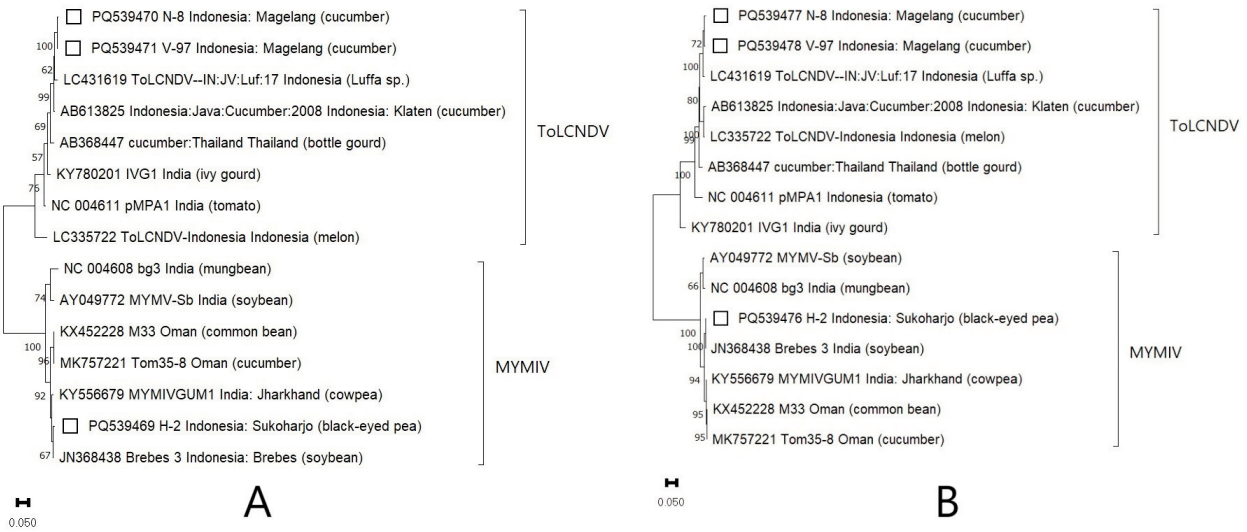

Figure 2. Maximum Likelihood phylogenetic trees generated in MEGA11 software based on nucleotide sequences of (A) ± 500 bp partial AV1 gene covered by Krusty/Homer primers and (B) ± 890 bp partial AC1 and AC2 genes covered by SPG1/SPG2 primers. Tamura-Nei-parameter's model with 1000 bootstrap replicates (only values >50% were shown) was applied for branches development. Three isolates reported in this study were highlighted with white squares. MYMIV = mungbean yellow mosaic India virus; ToLCNDV = tomato leaf curl New Delhi virus.

with six other MYMIV isolates, and N-8 and V-97 with six other ToLCNDV isolates thus confirmed species of the respective isolates (Figure 2). H-2 isolate had the closest genetic relationship with 'Brebes 3' isolated from soybean in Brebes Regency. Both Magelang and Brebes are in Central Java Province but they are geographically distant from each other. This indicated that the distribution of isolates could be widespread either facilitated by *B. tabaci* vector or seed transmission, as MYMIV was previously reported to be seed transmitted in yardlong bean (Mulyadi *et al.*, 2021).

At partial AV1 region, H-2 isolate shared 93.8−98.8%, and at partial AC1 and AC2 regions shared 94.6−99.8% nt identity to the other MYMIV isolates. Meanwhile, N-8 and V-97 isolates had 93.6− 97.7% and 91.7−98.4% nt identity at partial AV1 region and partial AC1 and AC2 regions to the other ToLCNDV isolates, respectively (Figure 3). Therefore, the SDT calculation were in accordance with phylogenetic analysis which showed that the H-2 isolate was MYMIV and N-8 and V-97 isolates were ToLCNDV.

Within the *Begomovirus* group, MYMIV and ToLCNDV were important pathogens affecting crops across Indonesia and other parts of Asia. MYMIV was an Old World *Begomovirus* primarily infecting legumes such as mungbean (*V. radiata*) and yardlong bean (*V. unguiculata* ssp. *sesquipedalis*) but had expanded its host range to include additional plant groups under favorable conditions (Kumar *et al.*, 2010). Several regions of Indonesia, such as West Java, Central Java, and Yogyakarta, have been reported to be affected by the virus (Sutrawati *et al.*, 2020; Mulyadi *et al.*, 2021; Santosa & Somowiyarjo, 2023). MYMIV was identified using SPG primers in yardlong bean grown in Sukoharjo (Supyani *et al.*, 2020) thus this study reported black-eyed pea as an additional host in the regency. Nurulita *et al.* (2015) detected MYMIV from yardlong bean in Magelang, Central Java. The virus has also been reported to cause major losses in legumes throughout South East (Tsai *et al.*, 2013) and South Asia (Singh *et al.*, 2020), where it disrupts agriculture and local food security.

ToLCNDV, a member of the New World begomoviruses, has a wide host range that includes solanaceous and cucurbitaceous plants. It is particularly damaging to tomato (*Solanum lycopersicum*) and cucumber, causing severe leaf curl symptoms that reduce yield and quality of both plant species (Jyothsna *et al.*, 2013). ToLCNDV has been reported to be identified in cucumber plants in West Java, Bali,

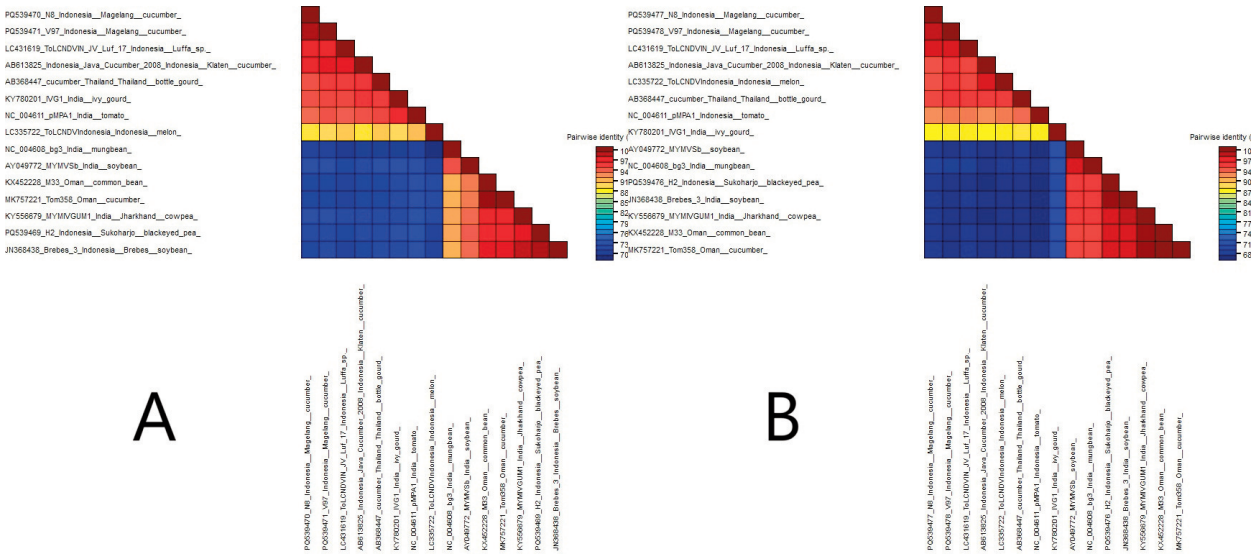

Figure 3. Percentage nucleotide identity of H-2, N-8, and V-97 isolates to other tested isolates at (A) partial AV1 gene and (B) partial AC1 and AC2 genes.

Central Java, East Java and Yogyakarta where its presence provides a challenge to cucumber production in the region (Mizutani *et al*., 2011; Wiratama *et al*., 2015; Haerunisa *et al*., 2016; Septariani *et al*., 2014; Listihani *et al*., 2019; Santosa & Somowiyarjo, 2023). While ToLCNDV had been reported in cucumber cultivated in Sukoharjo (Septariani *et al*., 2014), this was the first confirmation of the virus in cucumber in Magelang to the best of our knowledge. The virus has spread worldwide, affecting regions beyond Asia, including parts of Europe and the Mediterranean, where its adaptability has raised concerns about its potential to cause epidemics in new agricultural environments (Fortes *et al*., 2016; Moriones *et al*., 2017; Cai *et al*., 2023).

Genetically, N-8 and V-97 were shown to share closer relations with luffa (*Luffa acutangular*, *Cucurbitaceae*) and cucumber isolates from Java Island, a bottle gourd (*Lagenaria siceraria*, *Cucurbitaceae*) isolate from Thailand, and an ivy gourd (*Coccinia grandis*, *Cucurbitaceae*) isolate from India than a tomato (*Solanum lycopersicum*, *Solanaceae*) isolate from India following phylogenetic and identity analyses on both partial AV1, and AC1 and AC2 sequences. This suggested that hosts may have stronger influence on genome variation in ToLCNDV than geographic locations.

CONCLUSION

The black-eyed pea and cucumber samples from Sukoharjo and Magelang Regencies were respectively determined to be infected with two distinct species of begomovirus based on molecular detection. The viral species were identified as MYMIV for the black-eyed pea and ToLCNDV for both cucumber isolates. These were the firsts MYMIV in black-eyed pea grown in Indonesia, and ToLCNDV in cucumber Magelang Regency. The genetic relationship between isolates suggested long-distance distribution possibly through vector transmission. Additionally, the analysis indicated that host plants may have stronger influences on genome variation than geographic locations for ToLCNDV. This research provides new insights into the genetic diversity and distribution of both viruses, potentially triggering further studies at regional and national scales in Indonesia. Important genome recombination may also be observed when additional full ge-nome sequences of Indonesian isolates of MYMIV and ToLCNDV obtained in the future.

ACKNOWLEDGMENT

This research was funded by Lecturer and Student Research Collaboration Scheme of Faculty of

Agriculture, Universitas Gadjah Mada, contract number 31/UNI/FPN/KU/KU.02.05/2025. Authors do not have conflict of interest in regard to this research.

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