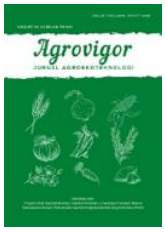
Open Access & Available Online

Agrovigor: Jurnal Agroekoteknologi
ISSN: 1979-5777 (*Print*), 2477-0353 (*Online*)

Journal homepage: https://journal.trunojoyo.ac.id/agrovigor



Article

Molecular Characterization of a Bean common mosaic virus Isolate Inciting Severe Symptoms on Yardlong Bean in Gunungkidul Regency, Indonesia

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ARTICLE INFO

Article history
Received: July 2026
Accepted: September 2026
Published: September 2026

Keywords
Fabaceae;
Nucleotide identity;
Phylogenetic tree;
Plant virus;
RT-PCR;

DOI:
https://doi.org/10.21107/agrovigor.v19i2.34067

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ABSTRACT

In 2023, an outbreak of viral disease on yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) was reported in Tanjungsari District of Gunungkidul Regency, Indonesia. Symptomatic leaves showing mosaic, chlorosis, severe blistering, and leaf malformation were collected as a composite sample and then molecularly tested with RT-PCR. The sample was positive for potyvirus infection using U341/Poty1 universal primer pair, and BLAST analysis on the obtained nucleotide sequence further specified bean common mosaic virus (BCMV, *Potyvirus phaseovulgaris*) as the causal agent of the disease. Sequence of 691 bp of 3'-end region of BCMV-GKD-Ida isolate was registered in NCBI GenBank with accession no. PZ118702. The phylogenetic tree constructed using the Maximum Likelihood method with Tamura 3-parameter substitution model clustered BCMV-GKD-Ida with other BCMV isolates from 17 countries but in a distinct monophyletic branch. A paired identity analysis using the Sequence Demarcation Tool (SDT) software version 1.3 estimated that BCMV-GKD-Ida shares a fairly low nucleotide identity of 86.5%-92.5% with the compared BCMV isolates, further indicating considerable genetic variation. BCMV is one of most important plant pathogens that threaten the productivity legumes. This research provides additional molecular data and contributes a basis for early diagnosis and control strategies for the virus in yardlong bean.

INTRODUCTION

Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) grows optimally at temperatures of 27–30 °C, and is relatively more tolerant to drought conditions than other legume crops (Quamruzzaman *et al*., 2022; Choi *et al*., 2024). The plant is a climbing annual that is commonly harvested at the immature stage as a green vegetable. It was first domesticated in West Africa, and has since expanded to other world regions, including Southeast Asia. It is now an important horticultural commodity in Indonesia. Yardlong bean cultivation faces several difficulties, particularly pest and disease infestations (Worrall *et al*., 2015).

One of the most important plant pathogens threatening yardlong bean productivity is bean common mosaic virus (BCMV, *Potyvirus phaseovulgaris*) (Arli-Sokmen *et al*., 2016). BCMV is known to possess a broad host range, infecting more than 100 plant species belonging to 44 genera. These hosts include several economically important legumes, such as common bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*), pigeonpea (*Cajanus cajan*), jack bean (*Phaseolus lunatus*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), white lupine (*Lupinus albus*), soybean (*Glycine max*), broad bean (*Vicia faba*), and sesame (*Sesamum indicum*) (Worrall *et al*., 2015; Mwaipopo *et al*., 2018; Yadav *et al*., 2021).

Taxonomically, BCMV belongs to the genus *Potyvirus* within the family *Potyviridae*, the largest family of plant RNA viruses. Members of the family *Potyviridae* are characterized by filamentous virions and single-stranded positive-sense RNA genomes (Valli *et al*., 2015; Wylie *et al*., 2017). BCMV is transmitted by aphid vector and mechanical contact, and can also be seed-borne in common bean, with transmission efficiencies reaching up to 80%, depending on the bean cultivar and viral strain (Aishwarya *et al*., 2020). Aphids represent the primary vectors responsible for the natural spread of BCMV. Several aphid species, including *Acyrthosiphon pisum*, *Aphis fabae*, *Myzus persicae*, and *Aphis craccivora*, have been identified as effective vectors (Worrall *et al*., 2015). The virus is evidently seed transmissible in cowpea (*Vigna unguiculata*) without significantly affecting the germination (Kareem *et al*., 2023). Usually, seed transmissions of BCMV were at relatively low rates ranged from 15% to 35% (Valli *et al*., 2015; Worrall *et al*., 2015). However, seed-transmitted viruses may persist in the seeds as long as they are viable and thus might transported for a long distance (Kareem *et al*., 2023).

Viral infections caused by phytopathogens, including BCMV, can lead to substantial losses in common bean production by reducing both yield and crop quality (Adak *et al*., 2026). The virus infection had been reported to induce total production losses on common bean, although yield reductions were usually between 6 and 98%, depending on cultivar sensitivity, virus strain, and environment suitability (Expósito-Goás *et al*., 2024). Symptoms on susceptible legume varieties could vary, including mosaic, stunting, chlorosis, and leaf curling (Arli-Sokmen *et al*., 2016).

The virus has been reported in 57 countries worldwide, including Tanzania, Spain, USA, India, China, Australia, South Korea, and Türkiye (Arli-Sokmen *et al*., 2016; Mwaipopo *et al*., 2018; Tang & Feng, 2022; Kareem *et al*., 2023; Expósito-Goás *et al*., 2024). In Indonesia, BCMV was first detected in yam bean (*Pachyrhizus erosus*) in Bogor, West Java, and Prembun, Central Java (Damayanti *et al*., 2008), although the isolate has now been determined to belong to the closely related yambean mosaic virus (YBMV, *Potyvirus pachyrhizus*) (Wida *et al*., 2026). During 2008–2009, outbreaks of yellow mosaic disease were widely reported in yardlong bean crops across several regions of West Java and Central Java, Indonesia. The predominant causal agent identified in affected areas of West Java, including Bogor, Karawang, Subang, Indramayu, and Cirebon, as well as in Central Java regions such as Tegal and Pekalongan, was BCMV strain black-eye cowpea (BCMV-BIC) (Damayanti *et al*., 2009). As recent, BCMV strain peanut stripe (BCMV-PSt) was identified as the causal of mottle disease on patchouli (*Pogostemon cablin*) in Java Island (Hartono & Kandito, 2024). BCMV-PSt isolates were also identified on groundnut (*A. hypogaea*) and aphids collected in South Sulawesi (Kasim et al., 2024)

According to CBSI (2025), the annual national production of yardlong bean reached 208,583 tons. Gunungkidul Regency contributes a meager 14.5 tons of the total of 1.732 tons harvested in the Special Region of Yogyakarta. Beside harsh environmental factors, viral diseases could be another major constraint in the cultivation of yardlong bean in Gunungkidul. In August 2023, farmers reported severe form of viral symptoms on the yardlong bean cultivated in Tanjungsari District. Molecular testing was performed to confirm BCMV infection in the collected samples. Partial genomic data obtained in this study also could enrich diversity of BCMV isolate in Indonesia.

Therefore, results of this study may incite formulation of management strategies that prevent further yield loss and BCMV dissemination, particularly in the observed area.

MATERIALS AND METHODS

Sample collection

Yardlong bean leaves showing mosaic, chlorosis, severe blisters, and leaf malformation (Figure 1) were observed in Tangjungsari District, Gunungkidul Regency, Special Region of Yogyakarta, Indonesia. Composite leaf samples from five symptomatic plants were taken to the Phytopathology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, and stored in a freezer at -20 °C to be used later in molecular identification of BCMV.

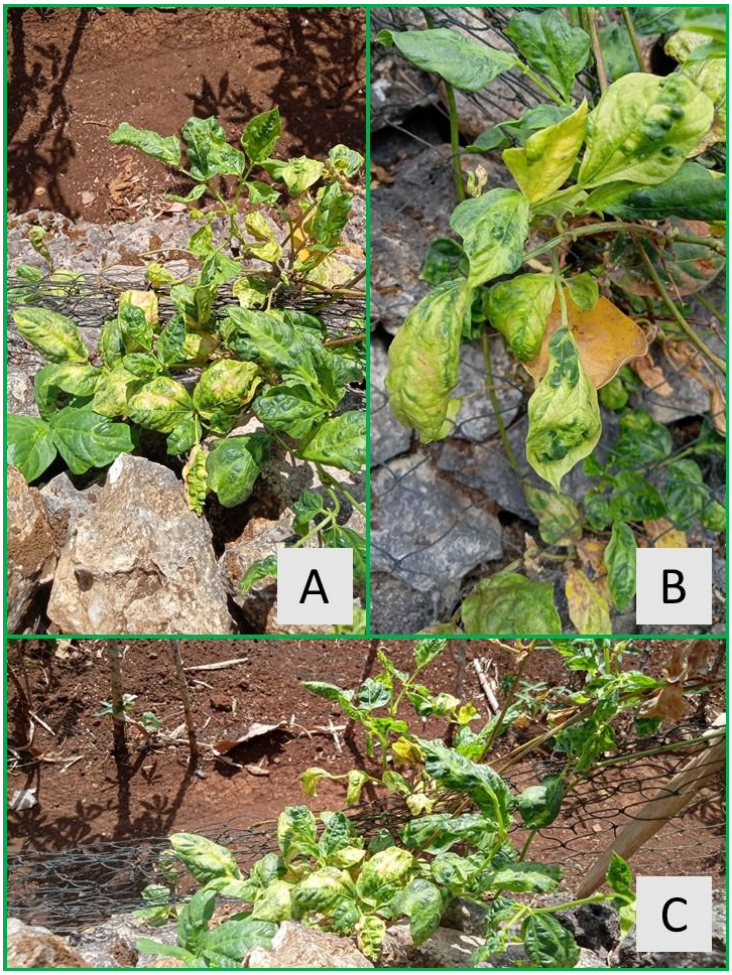


Figure 1. Symptoms of viral infection observed in yardlong bean plants in Tanjungsari District, Gunungkidul Regency: (A) mosaic and chlorosis, (B) chlorosis and leaf deformation, and (C) severe blistering and leaf curling.

Molecular Identification of BCMV

Symptomatic long bean leaf samples were extracted for RNA using the Genomic RNA Mini Kit for Plants (GeneAid Biotech Ltd., Taiwan). RNA was synthesized into cDNA with a total reaction volume of 10 µl consisting of 3.5 µl of RNAse-free H_2O, 2 µl of 5× RT Buffer, 1 µl of dNTP mix, 0.5 µl of Oligo (dt) primer, 0.5 µl of RNAse Inhibitor, 0.5 µl of ReverTraAce, and 2 µl of RNA template, using the ReverTra Ace kit (Toyobo Co., Ltd., Japan). The RT-PCR was conducted with reverse transcription step at 42 °C (20 minutes), inactivation of reverse transcriptase step at 99 °C (5 minutes), followed by temporary storage of the samples at 4 °C.

The cDNA was amplified to multiple copies of the intended DNA fragment using a universal potyvirus primer pair, U341-forward (5'-CCGGAATTCATGRTITGGTGYATIGAIAAYGG-3') and Poty1-reverse (5'GGATCCCGGGTTTTTTTTTTTTTTTTV-3'), which targets a 600–800 bp of the 3'-end (Langeveld *et al.*, 1991). The

PCR reaction was prepared with a total volume of 10 µL, consisting of 3 µL ddH_2O, 0.5 µL forward primer, 0.5 µL reverse primer, 5 µL ready mix (Bioline GmbH, Germany), and 4 µL cDNA. PCR was conducted with pre-denaturation at 95 °C (3 minutes), 35 cycles of denaturation at 95 °C (1 minute), annealing at 55 °C (1 minute), extension at 72 °C (1 minute), and final extension at 72 °C (7 minutes) (Santosa *et al*., 2024).

PCR product was visualized by electrophoresis on a 1% agarose gel containing Florosafe DNA Stain (1st BASE, Malaysia). The PCR products and DNA markers were loaded into 4 µL and electrophoresis was conducted at 80 V (30 minutes). Visualization of the DNA bands was performed using a UV transilluminator.

Sampel tested positive was sent to the Integrated Laboratory for Research and Testing (LPPT) of Universitas Gadjah Mada and dual-directions sequenced using Sanger method. The sequencing result was analyzed using BLAST nucleotide on the NCBI website (https://blast.ncbi.nlm.nih.gov) to identify the virus species. The nucleotide sequence was then listed in NCBI GenBank with certain accession number.

Phylogenetic Analysis and Percentage Identity

The nucleotide sequence from the obtained isolate was compared with related sequences of the same virus species listed in NCBI GenBank. The isolate sequences were aligned and trimmed using the ClustalW algorithm suited in MEGA software version 12.1.2 (Kumar *et al*., 2024). The phylogenetic tree was constructed using the Maximum Likelihood (ML) method with the Tamura 3-parameter (T92) substitution model (Tamura, 1992), and the inter-site rate variation used Gamma distribution with invariant sites (G + I). Evaluation of the phylogenetic tree branches used 1,000 bootstrap replicates. The observed sequences were also analyzed for percentage nucleotide identity using the Sequence Demarcation Tool (SDT) software version 1.3 (Muhire *et al*., 2014) for evolutionary analysis based on nucleotide composition.

RESULTS AND DISCUSSION

Molecular Identification of BCMV

The tested yardlong bean sample was positive for potyvirus, showing a target band of approximately 700 bp on agarose gel. Subsequent BLAST analysis on the obtained nucleotide sequence determined that the isolate belongs to BCMV. Similar to the field observation on yardlong bean of this study, symptoms induced by BCMV infection in common beans had been reported to include leaf mosaic patterns, chlorosis, deformation, etiolation, stunted plant growth, and local or systemic necrosis, which in severe cases may lead to plant death (Worrall *et al*., 2015; Mangeni *et al*., 2020). The severity and expression of these symptoms could be influenced by several factors, including the host genotype, environmental conditions, and the specific viral strain involved (Naderpour, 2010; Singh & Schwartz, 2010; Pasev *et al*., 2014). Furthermore, bean cultivars carrying the *I* resistance gene may develop systemic necrosis when exposed to high temperatures, a condition commonly referred to as "black root" disease (Collmer *et al*., 2000; Cadle-Davidson & Jahn, 2005). Due to varied symptom expression, yield losses associated with BCMV infection can reach up to 50% under severe conditions (Mangeni *et al*., 2020).

Phylogenetic Analysis and Percentage Identity

The partial coat protein + sequence was submitted to NCBI GenBank under the isolate name BCMV-GKD-Ida with accession number PZ118702. Phylogenetic analysis revealed that BCMV-GKD-Ida was clustered within the BCMV clade with isolates from 17 countries, including Indonesia, China, South Korea, Vietnam, Japan, Slovakia, Taiwan, Colombia, Brazil, Mexico, Iran, India, USA, Australia, the Netherlands, Peru, and Kenya. The other 14 Indonesian isolates tested in this study were clustered with eight PSt strain isolates in a separate sub clade (Figure 2). In agreement with result of this study, Hartono & Kandito (2024) also clustered an Indonesian PSt isolate (PP808493) with PSt isolates from China, South Korea, and USA.

The BCMV-GKD-Ida isolate showed tighter genetic relationship with other BCMV isolates than to the closely related potyviruses YBMV and bean common mosaic necrosis virus (BCMNV, *Potyvirus phaseoli*), but it formed a distinct monophyletic branch. Furthermore, the phylogenetic tree showed that the BCMV-GKD-Ida isolate was positioned closer to YBMV clade, and had a longer branch length compared with other BCMV isolates (Figure 2).

These results suggest the presence of considerable genetic variation between the BCMV-GKD-Ida and other known BCMV isolates.

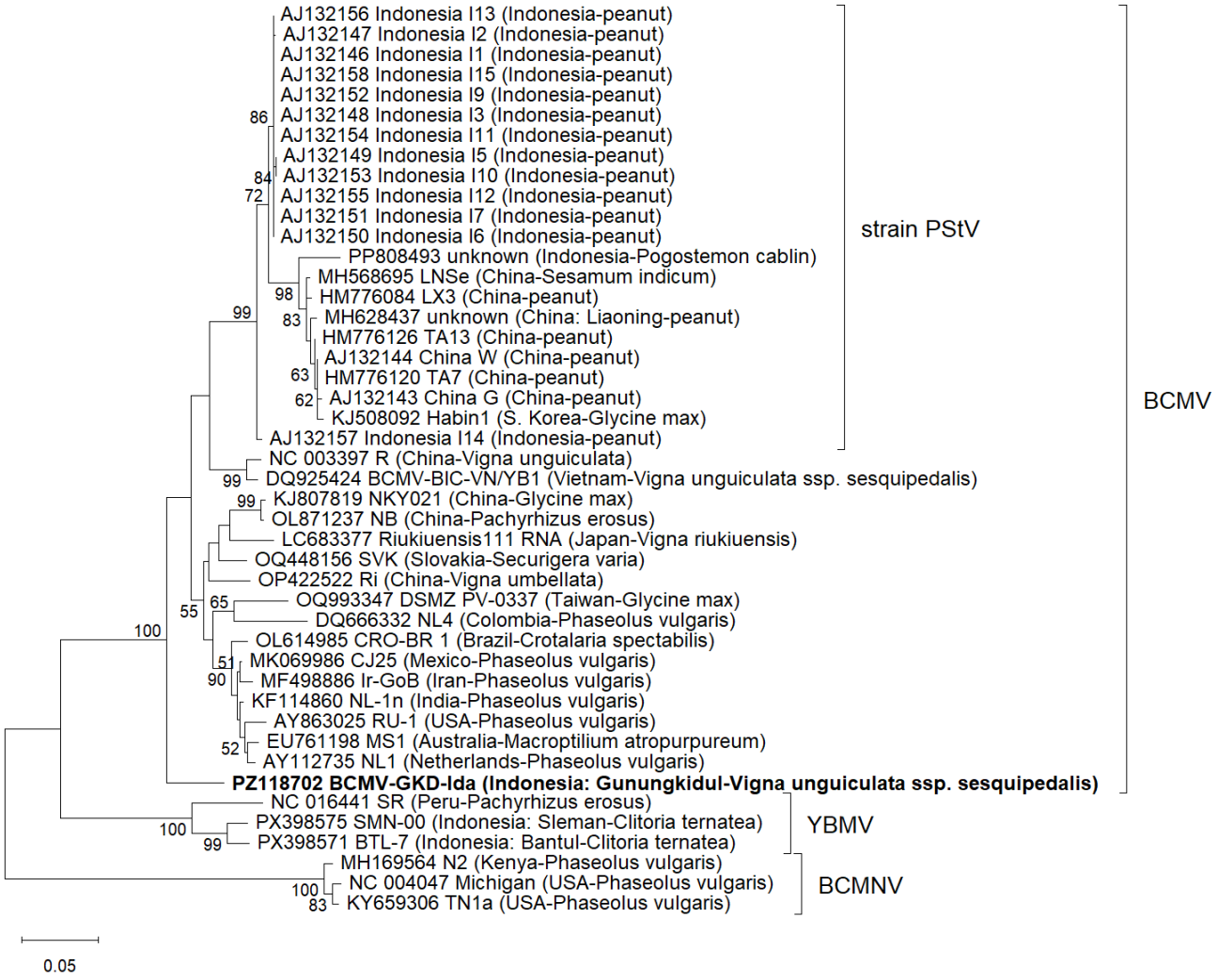


Figure 2. Phylogenetic trees based on nucleotide sequences of the 691 bp 3'-end region of bean common mosaic virus (BCMV) genome. Only bootstrap values > 50% were shown. BCMV-GKD-Ida isolate originated from Gunungkidul Regency was printed in bold. Three isolates of yambean mosaic virus (YBMV) and three isolates of bean common mosaic necrosis virus (BCMNV) were used as out-groups.

Pairwise identity analysis using SDT software demonstrated that the BCMV-GKD-Ida isolate, at 3'-end region, had nucleotide identity between 90.3%-92.5% with non-strain PSt BCMV isolates from various countries including NB from China (12345678), NKY021 from China (KJ807819), R from China (NC 003397), SVK from Slovakia (OQ448156), CJ25 from Mexico (MK069986), BCMV-BIC-VN/YB1 from Vietnam (DQ925424), Riukiuensis111 RNA from Japan (LC683377), NL1 from the Netherlands (AY112735), MS1 from Australia (EU761198), Ri from China (OP422522), NL-1n from India (KF114860), Ir-GoB from Iran (MF498886), CRO-BR 1 from Brazil (OL614985), DSMZ PV-0337 from Taiwan (OQ993347), and RU-1 from the USA (AY863025). The percentage of nucleotide identity of BCMV-GKD-Ida with BCMV strain PSt was 86.5%-90.1% with isolates from Indonesia, China, South Korea, and Colombia. These results indicate that BCMV-GKD-Ida shows a higher level of genetic identity to BCMV isolates of

non-PSt strain than to those of the PSt strain, although it remains closely related within the BCMV group and may represent genetic variation within the species (Figure 3).

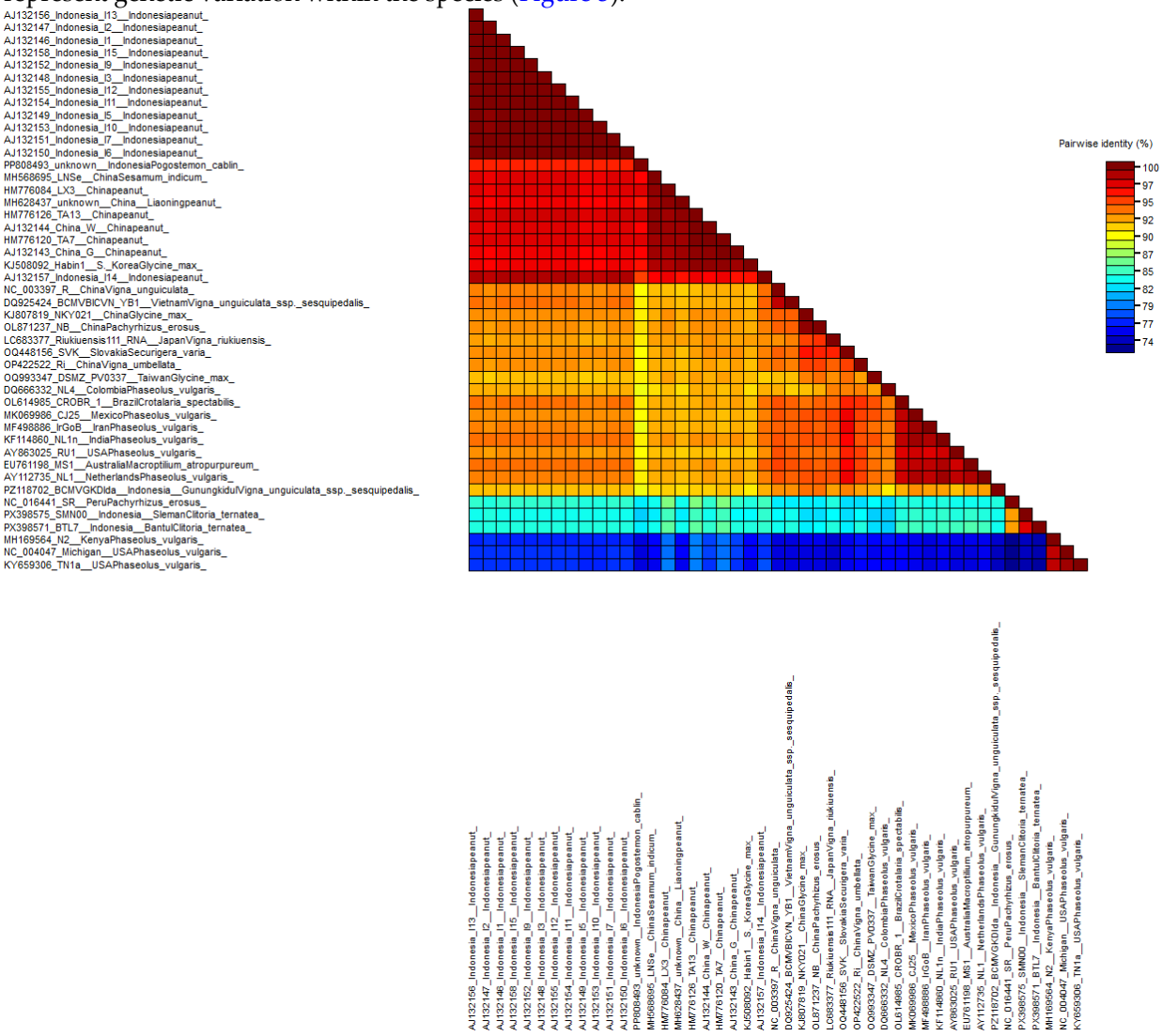


Figure 3. Schematic view of the results of percentage identity analysis by SDT v1.2 software on the obtained bean common mosaic virus isolate (BCMV-GKD-Ida) compared to 38 BCMV, 3 YBMV, and 3 BCMNV isolates in the NCBI GenBank database. Different colors represent pairwise sequence comparison based on the 691 bp 3′-end region.

Pairwise identity analysis testing between BCMV and other species from the *Potyvirus* genus estimated that the BCMV-GKD-Ida shares nucleotide identity of 84.7–85.7% with YBMV isolates. Meanwhile, the percentage of nucleotide identity of BCMV-GKD-Ida with the BCMNV isolates is 73.8%-75.6% (Figure 3). These results indicate that the BCMV-GKD-Ida has a nucleotide identity that is quite close to YBMV, but still lower than the BCMV isolate. According to the species demarcation criteria established by the International Committee on Taxonomy of Viruses, the nucleotide identity threshold for distinguishing species within the Potyvirus genus based on the coat protein gene is below 76–77% (Adams *et al*., 2005). Therefore, the BCMV-GKD-Ida isolate is classified as a BCMV species that distinct to YBMV and BCMNV based on phylogenetic relationships and nucleotide identity values among the isolates.

CONCLUSION

This study molecularly identified BCMV as the causal agent of a severe viral disease in yardlong bean cultivated in Tanjungsari District of Gunungkidul Regency, Indonesia. Results of the phylogenetic tree and nucleotide identity analyses showed considerable genetic variation between BCMV-GKD-Ida and other known BCMV isolates. Further epidemiological studies are needed to understand BCMV distribution and genetic diversity in Indonesia which may lead to accurate management strategies.

ACKNOWLEDGMENT

This research was funded by the Lecturer and Fasttrack Graduate Student Research Collaboration Grant by Faculty of Agriculture, Universitas Gadjah Mada, under contract number 13438/UN1/PN/TU/PT.01.03/2025.

AUTHORS CONTRIBUTIONS

RW, IAN, and ADSP collected leaf sample and performed molecular test. RW, AS, UAN, and S analyzed the data. AIS, E, and ANR interpreted results of analyses and wrote the draft manuscript. All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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