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Detection and Molecular Characterization of *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) Infecting Chili Pepper (*Capsicum annuum*) in Bengkulu, Indonesia

Deteksi dan Karakterisasi Molekuler *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) yang Menginfeksi Cabai Merah (*Capsicum annuum*) di Bengkulu, Indonesia

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ABSTRACT

Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV), an emerging *Begomovirus* in Southeast Asia, has not previously been confirmed in chili pepper (*Capsicum annuum*) in Bengkulu, Indonesia. This study provides the first molecular evidence of TYLCKaV infecting chili in this region and characterizes its genomic variation, viral accumulation dynamics, and predicted host-interaction profiles. PCR amplification of the AC1-IR-AV1 region confirmed TYLCKaV in all symptomatic samples. Sequence analysis revealed the highest nucleotide identity (99%) with Indonesian-Bengkulu and Philippines isolates (PP990580), whereas the lowest identity (92%) was observed with the Cambodia isolate (KR073086), indicating regional sequence divergence within Southeast Asian populations. Quantitative PCR demonstrated significant variation in viral accumulation among isolates ($p = 0.042$), with no consistent correlation between symptom severity and viral DNA levels. Comparative analysis of AC1-AC4 proteins identified amino acid substitutions across functional domains, particularly within central and C-terminal regions. Structural prediction and protein-protein interaction modelling suggested isolate-specific differences in predicted binding affinities to Histone-3, IMPA1, and BTF3. These findings confirm the presence of TYLCKaV in chili from Bengkulu and demonstrate genomic micro-variation and predicted differences in host interaction characteristics, supporting the importance of molecular surveillance for effective disease management.

Keywords: *Begomovirus*, *Capsicum annuum* , TYLCKaV, host interaction, viral accumulation

ABSTRAK

Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) merupakan virus dari kelompok *Begomovirus* yang baru muncul di Asia Tenggara yang sebelumnya belum pernah terkonfirmasi pada cabai (*Capsicum annuum* L.) di Bengkulu, Indonesia. Penelitian ini bertujuan untuk memberikan bukti molekuler pertama bahwa TYLCKaV menginfeksi cabai di wilayah ini serta mengkarakterisasi variasi genomiknya, dinamika akumulasi virus, dan prediksi profil interaksi inang. Amplifikasi PCR pada wilayah AC1-IR-AV1 mengonfirmasi keberadaan TYLCKaV pada semua sampel yang menunjukkan gejala. Analisis sikuensing menunjukkan homologi tertinggi (99%) dengan isolat dari Bengkulu, Indonesia, dan Filipina (PP990580), sedangkan homologi terendah (92%) diamati pada

isolat dari Kamboja (KR073086), yang mengindikasikan adanya vairiasi sikuen regional di antara populasi di Asia Tenggara. PCR kuantitatif menunjukkan variasi akumulasi virus yang signifikan dalam isolat ($p = 0.042$), tanpa adanya korelasi yang konsisten antara tingkat keparahan gejala dan kadar DNA virus. Analisis komparatif protein AC1-AC4 mengidentifikasi substitusi asam amino di seluruh domain fungsional terutama di wilayah tengah dan C-terminal. Prediksi struktural dan pemodelan interaksi protein-protein menunjukkan perbedaan spesifik isolat dalam afinitas pengikatan (*binding affinity*) terhadap Histone-3, IMPA1, dan BTF3. Temuan ini mengonfirmasi keberadaan TYLCKaV pada cabai dari Bengkulu serta menunjukkan adanya variasi mikro genomik dan perbedaan terprediksi dalam karakteristik interaksi dengan inang, sehingga mendukung pentingnya pengawasan molekuler untuk pengelolaan penyakit yang efektif.

Kata kunci: akumulasi virus, *Begomovirus*, *Capsicum annuum*, interaksi dengan inang, TYLCKaV

INTRODUCTION

Chili pepper (*Capsicum annuum*) is a high-value horticultural crop in Indonesia and is highly vulnerable to viral diseases. In Indonesia, chili is affected by several economically important viruses, including *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Pepper vein yellows virus* (PeVYV), *Cucumber mosaic virus* (CMV), *Chili veinal mottle virus* (ChiVMV), *Tobacco mosaic virus* (TMV), and *Pepper mild mottle virus* (PMMoV), belonging to the genera *Begomovirus*, *Polerovirus*, *Tobamovirus*, and *Orthotospovirus*, which induce symptoms such as mosaic, chlorosis, leaf curling, stunting, and yellow vein banding (Nyana *et al.* 2016; Temaja *et al.* 2022).

Among these, *Begomovirus* (family Geminiviridae) represents the most prevalent and destructive group, causing severe yield losses and widespread epidemics. These viruses possess circular single-stranded DNA genomes and exhibit high mutation and recombination rates, facilitating rapid adaptation and host expansion. Members of this genus possess DNA genomes that are either monopartite, consisting of a single genomic component, or bipartite, comprising two components, DNA-A and DNA-B. In bipartite viruses, each component is approximately 2.6-2.8 kb in size, whereas monopartite genomes consist of a single DNA component of comparable length that is sufficient to establish infection (Saeed 2007; Jamsari and Pedri 2013). These viruses are primarily transmitted by the whitefly *Bemisia tabaci* in a persistent manner, which plays a crucial role in their epidemiology. Their broad host range, high mutation and recombination rates, frequent mixed infections, and association with satellite DNA further complicate control strategies. Consequently, effective disease management largely relies on controlling the vector population to limit virus spread (Bhattacharyya *et al.* 2015; Khalil *et al.* 2020; Temaja *et al.* 2022).

Chili pepper viral infections occur in both lowland and mid-altitude regions, generally higher incidence and severity observed in lowland areas. In Aceh, *Begomovirus* infection reached 100% in 120 days old plants at approximately 30 m above sea level, whereas disease incidence was markedly lower at 485 m above sea level, indicating the influence of elevation on disease dynamics. In Bengkulu Province, variations in elevation, edaphic factors, and microclimatic conditions may further shape viral transmission and crop productivity, underscoring the importance of incorporating regional agroecological characteristics into resistant variety development and disease management strategies (Khalil *et al.* 2020; Jeger *et al.* 2023). *Begomovirus* outbreaks have been reported in Indonesia since the late 1990s and continue to pose a significant threat to vegetable production (Hidayat 1999; Sulandari *et al.* 2006).

Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) was first identified infecting tomato and eggplant in Thailand (Green *et al* 2003) and has subsequently been reported in Laos, Vietnam, Kyoto, and Myanmar (Ha *et al*. 2008; Koeda *et al.* 2015; Kwak *et al.* 2022). Its presence in Indonesia was first confirmed in 2013 on eggplant in Java through molecular detection (Kintasari *et al.* 2013). In Bengkulu, high *Begomovirus* incidence has been documented in chili (*C. annuum*) associated with PYLCIV, reaching 96.4-100% in Kepahiang District (Aulia *et al.* 2022). Additionally, cucumber has been reported infected by ToLCNDV with 96.04% nucleotide identity to North Sumatra isolates, representing the first record of this virus in the province. These findings suggest that Bengkulu may serve as a conducive environment for the emergence and establishment of diverse *Begomovirus* species (Sutrawati *et al.* 2022).

Despite increasing reports of TYLCKaV infecting solanaceous crops across Southeast Asia, no scientific evidence has yet confirmed its occurrence in chili pepper (C. *annuum*) in Bengkulu, Indonesia. Field observations have identified symptomatic chili plants exhibiting apical curling, leaf cupping, vein banding, and chlorosis, raising concerns about potential host

range expansion and a consequent threat to local production. However, the absence of molecular confirmation represents a critical knowledge gap regarding the epidemiology and host adaptation of TYLCKaV in this region.

This study aimed to (i) identify the nucleotide-based identity of TYLCKaV infecting chili from Bengkulu and its genetic variation based on DNA-A sequences, (ii) quantify viral accumulation levels from different disease severity, and (iii) predict structural virus protein and its host protein interaction in compare to corresponding isolates from other countries.

MATERIALS AND METHODS

Sample Collection

Samples were collected from the experimental field of the Department of Plant Protection, University of Bengkulu, Indonesia. The surveyed area covered approximately 350 m² and was located at an altitude of 10-15 m above sea level. The predominant cultivated variety was Lado F1, a hybrid curly chili pepper (*C. annuum*). Chili pepper plants exhibiting virus-like symptoms, including leaf mosaic, vein-associated chlorosis, yellowing, and mild curling, were identified during field observation. Symptomatic plants were selected using a random sampling approach within the same field area. A total of six symptomatic leaf samples were collected, individually labeled, and stored at -20 °C until further molecular analysis.

DNA Extraction and PCR Amplification

Total genomic DNA was extracted using a modified CTAB protocol following Doyle and Doyle (1987). PCR amplification targeted the AC1-IR-AV1 region of *Begomovirus* DNA-A using degenerate primers (Table 1), expected to generate an amplicon of approximately 1.2-1.3 kb.

The reaction composition followed the protocol described by Lee *et al.* (2020) with minor modifications. The amplification was performed in a total volume of 50 µL containing 5 µL of 10× PCR buffer, 1 µL of 10 mM dNTP mix (final concentration 200 µM each), 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM) (Table 1), 0.3 µL of Taq DNA polymerase (Protech Technology, Taipei, Taiwan), 1 µL of template DNA (~100 ng), and 41.7 µL of nuclease-free water.

The amplification conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min, as described by Lee *et al.* (2020). PCR products were separated by electrophoresis on a 0.8% agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination.

DNA Sequencing and Analysis

PCR amplicons of samples BKL-1 to BKL-6 were purified and analyzed using the Sanger sequencing method. The resulting chromatograms were visually inspected, trimmed, and assembled into consensus sequences using SeqBuilder software (DNASTAR, USA). Sequence comparison revealed high pairwise nucleotide identity (>99%) among all samples; therefore, one representative isolate (BKL-1) was selected for subsequent analyses.

The consensus nucleotide sequences were analyzed using the basic local alignment search tool (BLASTn) against the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) to determine nucleotide similarity and viral identity. Multiple sequence alignment was performed using the ClustalW algorithm.

Phylogenetic relationships were inferred using molecular evolutionary genetics analysis version X (MEGA X) software employing the neighbor-joining method with 1000 bootstrap replicates (Kumar *et al.* 2018).

Quantitative PCR

The quantitative PCR (qPCR) was performed to measure the virus accumulation level, following the procedures described by Chang (2022), with additional quality control steps included in this study. Total genomic DNA was first quantified using NanoDrop, and all samples were normalized to 50 ng DNA per reaction prior to amplification to avoid template loading bias. After normalization, DNA samples were diluted 20× uniformly across all treatments to minimize potential effects of contaminants while maintaining comparable viral and host DNA proportions.

Reactions were prepared using the iQ SYBR Green Supermix (Bio-Rad Laboratories) and run on a CFX Connect Real-Time PCR Detection System (Bio-Rad). The thermal profile consisted of 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 30 s. A melt-curve analysis (65-95 °C, 0.5 °C increments) was performed after amplification

Table 1 Primers used for amplification of the conserved DNA-A region (AC1-IR-AV1) of *Begomovirus* (Rojas *et al*. 2003)

Primer	Sequence	Size	Target
DNA-A Begomovirus	Forward: 5'-GCATCTGCAGGCCCACATYGTCTTYCCNGT -3' Reverse: 5'-GATTTCTGCAGTTDATRTTYTCRTCCATCCA-3'	1.2-1.3 kb	AC1-IR-AV1

to confirm single, specific products and the absence of primer dimers; reactions showing multiple peaks were excluded. In addition, representative PCR products were sequenced to validate target specificity.

Viral DNA accumulation was quantified using a TYLCV-specific primer pair (Chang 2022). The actin gene was used as an internal reference, and relative levels were calculated using the $2^{-\Delta\Delta Cq}$ method, with all reactions run in three biological replicates and three technical replicates. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test prior to statistical analysis. Statistical analyses were performed in CFX Maestro software (Bio-Rad) using one-way ANOVA followed by Tukey's test. Differences were considered statistically significant at $p < 0.05$.

Replication-associated Protein and Protein Structure Prediction of TYLCKaV

Replication-associated protein of *Begomovirus* plays a key role in viral replication, as previously described by Fondong (2013). In this study, the nucleotide sequences of AC1, AC2, AC3, and AC4 of TYLCKaV were obtained from infected chili pepper samples through PCR amplification followed by Sanger sequencing. PCR amplification was performed using *Begomovirus*-specific degenerate primers targeting conserved regions of the DNA-A component to amplify the AC1-IR-AV1 region, as described in Table 1. The resulting sequences were used as the local isolate data for subsequent analyses.

Reference sequences of the AC1, AC2, AC3, and AC4 genes from related Begomovirus isolates, such as ToLCNDV (GenBank accession no. GU180095), were downloaded from the NCBI database for comparative analysis. Multiple sequence alignment between the local TYLCKaV isolate and reference isolates was performed using CLUSTALW to identify sequence variation and conserved regions.

The nucleotide sequences were translated into amino acid sequences using SeqBuilder software (DNASTAR, USA). Protein structure prediction was performed using AlphaFold3. The predicted protein structures were visualized and analyzed using PyMOL software, and amino acid variations were annotated using color-coded visualization for structural comparison.

TYLCKaV Protein Interacting-host Protein Prediction

TYLCKaV protein-host protein interaction analysis was performed to predict putatively in silico protein-protein interactions between viral proteins and host cellular proteins. The amino acid sequences of AC1, AC2, AC3, and AC4, obtained from translated and sequence-confirmed TYLCKaV isolates, were submitted to ClusterPro software for in silico interaction analysis.

Host candidate proteins, including BTF3, IMPA1, and Histone H3, were selected based on their reported roles in transcriptional regulation, nuclear transport, and chromatin modification in plants (supported by previous studies and unpublished data). This analysis aimed to identify potential functional associations between viral replication-associated proteins and host cellular factors.

RESULTS

Molecular Detection of TYLCKaV

Infected chili pepper plants were identified in the experimental field based on characteristic visual symptoms. The predominant symptoms included leaf mosaic, vein-associated chlorosis, and general yellowing (Figure 1 a-d).

All symptomatic samples tested positive for *Begomovirus* using degenerate primers, yielding the expected 1.2-1.3 kb amplicon targeting the DNA-A region. However, this PCR assay was not specific for TYLCKaV. Species-level identification was subsequently confirmed through Sanger sequencing

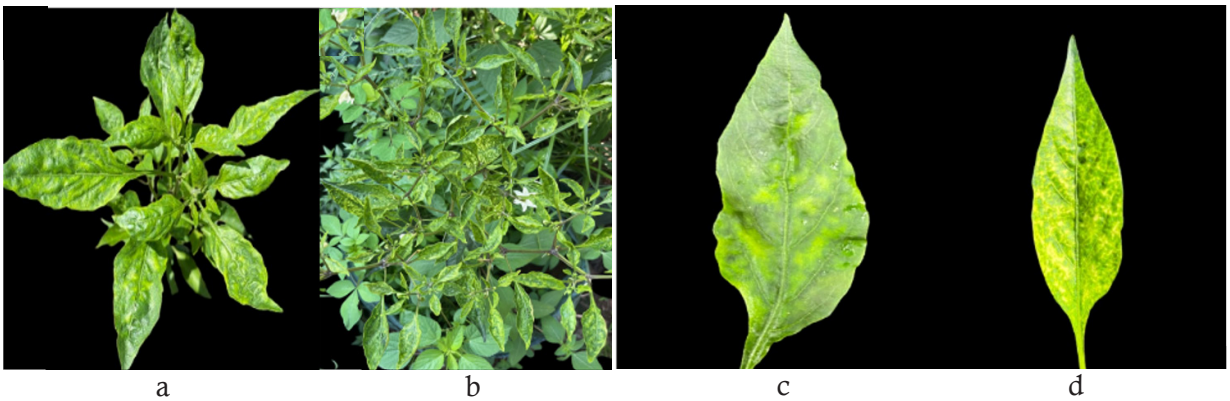

Figure 1 Symptoms of yellowing disease on chili pepper. a, Shortened stem (BKL-4-BKL-6); b, Leaf malformation (BKL-4-BKL-6); c, Mosaic patterns (BKL-1-BKL-3); and d, Chlorosis along leaf veins and general leaf yellowing (BKL-1-BKL-3).

of the amplified products followed by BLAST analysis against reference sequences in the NCBI GenBank database, which showed high nucleotide identity with TYLCKaV isolates.

Amplified DNA fragments matched the expected size (Figure 2), confirming successful amplification of *Begomovirus* DNA-A regions. The observed symptoms, including yellow mosaic, vein chlorosis, leaf curling, and dwarfing, were therefore associated with *Begomovirus* infection.

Sequence Identity and Phylogenetic Characterization of TYLCKaV

Phylogenetic analysis was conducted by comparing the obtained sequences with other TYLCKaV sequences available in the GenBank database. To ensure a more reliable identification, species confirmation of TYLCKaV was carried out through a more refined sequence analysis. PCR products were sequenced using the Sanger sequencing method and analyzed via NCBI BLASTn. The results revealed 12 closely related species with the highest percent identity (Table 2).

DNA analysis showed that the TYLCKaV isolate from Indonesia-Bengkulu shared 99% sequence identity with isolates from the Philippines, Indonesia, and China, mainly associated with tomato and eggplant, and 83% identity with other *Begomoviruses* such as *Ageratum yellow vein China virus* and *Sweet potato leaf curl Hubei virus*. These results indicate strong genetic relatedness among Southeast and East Asian TYLCKaV populations and notable divergence within the genus.

Accumulation Levels of TYLCKaV in Chili Pepper

Conventional PCR was employed solely to confirm the presence of TYLCKaV but did not provide quantitative information on viral load. Therefore, we proceeded with quantitative PCR (qPCR) using TYLCKaV specific primers. Quantification

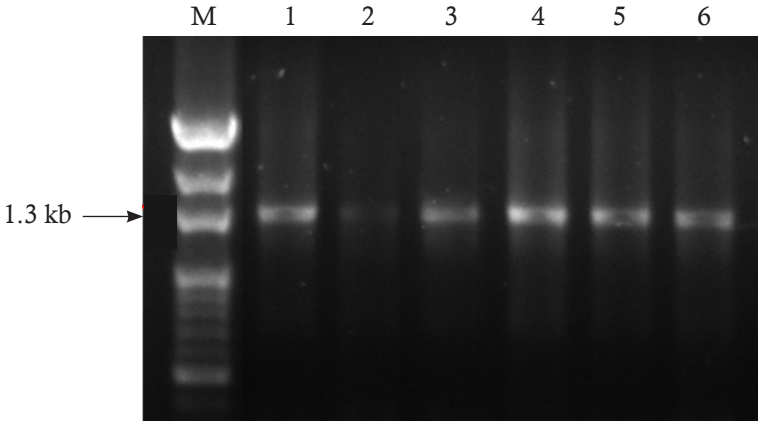

Figure 2 PCR amplification of the *Begomovirus* DNA-A conserved AC1-IR-AV1 region from symptomatic chili pepper plants, resolved on an agarose gel. M, 1.0 kb DNA ladder (Geneaid Biotech Ltd., Taiwan); Lanes 1–6, PCR-positive samples (BKL-1 to BKL-6) selected for subsequent Sanger sequencing (n = 6).

Table 2 Homology level of *Tomato yellow leaf curl Kanchanaburi virus* isolate IDN-BKL (BKL-1) against corresponding viruses in the NCBI GenBank database.

TYLCKaV isolate	Homology (%)	GenBank accesion number	Origin of the isolate	Host
TYLCKaV isolate TYLCVVIP6	99	PP990580	Philippines	Tomato
TYLCKaV isolate BA-B6	99	LC051116	Indonesia	Chili
TYLCKaV isolate E6	99	KF446667	Indonesia	Eggplant
TYLCKaV isolate Eggplant	99	MZ374359	China	Eggplant
TYLCKaV isolate AC21	99	KF446661	Indonesia	Eggplant
TYLCKaV isolate Thailand Kan2	98	AF511530	Thailand	Eggplant
TYLCKaV isolate Bohol-1	97	PV199024	Philippines	Tomato
TYLCKaV isolate terung422	97	PQ539480	Indonesia	Eggplant
TYLCKaV isolate Kulon Progo 2	96	LC815938	Indonesia	Chili
TYLCKaV isolate Cambodia	92	KR073086	Cambodia	Eggplant
Ageratum yellow vein China virus	83	OP851485	China	Tomato
Sweet potato leaf curl Hubei virus	83	MK951974	China	Potato

of TYLCKaV in chili pepper by qPCR revealed differences in viral accumulation between isolates. Six samples, comprising three biological replicate each of TYLCKaV-1 and TYLCKaV-2, were analyzed alongside a dH_2O negative control for each group. TYLCKaV-1 showed the highest relative viral accumulation (mean $\log_2$ fold change ≈ -4; replicate range ±0.7-6.5), correlating with severe leaf curling, chlorosis, and growth distortion. TYLCKaV-2 exhibited very low viral accumulation (average 0.1-0.2; all replicates <0.3), corresponding to mild or asymptomatic infections. The negative control showed values close to zero. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test prior to analysis. As both assumptions were met ($p > 0.05$), one-way ANOVA was performed (n = 3 biological replicates per isolate), revealing significant differences among isolates ($p = 0.042$) (Figure 3).

DNA-A Comparison, Replication Protein Structures, and Host Interactions of TYLCKaV in Chili Pepper

Comparative analysis of full-length DNA-A sequences demonstrated that the Indonesia-Bengkulu isolate shared 99% nucleotide identity with PP990580 (Philippines TYLCVVIP6) and 92% identity with KR073086 (Cambodia isolate) (Table 2). Multiple sequence alignment confirmed that the highest similarity occurred between the IDN-BKL isolate and PP990580, whereas greater divergence was observed with KR073086. The close relationship between IDN-BKL and PP990580 was supported by a limited number of single nucleotide polymorphisms (SNPs) and the absence of major genomic rearrangements among the three isolates. In contrast, comparison with KR073086 revealed a higher number of nucleotide substitutions and several small deletions. Detailed sequence examination indicated that these variations were distributed across both coding and intergenic regions, and the specific nucleotide substitutions identified (Table 3).

Comparative alignment of amino acid sequences among the Indonesia-Bengkulu (IDN-BKL), Cambodia (CMB), and Philippines (PHP; PP990580, TYLCVVIP6) isolates revealed that substitutions were distributed across the entire protein length, including the N-terminal, central, and C-terminal regions (Figure 4).

Prior to analysis, all translated sequences were verified to contain intact open reading frames (ORFs) without internal stop codons, ensuring that full-length protein sequences were used for comparative analysis. Sequence divergence was detectable from the N-terminal region and became more pronounced in the central region (approximately positions 120-300), with increased variability observed between positions 300-600, including point mutations and minor insertions/deletions. The C-terminal region exhibited the highest level of variability among isolates.

Overall, the alignment patterns at both nucleotide and amino acid levels suggest genetic differentiation among isolates, with the Philippines and Cambodia isolates showing higher sequence variability compared to the (IDN-BKL) isolate. Predicted structural differences among TYLCKaV proteins were analyzed to explore interactions of three isolates (IDN-BKL), Philippines PP990580 (TYLCVVIP6), and Cambodia

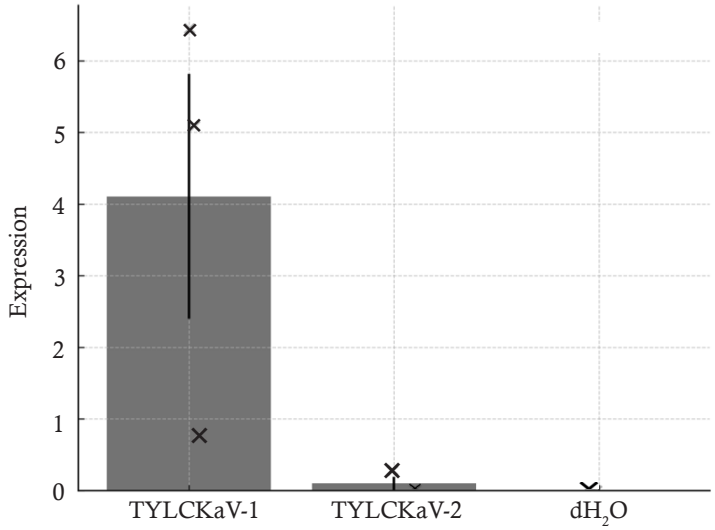

Figure 3 Virus accumulation levels of TYLCKaV-1 and TYLCKaV-2 in chili plants, as determined by ANOVA ($p = 0.042$), compared to the control (dH_2O). The height of the bars indicates the average level of viral accumulation, while the vertical lines/marks (×) indicate the distribution of the sample data. There was a statistically significant difference ($p < 0.05$) between plants infected with TYLCKaV-1 (high expression, symptoms in figure 1 a-b), TYLCKaV-2 (low expression, symptoms in figure 1 c-d), and the control (dH_2O).

Table 3 Nucleotide substitutions among *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) isolates with representative reference sequences from GenBank

Position	IDN-BKL (Indonesia)	PP990580.1 (Philippines)	KR073086.1 (Cambodia)	Mutation Pattern
78	C	T	C	C78T (PP only)
156	A	G	G	A156G (PP & KR)
241	T	C	C	T241C (PP & KR)
389	G	A	C	G389A (PP), G389C (KR)
512	G	G	A	G512A (KR only)
744	T	T	C	T744C (KR only)
1032	C	C	-	Deletion in KR073086.1

Note: PP refers to the Philippine isolate (PP990580.1), and KR refers to the Cambodian isolate (KR073086.1). Mutation patterns represent nucleotide substitutions or deletions identified relative to the Indonesian isolate (IDN-BKL), indicating whether mutations are specific to PP, KR, or shared between them.

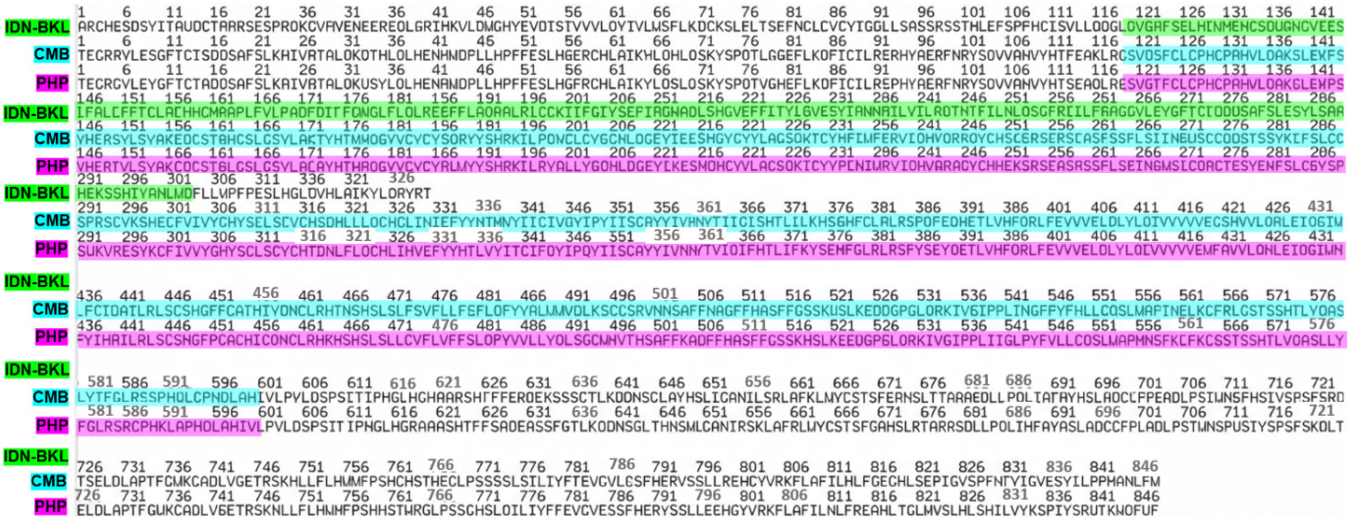

Figure 4 Amino acid alignment of AC proteins among TYLCKaV isolates showing substitutions across the entire protein (positions 1-600). Higher variability occurs in the central (120-300) and C-terminal (300-600) regions, with the IDN-BKL isolate exhibiting distinct motifs at specific positions compared to Cambodia and Philippines isolates.

KR073086 with host genes Histone 3, IMPA1, and BTF3. Interaction energy calculations indicated that the Cambodia isolate had the strongest interaction with IMPA1 (-2757.1), suggesting stable protein-host complexes. The Philippines isolate showed dominant interactions with BTF3 (-2253.3) and Histone 3 (-1854.1), while the IDN-BKL isolate exhibited lower interaction energies with BTF3 (-1725.9), IMPA1 (-2073.4), and Histone 3 (-1297.6), reflecting comparatively less stable protein-protein complexes. These results highlight isolate-specific differences in predicted TYLCKaV protein interactions with host factors.

DISCUSSION

This study demonstrates a host-dependent pathogenic pattern of TYLCKaV in chili, revealing a clear decoupling between symptom severity and viral DNA accumulation. Although TYLCKaV was originally reported infecting tomato in Thailand (Green *et al.* 2003) land subsequently spread across Southeast Asia (Ha *et al.* 2008; Khan *et al.* 2013; Kintasari *et al.* 2013; Zhang *et al.* 2018; Kwak *et al.* 2022), its pathogenic behavior in different hosts remains incompletely understood. Reports from Indonesia have identified closely related isolates in eggplant (>99% identity) (Subiastuti *et al.* 2019), where disease severity is generally associated with active replication (Singh *et al.* 2015). In contrast, our data show that visually comparable symptoms in chili—mosaic, vein chlorosis, and curling—mask significant quantitative differences in viral accumulation. Consistent with previous observations that minor DNA-A variations can generate marked phenotypic divergence (Lett *et al.* 2011), qPCR analysis revealed statistically significant variation among isolates ($p = 0.042$), indicating that disease severity is not a reliable representation for replication efficiency in this host.

A notable finding of this study is that severe phenotypes (e.g., BKL-2) did not correspond to maximal viral load. While the TYLCKaV-1 group

showed the lowest Ct values and highest relative accumulation, BKL-2 exhibited only moderate viral levels despite pronounced deformation and chlorosis. Conversely, mild phenotypes harbored substantially lower viral loads. These data suggest that disease severity in chili is not solely replication-driven but is strongly influenced by host regulatory responses. Environmental modulation and host genetic background may further contribute to this variability (Wiridannisaa *et al.* 2025).

From an epidemiological perspective, viral accumulation directly affects vector acquisition efficiency by *B. tabaci*. High-accumulating isolates may therefore pose greater outbreak risk due to enhanced transmission potential (Temaja *et al.* 2022). The observed heterogeneity among Bengkulu isolates underscores the importance of quantitative diagnostics rather than reliance on symptom-based assessment.

At the molecular level, variation within the AC1-IR-AV1 region likely underpins the observed phenotypic differences. AC1 (Rep) controls rolling-circle replication, AC2 regulates transcription and acts as a silencing suppressor, AC4 contributes to symptom development, and AV1 encodes the coat protein responsible for encapsidation and vector transmission.

Protein-level analysis is essential in this context because nucleotide variations alone do not directly reflect functional consequences. Amino acid substitutions in AC1-AC4 may alter protein conformation, stability, and interaction with host factors, thereby influencing viral replication efficiency and pathogenicity. Consistent with this, amino acid substitutions identified in AC1-AC4 may modify Rep protein function, particularly in origin recognition and host factor recruitment, as even subtle changes in Rep have been reported to significantly affect viral replication dynamics (Fondong 2013). The higher viral accumulation observed in TYLCKaV-1 plausibly reflects enhanced replication competency driven by these structural differences, whereas the BKL-2 profile suggests a differential balance between replication efficiency and symptom-associated pathways.

Predicted protein-host interaction analyses further support a host-regulatory model of pathogenicity. Viral association with histone components has been reported to enhance replication accessibility (Zhou *et al.* 2011), whereas epigenetic interference can suppress host defence signalling (Sahu *et al.* 2014). In this study, comparative interaction strengths with Histone-3, IMPA1, and BTF3 differed among isolates, indicating potential variation in chromatin engagement and nuclear transport efficiency. Importin-α-mediated nuclear trafficking of Rep is essential for replication (Zhao *et al.* 2020), and altered interaction dynamics could independently influence intracellular viral amplification independent of systemic symptom severity.

Host transcriptional profiling, a key method for understanding gene expression changes, strengthens this interpretation. Plants exhibiting high viral accumulation showed coordinated modulation of Histone-3 and BTF3 expression, whereas severely symptomatic plants with moderate viral load displayed disproportionate transcriptional shifts. BTF3 has been implicated as a regulator of basal immunity that viruses may exploit to attenuate defence responses (Huh *et al.* 2012; Viswanath *et al.* 2023). Together, these findings suggest that symptom expression in chili reflects the balance between viral replication capacity and host chromatin-associated regulatory disruption rather than viral copy number alone.

The TYLCKaV Bengkulu (IDN-BKL) isolate shares high nucleotide identity with Southeast Asian strains but displays measurable structural and interaction divergence, indicating adaptive micro-variation at the protein level. Such fine-scale variation may reflect ongoing host-associated differentiation. Although *Begomoviruses* are traditionally considered non-seed-transmitted, low-frequency embryo detection has been reported in related systems (Kothandaraman *et al.* 2016; Kil *et al.* 2018), warranting precautionary surveillance alongside vector control.

Integrated management must be reinforced through coordinated short and long-term strategies. Optimized agronomic practices, biological control using *Encarsia formosa*, and evidence-based insecticide rotation are essential to address widespread resistance in *B. tabaci* (Li *et al.* 2020). Future priorities include functional validation of host susceptibility genes, durability assessment of resistant cultivars, and field deployment of RNAi- and CRISPR-based targeting of viral determinants (Roy and Bagewadi 2020). Integrating genomic surveillance with precision vector management will be critical to prevent adaptive outbreaks and sustain chili production.

This study provides molecular and quantitative evidence of TYLCKaV infection in chili pepper from Bengkulu, Indonesia, and demonstrates significant host-dependent variation in viral accumulation and symptom expression among isolates. By integrating sequence characterization with relative viral load analysis, this study advances current understanding of pathogenic variability within the *Begomovirus* complex. The findings establish a quantitative framework for evaluating infection dynamics and epidemiological risk in emerging chili production areas. They may inform future surveillance programs, disease management strategies, and breeding efforts to manage resistance in tropical agroecosystems.

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