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Genomic Characterization and Comparative Evolutionary Analysis of Pepper Yellow Leaf Curl Viruses in Southeast Asia

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Abstract

Pepper yellow leaf curl Indonesia virus (PepYLCIV), a bipartite begomovirus transmitted by *Bemisia tabaci*, is a major viral pathogen affecting chili pepper production in Indonesia. Although complete genome sequences are available for several pepper yellow leaf curl viruses, comparative evolutionary analyses across all recognized species remain limited. This study generated complete genome sequences for 3 PepYLCIV isolates using Oxford Nanopore sequencing and analyzed them together with publicly available genomes representing 4 recognized pepper yellow leaf curl virus species. The 3 isolates displayed the typical bipartite genome organization of PepYLCIV. Comparative genome analyses detected 14 recombination events in DNA-A and 9 in DNA-B, indicating that recombination has contributed substantially to genome diversification. Phylogenetic reconstruction and pairwise nucleotide identity analyses consistently placed the newly sequenced isolates within the PepYLCIV clade and resolved the 4 virus species as distinct evolutionary groups. Among the species examined, PepYLCIV showed the highest level of nucleotide diversity. Selection analyses indicated stronger purifying selection acting on DNA-A (ω = 0.049–0.111) than on DNA-B (ω = 0.768–0.839), consistent with the greater sequence variability of DNA-B. Population genetic analyses also revealed marked genetic differentiation among species ($^{\beta}F_{ST}$ = 0.765–0.991 for DNA-A and 0.751 for DNA-B). These findings expand the genomic resources available for PepYLCIV, provide new insights into the evolution of pepper yellow leaf curl viruses, and support future epidemiological surveillance, molecular detection, and resistance breeding in chili pepper.

Keywords: Begomovirus; Oxford Nanopore sequencing; population genetics; purifying selection; recombination

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INTRODUCTION

Pepper yellow leaf curl disease has become a major threat to chili pepper cultivation throughout Indonesia and much of Southeast Asia. The disease is associated with members of the genus *Begomovirus* (family *Geminiviridae*), which are naturally transmitted by the whitefly

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Bemisia tabaci. Begomovirus genomes consist of circular single-stranded DNA and occur in either monopartite or bipartite forms. In bipartite species, the DNA-A component carries genes required for viral replication and particle formation, whereas DNA-B encodes proteins responsible for virus movement within infected plants and the development of disease symptoms (Jackel et al., 2016; Fiallo-Olivé et al., 2021; Ruhel et al., 2021).

Among the begomoviruses infecting chili pepper, Pepper yellow leaf curl Indonesia virus (PepYLCIV) is the predominant species in Indonesia. Originally described by Hidayat et al. (1999) and later recognized as a distinct species by Sakata et al. (2008), PepYLCIV has since been reported throughout the country's major chili-producing regions (Koeda et al., 2016; Selangga and Listihani, 2021; Wahyono et al., 2023; Santosa et al., 2024). Recent surveys have further demonstrated its broad geographic distribution and substantial genetic diversity in provinces such as West Java and East Java (Nurenik and Rastono, 2025; Polosoro et al., 2026). Infection typically causes leaf yellowing, mosaic, curling, and plant stunting, as well as reduced fruit production, often leading to considerable yield losses (Temaja et al., 2022; Sayekti et al., 2023; Noviana et al., 2024).

Besides PepYLCIV, several closely related begomoviruses have been identified in Southeast Asia. These include Pepper yellow leaf curl Aceh virus (PepYLCAV), reported from Indonesia and Malaysia (Kesumawati et al., 2019; Sau et al., 2020); Pepper yellow leaf curl Thailand virus (PepYLCThV), detected in Thailand, Vietnam, and Laos (Chiemsombat et al., 2018; An et al., 2021; Nguyen et al., 2024); and Pepper yellow leaf curl virus (PepYLCV), described from China (Li et al., 2021). The International Committee on Taxonomy of Viruses (ICTV) currently recognizes these 4 viruses as distinct species associated with yellow leaf curl disease of chili pepper.

Although complete genome sequences are available for the recognized pepper yellow leaf curl virus species, previous studies have mainly focused on individual species or a limited number of isolates. Consequently, evolutionary relationships across the pepper yellow leaf curl virus complex remain incompletely understood, particularly regarding recombination, genetic diversity, selection pressure, and population genetic structure. Comparative analyses based on complete genome sequences representing all 4 recognized species are needed to clarify the evolutionary processes driving diversification within this virus complex.

Whole-genome sequencing has become an effective approach for investigating virus evolution because it captures complete genomic variation that cannot be resolved using partial genome sequences. Oxford Nanopore sequencing has been successfully applied to characterize several begomoviruses, demonstrating its suitability for rapid and accurate whole-genome analysis (Chalupowicz et al., 2019; Naito et al., 2019; Leiva et al., 2020; Martins et al., 2021). The availability of complete genome sequences enables integrated analyses of genome organization, recombination, phylogenetic relationships, selection pressure, and population genetic diversity.

In this study, researchers characterized 3 PepYLCIV isolates collected from chili peppers using Oxford Nanopore sequencing. The newly generated genomes were analyzed alongside publicly available genomes of PepYLCIV, PepYLCAV, PepYLCV, and PepYLCThV to investigate genome organization, recombination patterns, phylogenetic relationships, genetic diversity, selection pressure, and population structure. The findings enhance the genomic resources accessible for PepYLCIV and deepen our comprehension of the evolution of pepper yellow leaf curl viruses in Southeast Asia.

MATERIALS AND METHOD

Virus inoculation

An isolate of PepYLCIV maintained at the Plant Virology Laboratory, IPB University, was used in this study. The isolate was routinely propagated on the susceptible chili cultivar 'Bara' (*Capsicum annuum*) (Saputra, 2025). To obtain fresh infected plant material for molecular analyses, the virus was transmitted to 3 susceptible chili genotypes by the whitefly *B. tabaci*: 'Bara' (*C. annuum*), 'Red Habanero' (*Capsicum chinense*), and the breeding line F8 012328-6-2-1-1-3-1 (*C. annuum*). 'Bara' was included as the susceptible control and maintenance host, whereas 'Red Habanero' and the F8 breeding line were selected based on their reported susceptibility to PepYLCIV (Saputra, 2025). The method outlined by Ganefianti et al. (2017) was used for virus transmission.

Adult whiteflies were allowed a 24-hour acquisition access period to infected source plants, after which they were allowed a 48-hour

inoculation access period on healthy plants with 10 whiteflies per plant. Each chili genotype consisted of 3 biological replicates, with 10 plants per replicate. Thirty days after inoculation, symptomatic leaves from all 10 plants in each biological replicate were pooled to form a single composite sample. About 100 mg of the pooled leaf tissue was utilized to extract the genomic DNA.

DNA extraction

Genomic DNA was isolated from approximately 100 mg of leaf tissue using a modified phenol extraction protocol adapted from Haible et al. (2006). Leaf samples were ground in liquid nitrogen with a micropestle before the addition of 800 µl of extraction buffer (100 mM Tris-HCl, pH 7.5; 1 mM EDTA; 100 mM NaCl; 100 mM DTT; and 0.6% SDS) together with 20 µl of RNase A (10 mg ml^{-1}). The homogenate was extracted with an equal volume of phenol:chloroform (9:1), incubated for 15 minutes at room temperature, and centrifuged at 14,000 rpm for 10 minutes. Approximately 700 µl of the aqueous phase was transferred to a fresh 1.5 ml microcentrifuge tube, mixed with an equal volume of chloroform:isoamyl alcohol (24:1), and centrifuged again under the same conditions. About 600 µl of the resulting supernatant was recovered, and genomic DNA was precipitated by adding an equal volume of isopropanol. The DNA pellet was collected by centrifugation at 14,000 rpm for 5 minutes, washed twice with 80% ethanol, air-dried, and dissolved in 20 µl of TE buffer. Before PCR amplification and rolling circle amplification (RCA), the DNA solution was incubated at 65 °C for 60 minutes.

PepYLCIV detection

PCR amplification was carried out with NZYTaq II 2× Green Master Mix (NZYtech, Portugal). Each reaction (10 µl) consisted of 6 µl of master mix, 2 µl of template DNA (50 ng $µl^{-1}$), and 1 µl each of the forward and reverse primers (10 µM). The DNA-A component was amplified using the universal begomovirus primer pair SPG-1 (5′-CCCCKGTGCGWRAATCCAT-3′) and SPG-2 (5′-ATCCVAAYWTYCAGGGAG CTAA-3′) (Li et al., 2004), which yields an amplicon of approximately 912 bp. DNA-B amplification employed the PepYLCIV-specific primers PepYLCIV DNA-B F (5′-TGTCCTCAT CGTAGTCACACA-3′) and PepYLCIV DNA-B R (5′-GAAGATAGTCTGTACCGTCATGTAC-3′) (Koeda et al., 2018), producing an expected fragment of approximately 385 bp.

Amplification of DNA-A was carried out with an initial denaturation step at 94 °C for 2 minutes, followed by 35 amplification cycles consisting of denaturation at 94 °C for 1 minute, primer annealing at 59 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension was performed at 72 °C for 10 minutes. For DNA-B, PCR began with an initial denaturation at 94 °C for 2 minutes, followed by 45 cycles of 94 °C for 30 seconds, 68 °C for 30 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 3 minutes. Amplified DNA fragments were resolved on 1% agarose gels containing 0.004% FluoroSafe DNA Stain (1st BASE, Singapore).

Rolling circle amplification (RCA)

RCA was carried out with phi29 DNA polymerase (10 U $µl^{-1}$; Thermo Fisher Scientific Baltics, Lithuania). Genomic DNA (200 ng) was mixed with 1 µl of 10× reaction buffer, 1 µl of 100 µM random hexamer primers, and nuclease-free water to a volume of 15 µl. The mixture was heated at 95 °C for 5 minutes and rapidly cooled on ice for 3 to 5 minutes to denature the DNA and facilitate primer annealing. After cooling, an additional 1 µl of 10× reaction buffer, 1 µl of 100 µM random hexamer primers, 2 µl of dNTP mix, 1 µl of phi29 DNA polymerase, and nuclease-free water were added to achieve a final reaction volume of 20 µl. Amplification was performed at 36 °C for 18 hours, after which the enzyme was inactivated by incubation at 65 °C for 10 minutes. The amplified DNA was stored at -20 °C until subsequent analyses. To verify successful amplification, RCA products were digested with *Eco*RI, which linearizes circular viral DNA for electrophoretic analysis. Both digested and undigested RCA products were resolved on 1.5% agarose gels prepared in 1× TBE buffer and electrophoresed at 45 V for 120 minutes.

Sequencing

The identity of PepYLCIV was verified by Sanger sequencing of PCR amplicons prior to whole-genome sequencing. For each chili genotype, equal amounts of RCA products from 3 biological replicates were pooled and analyzed on the PromethION 24 platform (Oxford Nanopore Technologies) at the Sequencing Laboratory, Genomic Building, KST Soekarno, National Research and Innovation Agency (BRIN), Cibinong, Indonesia.

Genome assembly and annotation

Whole-genome sequencing data were processed on the Galaxy Europe platform

following the workflow described by Sun et al. (2022). Sequence quality was initially assessed using NanoPlot, followed by adapter trimming with Porechop and quality filtering using fastp. High-quality long reads were assembled using Flye and subsequently aligned to the reference genomes using Minimap2 to facilitate reference-guided consensus polishing. Reference sequences for DNA-A and DNA-B were downloaded from the NCBI RefSeq assembly GCF_000867505.1 (NC_008283.1 and NC_008284.1, respectively). Consensus genome sequences were generated with the Medaka consensus pipeline and compared with sequences in the NCBI GenBank database through BLASTn to identify the closest matches. Genome annotation and visualization were performed using SnapGene v8.0.2.

The complete DNA-A and DNA-B genome sequences generated in this study have been submitted to the NCBI GenBank database under accession numbers PX237379 (F8 DNA-A), PX237380 (F8 DNA-B), PX237381 (Red Habanero DNA-A), PX237382 (Red Habanero DNA-B), PX237383 (Bara DNA-A), and PX237384 (Bara DNA-B). Raw Oxford Nanopore sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1497540.

Comparative genome analysis

Complete genome sequences of pepper yellow leaf curl viruses available in the NCBI GenBank database up to January 31, 2025, were retrieved for comparative analyses (Supplementary Table S1). The dataset consisted of 8 PepYLCIV, 7 PepYLCAV, 3 PepYLCV, and 4 PepYLCThV isolates. These reference genomes were aligned together with the 3 PepYLCIV genomes generated in the present study using the ClustalW multiple-sequence alignment algorithm implemented in MEGA11 (Tamura et al., 2021). Because PepYLCV possesses a monopartite genome, DNA-B sequences were unavailable for the APWS and TDWS-21 isolates.

Putative recombination events were screened using the Recombination Detection Program version 5 (RDP5 v5.30) with the 7 detection algorithms: RDP, GENECONV, Bootscan, MaxChi, Chimera, SiScan, and 3Seq (Martin et al., 2021). Only recombination events supported by at least 5 algorithms with a Bonferroni-adjusted *p*-value below 0.05 were retained for subsequent analyses. Phylogenetic relationships of the DNA-A and DNA-B datasets were reconstructed independently using the maximum-likelihood algorithm in MEGA11. The Tamura 3-parameter substitution model (Tamura, 1992) was selected because it provides an appropriate compromise between model fit and computational efficiency for closely related nucleotide sequences. Confidence in the inferred topology was evaluated from 1,000 bootstrap pseudoreplicates. Pairwise nucleotide sequence identities were estimated with the Sequence Demarcation Tool (SDT v1.2) (Muhire et al., 2014).

Population genetics and selection analyses

Population genetic analyses were conducted using DnaSP v6.12.03 (Rozas et al., 2017), with each virus species considered a separate population. Genetic diversity was characterized using haplotype number (*h*), haplotype diversity (*Hd*), variable sites (*S*), total mutational events (η), mean pairwise nucleotide differences (*k*), and nucleotide diversity (π). Selective pressure was inferred from the ratio of nonsynonymous to synonymous substitutions ($\omega = dN/dS$), where dN and dS represent nonsynonymous and synonymous substitution rates, respectively. Values of $\omega > 1$ indicate positive selection, $\omega = 1$ is consistent with neutral evolution, and $\omega < 1$ reflects purifying selection. Neutrality was examined using Tajima's D together with Fu and Li's *D** and *F** statistics (Tajima, 1989; Fu and Li, 1993). For datasets meeting the required sample size, neutrality profiles were additionally evaluated using a sliding-window approach with a window length of 100 bp and a step size of 25 bp.

Population differentiation among the virus species was assessed in DnaSP using the statistics K_S*, K_{ST}*, *Z**, S_{nn}, and F_{ST} (Hudson, 2000). The fixation index (F_{ST}) provides a quantitative measure of genetic differentiation, where values approaching 0 indicate little or no differentiation and values approaching 1 indicate highly differentiated populations (Hudson et al., 1992; Hudson, 2000). In general, F_{ST} values exceeding 0.33 are interpreted as evidence of restricted gene flow and substantial population differentiation (Morca et al., 2024).

RESULTS AND DISCUSSION

PepYLCIV detection and partial genome sequencing

Following transmission by *B. tabaci*, the virus isolates established successful infections in all 3 healthy chili pepper genotypes and induced symptoms characteristic of begomovirus disease,

including chlorosis, mosaic, leaf curling, and leaf distortion (Figure 1A–C). Disease severity differed among the tested genotypes. 'Bara' exhibited pronounced yellow mosaic, severe leaf curling, and stunted growth, whereas 'Red Habanero' developed yellow mosaic accompanied by reduced leaf size. The F8 breeding line (012328-6-2-1-1-3-1) showed yellow mosaic leaf together with downward leaf curling and leaf crinkling. These phenotypic differences most likely reflect variation in host responses to PepYLCIV infection and are consistent with symptom variability previously documented for chili pepper-begomovirus interactions (Paradisa et al., 2022; Wahyono et al., 2023).

PCR amplification using the universal begomovirus primers SPG1/SPG2 generated the expected ~912 bp DNA-A fragment from all 3 samples, whereas PepYLCIV-specific primers amplified the expected ~385 bp DNA-B fragment (Figure 2A and 2B). SPG1/SPG2 amplified the conserved DNA-A region, whereas the PepYLCIV-specific primers amplified DNA-B, confirming the presence of both genomic components. BLASTn analysis identified all amplicons as PepYLCIV. The successful amplification of both genomic components, together with BLASTn analysis, confirmed that all 3 isolates were PepYLCIV, a bipartite begomovirus. These confirmed PepYLCIV isolates were subsequently subjected to whole-genome sequencing, followed by comparative evolutionary and population genetic analyses.

Genome sequencing and genome organization

Complete genome sequences were obtained from the 3 PepYLCIV isolates following RCA and Oxford Nanopore sequencing. Comparison

Figure 1. Symptoms of PepYLCIV infection in (A) *Capsicum annuum* 'Bara', (B) *Capsicum chinense* 'Red Habanero', and (C) *Capsicum annuum* F8 012328-6-2-1-1-3-1

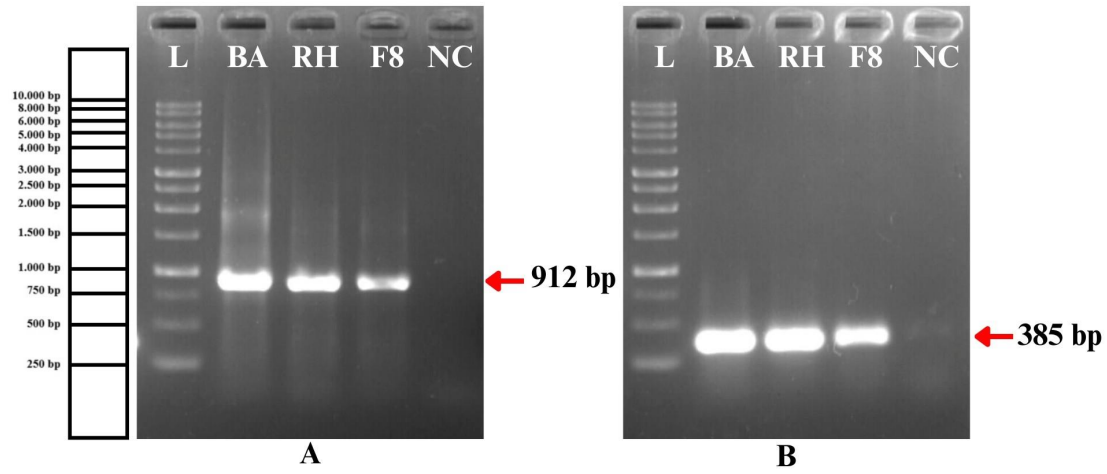

Figure 2. Agarose gel electrophoresis of PCR amplicons corresponding to PepYLCIV DNA-A (A) and DNA-B (B)

Note: Samples represented chili pepper genotypes of 'Bara' (BA), 'Red Habanero' (RH), and F8 012328-6-2-1-1-3-1 (F8). Negative control of the PCR reaction was represented as NC. Lane L contained the DM3100 ExcelBand™ 1 kb DNA Ladder (0.25–10 kb)

of undigested genomic DNA (gDNA), *Eco*RI-digested gDNA, undigested RCA products, and *Eco*RI-digested RCA products verified the successful enrichment of circular DNA molecules (Figure 3). After *Eco*RI digestion, the RCA products consistently yielded 3 fragments of approximately 1.2, 1.5, and 2.5 kb (Figure 3D), whereas the digested gDNA appeared as a continuous smear because the host genome contains numerous *Eco*RI restriction sites (Figure 3C). The occurrence of multiple fragments following RCA suggests that several circular DNA molecules were present in the infected samples. The largest fragment most likely corresponds to the viral genomic components (DNA-A or DNA-B), whereas the smaller fragments may represent other circular DNA molecules whose identities remain to be determined. Comparable RCA fragmentation patterns have previously been described for several begomoviruses (Haible et al., 2006; Lozano et al., 2016).

Whole-genome sequencing confirmed the preliminary PCR and Sanger sequencing results, identifying all 3 isolates as PepYLCIV. Oxford Nanopore sequencing generated between 243,023 and 434,239 raw reads per isolate, with mean read lengths ranging from 2,534 to 4,582 bp and read N50 values ranging from 4,248 to 8,986 bp. Following quality filtering, 54.98 to 90.48% of the reads were retained, providing 0.582 to 1.204 Gb of high-quality sequence data for genome assembly (Supplementary Table S2). The complete DNA-A genomes ranged from 2,750 to 2,763 nt, whereas DNA-B ranged from 2,748 to 2,764 nt (Figure 4; Supplementary Table S3). Genome annotation identified 6 coding sequences in DNA-A (*AV1*, *AV2*, *AC1*, *AC2*, *AC3*, and *AC4*), while DNA-B encoded the *BV1* nuclear shuttle protein and *BC1* movement protein. Both genomic components contained the conserved stem-loop motif harboring the nonanucleotide sequence TAATATTAC, which functions as the origin of rolling-circle replication in begomoviruses (Hanley-Bowdoin et al., 2013). The overall genome architecture of the 3 isolates closely matched that reported previously for bipartite begomoviruses (Roshan et al., 2017; Fiallo-Olivé and Navas-Castillo, 2023).

Despite the widespread distribution and economic importance of PepYLCIV in Indonesia, relatively few complete genome sequences have been available in GenBank. The 3 complete genomes generated in this study substantially expand the genomic resources available for this emerging virus. Recently, Polosoro et al. (2026) reported considerable genetic diversity among PepYLCIV isolates collected from major chili-producing areas in West Java, based primarily on coat protein gene sequences. Although that study analyzed partial rather than complete viral genomes, its results are consistent with the extensive genomic variation observed here and further highlight the value of expanding the collection of complete genome sequences. A larger collection of complete genomes will improve the accuracy of recombination analyses, phylogenetic reconstruction, and population genetic inference by providing more representative sampling across virus populations (Rozas et al., 2017; Fiallo-Olivé and Navas-Castillo, 2023). The genome sequences generated in this study constitute an additional

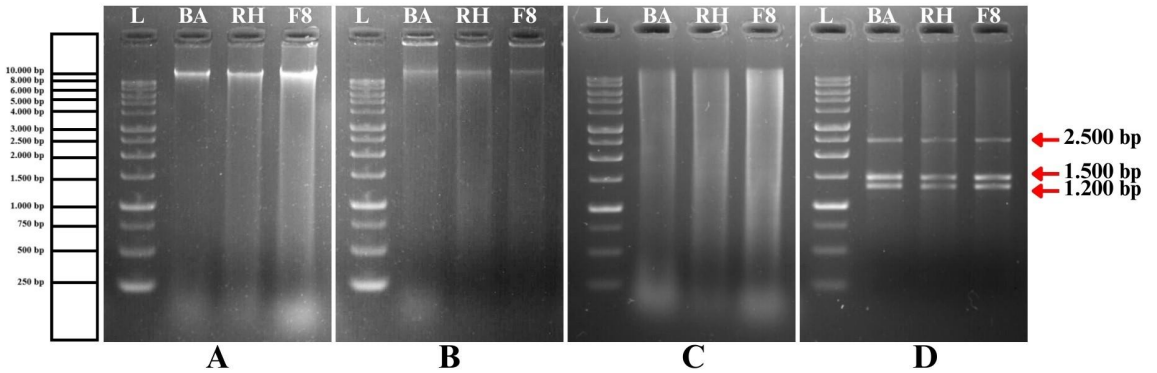

Figure 3. Agarose gel electrophoresis of genomic DNA (gDNA) RCA products. (A) Undigested gDNA, (B) Undigested RCA products, (C) *Eco*RI-digested gDNA, and (D) *Eco*RI-digested RCA products separated on a 1% agarose gel

Note: Lane L contains the DM3100 ExcelBand™ 1 kb DNA Ladder (0.25–10 kb). Lanes BA, RH, and F8 correspond to PepYLCIV isolates recovered from the 'Bara', 'Red Habanero', and F8 012328-6-2-1-1-3-1 genotypes, respectively

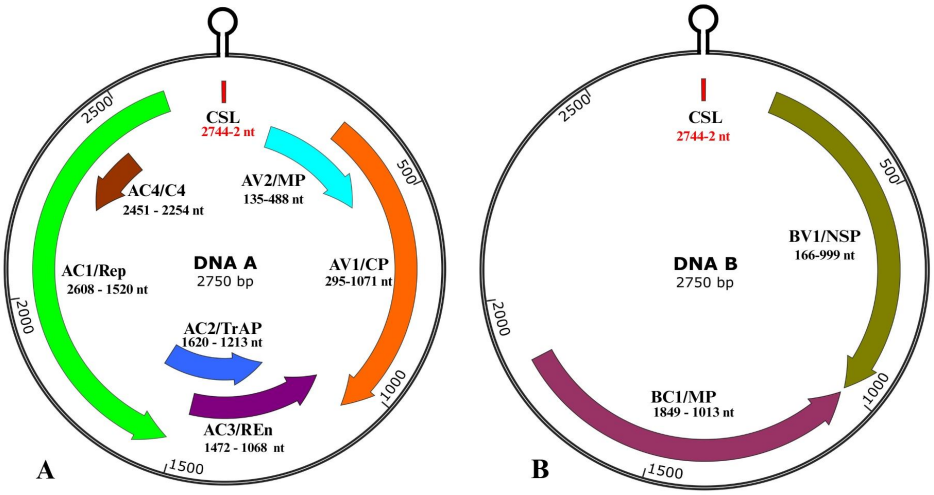

Figure 4. Schematic representation of the DNA-A (A) and DNA-B (B) genomes of PepYLCIV from the 'Bara', 'Red Habanero', and F8 012328-6-2-1-1-3-1 isolates

Note: The diagrams were constructed from the consensus genome sequences of the 3 isolates using SnapGene v8.0.2. Predicted open reading frames are shown as directional arrows, with genes encoded on the virion-sense strand positioned above the genome and those on the complementary-sense strand below. The location of the conserved stem-loop (CSL) region is also indicated

genomic resource for future epidemiological investigations, evolutionary analyses, and improved molecular detection of PepYLCIV.

Recombination analysis

Recombination analysis revealed extensive genetic exchange among pepper yellow leaf curl viruses. Among the 25 DNA-A sequences, 14 recombination events were detected involving 10 isolates, whereas analysis of the 20 DNA-B sequences identified 9 events affecting 7 isolates (Supplementary Table S4). These findings highlight the substantial contribution of recombination to the evolutionary diversification of both genomic components.

Several recombination events were detected among closely related PepYLCV species, indicating that genetic material has been exchanged repeatedly during their evolutionary history in Southeast Asia. Earlier work suggested that the genome of PepYLCAV arose through recombination, with PepYLCIV acting as the principal parental lineage. In contrast, Pumpkin yellow mosaic virus (PuYMV) from Malaysia and Tomato leaf curl New Delhi virus (ToLCNDV) from Indonesia contributed to different genomic components (Kesumawati et al., 2019). This research analysis further supports the importance of recombination by showing that PepYLCAV isolates BATo-50 and APWS acquired genomic regions from PepYLCIV. In contrast, PepYLCIV isolates NC_008283 and TDWS-2 appear to have inherited genomic segments from PepYLCAV BATo-46. Additional recombination events involved parental viruses originating from different countries, including PepYLCV from China and PepYLCThV from Thailand, highlighting the regional evolutionary connectivity of pepper yellow leaf curl viruses across Southeast and East Asia.

The divergent isolate PepYLCIV-[IN:Bali:Tom:19] deserves particular attention. Although no recombination event was detected for this isolate in the present analysis, it shared only 89.9 to 91.6% nucleotide identity with other PepYLCIV isolates. It exhibited 79.6% nucleotide identity with Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) from Cambodia. This observation supports previous reports describing PepYLCIV-[IN:Bali:Tom:19] as a highly divergent PepYLCIV isolate (Neriya et al., 2020) and suggests that additional unsampled parental lineages may have contributed to its evolutionary history.

Comparable patterns of inter-group recombination were also detected in DNA-B. For example, PepYLCAV BATa-10 appears to have originated through recombination between PepYLCIV BA_C1 from Indonesia and PepYLCThV Sava01 from Laos. Collectively, these findings identify recombination as the principal mechanism underlying genome diversification within the PepYLCV complex. Recombination is a hallmark of begomovirus evolution and frequently occurs when mixed infections allow template switching during rolling-circle replication (Lefeuvre et al., 2019;

Fiallo-Olivé and Navas-Castillo, 2023). The widespread cultivation of pepper, overlapping host ranges, and efficient transmission by *B. tabaci* likely increase opportunities for repeated encounters among divergent begomoviruses throughout Southeast Asia, thereby facilitating genetic exchange among co-circulating viruses. Consequently, recombination provides an efficient evolutionary mechanism for generating novel genetic variants while preserving essential genome organization, accelerating viral adaptation, and contributing to the continued emergence of genetically distinct virus lineages across the region.

Phylogenetic relationships and nucleotide sequence identity

Phylogenetic reconstruction using complete DNA-A sequences consistently placed the 3 newly characterized isolates within the PepYLCIV clade together with previously reported PepYLCIV isolates (Figure 5A). The other sequences resolved into 3 strongly supported clades representing PepYLCAV, PepYLCV, and PepYLCThV, confirming the genetic separation of the 4 recognized species. Interestingly, 2 isolates previously designated as PepYLCV showed incongruent phylogenetic positions. TDWS-21 clustered within the PepYLCIV clade, whereas APWS grouped with PepYLCAV, suggesting that their original classifications should be reconsidered based on complete genome analyses.

Phylogenetic reconstruction using DNA-B sequences resolved 2 major lineages (Figure 5B). Group 1 comprised DNA-B sequences associated with PepYLCIV and PepYLCAV, whereas Group 2 consisted exclusively of PepYLCThV isolates. Within Group 1, PepYLCAV DNA-B sequences formed a distinct subclade, while PepYLCIV isolates occupied several basal positions, indicating greater genetic diversity within PepYLCIV than within PepYLCAV. The close phylogenetic relationship between PepYLCIV and PepYLCAV likely reflects their shared evolutionary history and overlapping geographic distributions in Indonesia and neighboring Malaysia. This close relationship is further supported by the extensive recombination detected between the 2 species, suggesting repeated historical genetic exchange before their divergence into distinct evolutionary lineages. Nevertheless, because recombination can influence phylogenetic inference by generating mosaic genomes with different evolutionary histories, the inferred relationships should be interpreted in conjunction with the recombination analyses.

Future studies incorporating larger datasets and recombination-aware phylogenetic approaches will help further resolve the evolutionary history of pepper yellow leaf curl viruses. In contrast, PepYLCThV isolates from Thailand, Laos, and Vietnam clustered as a distinct lineage, suggesting a prolonged period

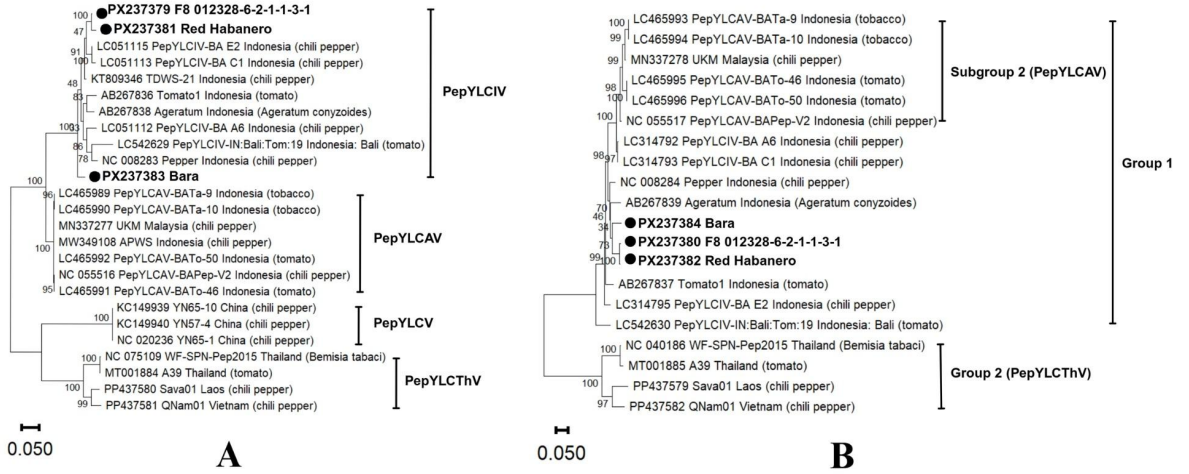

Figure 5. Maximum-likelihood phylogenetic trees reconstructed from complete DNA-A (A) and DNA-B (B) genome sequences of pepper yellow leaf curl viruses

Note: Trees were generated in MEGA11 using the Tamura 3-parameter nucleotide substitution model with uniform evolutionary rates across sites. Node supports were estimated from 1,000 bootstrap replicates. Three PepYLCIV isolates characterized in this study are marked with black circles. Four virus species are represented in the phylogeny: PepYLCV, PepYLCIV, PepYLCAV, and PepYLCThV

of evolutionary separation accompanied by limited genetic exchange. These observations collectively suggest that geographical distribution, shared host range, and historical recombination have jointly shaped the present genetic structure of pepper yellow leaf curl viruses across Southeast Asia, in agreement with previous studies on the evolutionary dynamics of begomoviruses (Lefeuvre et al., 2019; Fiallo-Olivé and Navas-Castillo, 2023). The clear separation of the 2 DNA-B groups further supports the genetic divergence between these virus lineages.

Sequence Demarcation Tool (SDT) analysis was consistent with the phylogenetic reconstruction (Figures 6 and 7). DNA-A sequences of PepYLCAV and PepYLCV shared more than 99% nucleotide identity within each species, reflecting their relatively low intraspecific diversity. PepYLCThV isolates exhibited greater variation in nucleotide identity (> 91%), largely attributable to the recombinant isolate QNam01, which showed lower sequence identity (90.8 to 94.8%) than the remaining isolates. Most PepYLCIV isolates shared more than 93% nucleotide identity; however, the divergent isolate PepYLCIV-[IN:Bali:Tom:19] displayed only 89.9 to 91.6% identity with other PepYLCIV isolates, consistent with its distinct phylogenetic placement. Similar patterns were observed for DNA-B, where Group 1 isolates shared more than 92% nucleotide identity, except for the recombinant PepYLCIV-[IN:Bali:Tom: 19], which exhibited only 86.8 to 88.5% identity with other Group 1 members. In contrast, nucleotide identity between Groups 1 and 2 ranged from only 62.1 to 64.6%, indicating substantial evolutionary divergence between the 2 DNA-B lineages.

Among the 3 newly sequenced isolates, 'Red Habanero' and F8 (012328-6-2-1-1-3-1) shared approximately 98% nucleotide identity, whereas 'Bara' exhibited approximately 95% identity with both isolates. Despite this level of intraspecific variation, all 3 clustered robustly with the previously reported PepYLCIV genomes, including the first complete genomes described by Sakata et al. (2008). The reclassification of APWS isolate as PepYLCAV, rather than PepYLCV as previously proposed (Cania et al., 2021), further demonstrates the value of complete genome sequencing for accurate begomovirus taxonomy.

The phylogenetic reconstruction and pairwise nucleotide identity analyses consistently identified PepYLCIV and PepYLCAV as sister taxa within the PepYLCV complex, likely reflecting their shared evolutionary history and geographic distribution in Indonesia and Malaysia. By comparison, PepYLCThV from Thailand, Laos, and Vietnam, and PepYLCV from China form more divergent evolutionary lineages. These findings reveal substantial genetic diversification of pepper yellow leaf curl viruses across Southeast and East Asia and provide a robust genomic framework for understanding virus emergence, regional dispersal, and species differentiation.

Selection pressure, genetic diversity, and neutrality analyses

Population genetic analyses performed in DnaSP revealed marked differences in sequence diversity among the 4 pepper yellow leaf curl virus species (Table 1). PepYLCAV and PepYLCV showed relatively low levels of genetic variation within DNA-A, as reflected by fewer segregating sites (*S*), lower numbers of mutational events (η), reduced mean pairwise nucleotide differences (*k*), and lower nucleotide diversity (π). This pattern is consistent with virus populations experiencing strong evolutionary constraints or relatively recent dispersal (Lefeuvre et al., 2019). In contrast, PepYLCIV and PepYLCThV exhibited substantially higher estimates for these diversity parameters, indicating greater genetic heterogeneity within their populations. The wider geographic distribution of PepYLCIV, together with its extensive history of recombination, probably contributed to the elevated level of genetic diversity. Comparable relationships between geographic range and genetic diversity have been reported for begomoviruses as well as other plant virus groups (Neoh et al., 2023; Crespo-Bellido et al., 2024). Recombination has likewise been recognized as an important evolutionary mechanism generating genetic diversity in begomoviruses (Kumar et al., 2017).

Across both DNA-B groups, values of *S*, η, and *k* were consistently higher than those observed for DNA-A, indicating greater sequence divergence in the genomic component encoding movement-associated functions. This pattern agrees with previous reports that DNA-B generally exhibits greater sequence variability than DNA-A because it is subject to relatively weaker functional constraints while encoding proteins involved in virus movement and host adaptation (Nigam, 2021; Fiallo-Olivé and Navas-Castillo, 2023). Consistent with these observations, haplotype diversity (*Hd*) reached a maximum of 1.0 across

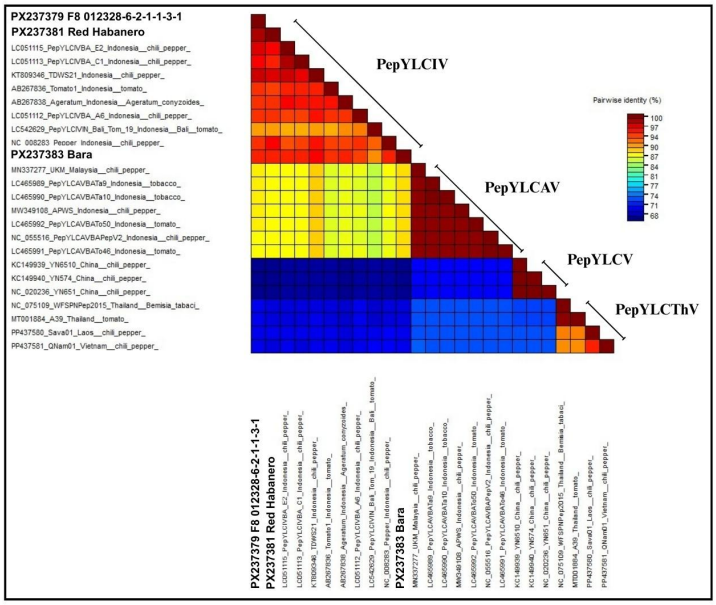

Figure 6. Matrix of pairwise sequence similarities calculated from the complete DNA-A genome sequences of 4 pepper yellow leaf curl virus species: PepYLCV, PepYLCIV, PepYLCAV, and PepYLCThV

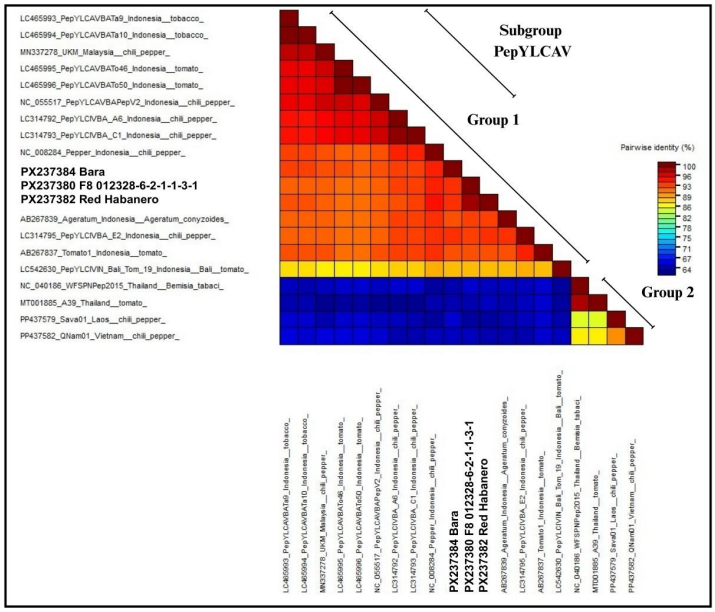

Figure 7. Matrix of pairwise sequence similarities calculated from the entire DNA-B genome sequences of PepYLCIV, PepYLCAV, and PepYLCThV

all analyzed populations, demonstrating that each isolate represented a unique haplotype and highlighting extensive genetic differentiation within the pepper yellow leaf curl virus complex.

Selection pressure analyses revealed distinct evolutionary constraints acting on the 2 genomic components. Estimates of the dN/dS ratio showed that DNA-A evolved under much stronger selective constraint (ω = 0.049 to 0.111) than DNA-B (ω = 0.768 to 0.839), indicating that nonsynonymous substitutions were removed more efficiently from DNA-A (Table 1). This difference is consistent with the functional organization of the begomovirus genome. DNA-A carries genes required for essential viral processes, including genome replication, gene expression regulation, and virion formation, whereas DNA-B encodes movement-associated proteins responsible for cell-to-cell and long-distance transport within the host (Fiallo-Olivé and Navas-Castillo, 2023). The comparatively relaxed selective constraint on DNA-B may

provide greater evolutionary flexibility, facilitating adaptation to different host species and vector populations and thereby promoting the ecological diversification of begomoviruses.

Across most populations, Tajima's *D*, together with Fu and Li's *D* and *F*, produced mainly negative values (Table 2). Such a distribution of neutrality statistics is characteristic of an excess of rare polymorphic sites and is commonly interpreted as evidence of purifying selection or recent population expansion (Tajima, 1989; Fu and Li, 1993). However, none of these statistics were significant, likely due to the relatively small sample sizes and limited statistical power (Rozas et al., 2017). Therefore, the neutrality tests alone provide only limited evidence for recent demographic changes.

Collectively, these results demonstrate that the evolutionary history of pepper yellow leaf curl viruses reflects the combined effects of 2 complementary processes. Frequent recombination generates new genetic variation, particularly within PepYLCIV, whereas strong purifying selection maintains the integrity of essential viral functions encoded primarily by DNA-A. By contrast, the relatively relaxed selective constraints acting on DNA-B permit greater sequence divergence, providing evolutionary flexibility for adaptation to different host plants, vector populations, and ecological conditions. Successful single-stranded DNA plant viruses often evolve through a balance between recombination, which introduces novel genetic variation, and purifying selection, which preserves essential genome functions. This evolutionary pattern probably contributes to the long-term persistence and adaptability of the pepper yellow leaf curl virus complex (Lefeuvre et al., 2019; Fiallo-Olivé and Navas-Castillo, 2023).

Population differentiation and gene flow

Population differentiation analysis using DnaSP provided quantitative support for the phylogenetic relationships reconstructed from the complete viral genomes (Table 3). All pairwise comparisons among the 4 pepper yellow leaf curl virus species exhibited high values of K_S*, K_{ST}*, and *Z**, together with a maximum nearest-neighbor statistic (S_{nn} = 1.000) and significant *p*-values, indicating strong genetic differentiation among the virus populations. These results

Table 1. Population genetic diversity indices and polymorphism statistics of complete genomes from 4 pepper yellow leaf curl viruses

Species	*N*	*h*	*Hd*	*S*	*η*	*k*	π	dS	dN	ω
DNA-A										
PepYLCIV	11	11	1.000	476	552	160.455	0.059	0.172	0.009	0.052
PepYLCAV	7	7	1.000	29	31	10.000	0.004	0.009	0.001	0.111
PepYLCV	3	3	1.000	8	8	5.333	0.002	nd	nd	nd
PepYLCThV	4	4	1.000	311	327	188.333	0.069	0.181	0.009	0.049
DNA-B										
Group 1	16	16	1.000	701	863	195.033	0.073	0.031	0.026	0.839
Group 2	4	4	1.000	556	608	334.333	0.125	0.069	0.053	0.768

Note: *N* = number of isolates; *h* = haplotype count; *Hd* = haplotype diversity; *S* = variable sites; *η* = total mutations; *k* = mean number of nucleotide differences among sequences; π = nucleotide diversity per site; dN = nonsynonymous nucleotide diversity; dS = synonymous nucleotide diversity; ω = dN/dS; nd = not determined, analysis not performed because fewer than 4 isolates were available

Table 2. Neutrality test statistics for complete genomes of 4 pepper yellow leaf curl viruses

Species	Fu and Li's *D**	Fu and Li's *F**	Tajima's *D*
DNA-A			
PepYLCIV	-0.616^{ns}	-0.730^{ns}	-0.721^{ns}
PepYLCAV	-1.147^{ns}	-1.280^{ns}	-1.198^{ns}
PepYLCV	nd	nd	nd
PepYLCThV	0.744^{ns}	0.771^{ns}	0.587^{ns}
DNA-B			
Group 1	-1.036^{ns}	0.360^{ns}	-1.091^{ns}
Group 2	-1.217^{ns}	0.335^{ns}	0.086^{ns}

Note: nd = not determined, analysis not performed because fewer than 4 isolates were available; ^{ns} = not significant (*p*-value > 0.10)

Table 3. Estimates of genetic differentiation among the 4 begomoviruses examined in this study, derived from pairwise comparisons of complete genome sequences

Comparisons	$^{\alpha}K_S$*	$^{\alpha}K_{ST}$*	*p*-value	$^{\alpha}Z$*	*p*-value	S_{nn}	*p*-value	$^{\beta}F_{ST}$
DNA-A								
PepYLCIV (n = 11) vs PepYLCAV (n = 7)	4.046	0.203	0.000***	3.240	0.000***	1.000	0.000***	0.765
PepYLCIV (n = 11) vs PepYLCV (n = 3)	4.674	0.159	0.002**	2.983	0.002**	1.000	0.003**	0.913
PepYLCIV (n = 11) vs PepYLCThV (n = 4)	4.980	0.130	0.000***	3.159	0.000***	1.000	0.000***	0.797
PepYLCAV (n = 7) vs PepYLCV (n = 3)	2.277	0.479	0.009**	2.239	0.004**	1.000	0.009**	0.991
PepYLCAV (n = 7) vs PepYLCThV (n = 4)	3.098	0.358	0.000***	2.467	0.000***	1.000	0.000***	0.872
PepYLCV (n = 3) vs PepYLCThV (n = 4)	3.879	0.288	0.007**	1.422	0.037*	1.000	0.037*	0.873
DNA-B								
Group 1 (n = 16) vs Group 2 (n = 4)	5.137	0.099	0.000***	3.895	0.000***	1.000	0.001**	0.751

Note: ** 0.001 < *p*-value > 0.01; *** *p*-value < 0.001. $^{\alpha}K_S$*, K_{ST}*, *Z**, and S_{nn} are statistical tests to evaluate genetic differentiation among populations. $^{\beta}F_{ST}$ indicates the degree of gene differentiation between populations

demonstrate that isolates were consistently assigned to their respective evolutionary lineages with little evidence of genetic overlap.

F_{ST} further confirmed the marked genetic separation among the 4 virus species. DNA-A comparisons yielded F_{ST} values ranging from 0.765 to 0.913, indicating very limited gene flow and substantial population subdivision. Similarly, the comparison of DNA-B between Groups 1 and 2 yielded a high F_{ST} value of 0.751, supporting the evolutionary divergence of the 2 major DNA-B lineages. According to the criterion adopted in this study, F_{ST} values exceeding 0.33 suggest increasing genetic differentiation among populations (Morca et al., 2024). Therefore, the values observed in this study reflect highly structured virus populations with minimal genetic exchange.

The population genetic analyses were fully consistent with the phylogenetic reconstruction and nucleotide identity analyses. The concordance among these independent approaches provides robust evidence that PepYLCIV, PepYLCAV, PepYLCV, and PepYLCThV represent genetically distinct evolutionary lineages rather than geographically distributed variants of a single virus species. Notably, the combination of S_{nn} = 1.000 and an F_{ST} value of 0.765 between PepYLCIV and PepYLCAV demonstrates that, despite their relatively close evolutionary relationship, these viruses constitute separate species with restricted gene flow.

Together, these findings demonstrate that pepper yellow leaf curl viruses comprise well-defined and genetically isolated evolutionary populations across Southeast Asia. The consistently high F_{ST} values and maximum S_{nn} statistics indicate that gene flow among species is extremely limited, allowing each lineage to follow an independent evolutionary trajectory. Consequently, adaptive mutations arising within one species are unlikely to spread extensively into other species unless facilitated by future recombination events. These results reinforce the current taxonomic classification of the virus complex while highlighting recombination, rather than routine gene flow, as the main mechanism linking otherwise distinct evolutionary lineages. Recombination can create new viral variants that may bypass current host defenses or broaden the host range. Therefore, ongoing genomic monitoring of PepYLCIV populations is necessary (Lefeuvre et al., 2019; Fiallo-Olivé and Navas-Castillo, 2023). Complete genome sequences obtained in this research provide an important baseline for monitoring future evolutionary changes and supporting pepper resistance breeding programs.

CONCLUSIONS

Three complete genomes of PepYLCIV were sequenced and compared with the available genomes of related pepper yellow leaf curl viruses. The findings suggest that recombination

has been a crucial factor in the evolution of these viruses, whereas DNA-A exhibits greater conservation than DNA-B as a result of more intense purifying selection. Phylogenetic and population genetic analyses clearly differentiated the 4 pepper yellow leaf curl virus species. These results expand the genomic information available for PepYLCIV and enrich our knowledge about the evolution of pepper yellow leaf curl viruses in Southeast Asia. The genomic resources obtained in this study can also facilitate the development of molecular diagnostic tools and breeding programs to enhance resistance to pepper yellow leaf curl viruses.

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DECLARATION OF GENERATIVE AI USE

The authors utilized OpenAI's ChatGPT (GPT-5.5) exclusively for language editing and manuscript refinement.

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Supplementary Table S1. Reference isolates included in the comparative genomic analyses

No.	Virus	Isolate	Origin	Host	Accession number		Reference
					DNA-A	DNA-B	
1	PepYLCIV	Pepper	Indonesia	Chili pepper	NC008283	NC008284	Sakata et al. (2008)
2	PepYLCIV	Tomato1	Indonesia	Tomato	AB267836	AB267837	Sakata et al. (2008)
3	PepYLCIV	Ageratum	Indonesia	Ageratum	AB267838	AB267839	Sakata et al. (2008)
4	PepYLCIV	TDWS-21	Indonesia	Chili pepper	KT809346	-	Jamsari and Pedri (2013)
5	PepYLCIV	PepYLCIVBA A6	Indonesia	Chili pepper	LC051112	LC314792	Koeda et al. (2016, 2018)
6	PepYLCIV	PepYLCIVBA C1	Indonesia	Chili pepper	LC051113	LC314793	Koeda et al. (2016, 2018)
7	PepYLCIV	PepYLCIVBA E2	Indonesia	Chili pepper	LC051115	LC314795	Koeda et al. (2016, 2018)
8	PepYLCIV	Bali:Tom:19	Indonesia	Tomato	LC542629	LC542630	Neriya et al. (2020)
9	PepYLCAV	PepYLCAVBATa9	Indonesia	Tobacco	LC465989	LC465993	Kesumawati et al. (2019)
10	PepYLCAV	PepYLCAVBATa10	Indonesia	Tobacco	LC465990	LC465994	Kesumawati et al. (2019)
11	PepYLCAV	PepYLCAVBATo46	Indonesia	Tomato	LC465991	LC465995	Kesumawati et al. (2019)
12	PepYLCAV	PepYLCAVBATo50	Indonesia	Tomato	LC465992	LC465996	Kesumawati et al. (2019)
13	PepYLCAV	PepYLCAVBAPepV2	Indonesia	Chili pepper	NC055516	NC055517	Kesumawati et al. (2019)
14	PepYLCAV	UKM	Malaysia	Chili pepper	MN337277	MN337278	Sau et al. (2020)
15	PepYLCAV	APWS	Indonesia	Chili pepper	MW349108	-	Cania et al. (2021)
16	PepYLCV	YN65-10	China	Chili pepper	KC149939	-	Li et al. (2021)
17	PepYLCV	YN57-4	China	Chili pepper	KC149940	-	Li et al. (2021)
18	PepYLCV	YN65-1	China	Chili pepper	NC020236	-	Li et al. (2021)
19	PepYLCThV	A39	Thailand	Tomato	MT001884	MT001885	An et al. (2021)
20	PepYLCThV	WF-SPN-Pep2015	Thailand	Whitefly	NC075109	NC040186	Yule et al. (2019)
21	PepYLCThV	Sava01	Laos	Chili pepper	PP437580	PP437579	Nguyen et al. (2024)
22	PepYLCThV	QNam01	Vietnam	Chili pepper	PP437581	PP437582	Nguyen et al. (2024)

Supplementary Table S2. Summary of Oxford Nanopore sequencing quality metrics for the three PepYLCIV isolates

Metric	Bara	F8 (012328-6-2-1-1-3-1)	Red Habanero
Raw reads	401,006	243,023	434,239
Raw bases (Gb)	1.31	1.11	1.10
Mean read length (bp)	3,275	4,582	2,534
Median read length (bp)	1,112	2,605	1,587
N50 (bp)	8,986	8,588	4,248
Mean quality score (Q)	12.70	11.2	10.80
Reads ≥ Q10 (%)	92.50	79.2	75.20
Reads ≥ Q15 (%)	38.10	15.4	8.80
Reads retained after filtering (%)	90.48	62.54	54.98
High-quality bases after filtering (Gb)	1.204	0.70	0.58
Q20 bases after filtering (%)	74.04	62.63	60.34
Q30 bases after filtering (%)	54.13	18.09	18.20
GC content after filtering (%)	35.58	35.61	35.00

Supplementary Table S3. Genomic features of the DNA-A and DNA-B components of the three PepYLCIV isolates

No.	Isolate	F8 012328-6-2-1-1-3-1	Red Habanero	Bara
DNA-A	Accession number	PX237379	PX237381	PX237383
1	*AV1*	295-1071 nt	295-1071 nt	295-1071 nt
2	*AV2*	135-448 nt	135-448 nt	135-448 nt
3	*AC1*	2608-1520 nt	2608-1520 nt	2608-1520 nt
4	*AC2*	1620-1213 nt	1620-1213 nt	1620-1213 nt
5	*AC3*	1472-1068 nt	1472-1068 nt	1472-1068 nt
6	*AC4*	2451-2254 nt	2451-2254 nt	2451-2254 nt
7	*CSL*	2744-2 nt	2744-2 nt	2757-2 nt
DNA-B	Accession number	PX237380	PX237382	PX237384
1	*BV1*	167-1000 nt	166-999 nt	167-1000 nt
2	*BC1*	1850-1014 nt	1849-1013 nt	1850-1014 nt
3	*CSL*	2742-2 nt	2744-2 nt	2758-2 nt

Supplementary Table S4. Putative recombination events detected in DNA-A and DNA-B genomes of pepper yellow leaf curl viruses using RDP5 v5.30

No.	Recombinant isolate	Major/Minor parents	Beginning/Ending breakpoint[1]	RDP implemented method (*p*-value)
DNA-A				
1	PepYLCAV-BATo-50 (PepYLCAV, Indonesia)	PepYLCIV-[IN:Bali:Tom:19] (PepYLCIV, Indonesia)/ TDWS-21 (PepYLCIV, Indonesia)	36/1925	R (1.349×10^{-18}) G (3.5×10^{-16}) B (6.212×10^{-20}) M (2.031×10^{-21}) C (1.263×10^{-20}) S (6.274×10^{-20}) 3S (1.173×10^{-43})
2	Red Habanero (PepYLCIV, Indonesia)	F8 012328-6-2-1-1-3-1 (PepYLCIV, Indonesia)/NC_008283 (PepYLCIV, Indonesia)	1/667	G (1.124×10^{-5}) B (1.111×10^{-6}) M (2.262×10^{-10}) C (3.799×10^{-10}) S (6.847×10^{-26}) 3S (1.446×10^{-24})
3	NC_008283 (PepYLCIV, Indonesia)	PepYLCAV-BATo-46	1474/2750	R (7.609×10^{-6}) G (2.235×10^{-4}) B (2.004×10^{-2})

Supplementary Table S4. Continued

No.	Recombinant isolate	Major/Minor parents	Beginning/Ending breakpoint[1]	RDP implemented method (*p*-value)
		(PepYLCAV, Indonesia)/PepYLCIV-BA_C1 (PepYLCIV, Indonesia)		M (7.067×10^{-6}) C (5.667×10^{-3}) S (1.414×10^{-10}) 3S (5.967×10^{-6})
4	TDWS-21 (PepYLCIV, Indonesia)	PepYLCAV-BATo-46 (PepYLCAV, Indonesia)/PepYLCIV-BA_E2 (PepYLCIV, Indonesia)	381/1104	R (2.030×10^{-6}) G (1.773×10^{-5}) M (7.472×10^{-8}) C (6.856×10^{-8}) S (2.362×10^{-12}) 3S (4.994×10^{-9})
5	TDWS-21 (PepYLCIV, Indonesia)	PepYLCIV-BA_C1 (PepYLCIV, Indonesia)/PepYLCIV-BA_A6 (PepYLCIV, Indonesia)	1419/1870	R (4.906×10^{-2}) M (6.461×10^{-6}) C (4.693×10^{-4}) S (1.605×10^{-5}) 3S (2.514×10^{-3})
6	PepYLCIV-BA_C1 (PepYLCIV, Indonesia)	Red Habanero (PepYLCIV, Indonesia)/PepYLCIV-BA_E2 (PepYLCIV, Indonesia)	1009/2748	G (2.750×10^{-2}) B (8.259×10^{-3}) M (7.009×10^{-7}) C (7.030×10^{-6}) S (7.359×10^{-17}) 3S (5.131×10^{-9})
7	Tomato1 (PepYLCIV, Indonesia)	PepYLCIV-BA_E2 (PepYLCIV, Indonesia)/F8 012328-6-2-1-1-3-1 (PepYLCIV, Indonesia)	1/1266 and 2260/2747	R (3.260×10^{-2}) G (2.735×10^{-2}) M (6.617×10^{-8}) C (1.106×10^{-7}) S (1.685×10^{-13}) 3S (8.861×10^{-6})
8	YN-65-1 (PepYLCV, China)	WF-SPN-Pep2015 (PepYLCThV, Thailand)/Ageratum (PepYLCIV, Indonesia)	2170/2558	R (5.896×10^{-6}) G (1.118×10^{-3}) B (5.147×10^{-5}) M (3.000×10^{-7}) C (5.024×10^{-6}) S (1.335×10^{-49}) 3S (2.914×10^{-6})
9	YN-65-1 (PepYLCV, China)	WF-SPN-Pep2015 (PepYLCThV, Thailand)/Bara (PepYLCIV, Indonesia)	2178/2308	R (1.791×10^{-2}) M (3.538×10^{-3}) C (2.228×10^{-3}) S (4.705×10^{-9}) 3S (1.839×10^{-3})
10	PepYLCIV-BA_E2 (PepYLCIV, Indonesia)	Bara (PepYLCIV, Indonesia)/F8 012328-6-2-1-1-3-1 (PepYLCIV, Indonesia)	1/1029 and 2267/2749	R (2.191×10^{-2}) G (3.389×10^{-3}) B (3.667×10^{-2}) M (9.472×10^{-8}) C (9.661×10^{-6}) S (2.563×10^{-11})
11	QNam01 (PepYLCThV, Vietnam)	WF-SPN-Pep2015 (PepYLCThV, Thailand)/PepYLCAV-BATo-46	2026/2127	R (1.940×10^{-5}) G (4.36×10^{-3}) M (4.275×10^{-3}) C (6.224×10^{-3})

Supplementary Table S4. Continued

No.	Recombinant isolate	Major/Minor parents	Beginning/Ending breakpoint[1]	RDP implemented method (*p*-value)
		(PepYLCAV, Indonesia)		3S (7.779×10^{-4})
12	APWS (PepYLCAV, Indonesia)	YN-65-1 (PepYLCV, China)/Bara (PepYLCIV, Indonesia)	2272/2578	G (3.772×10^{-2}) M (7.234×10^{-4}) C (2.718×10^{-3}) S (2.045×10^{-2}) 3S (9.629×10^{-4})
DNA-B				
1	PepYLCAV-BATa-10 (Group 1)	PepYLCIV-BA_C1 (Group 1)/Sava01 (Group 2)	1/33 and 2606/2732	R (6.803×10^{-26}) G (1.911×10^{-6}) B (1.911×10^{-18}) M (2.841×10^{-13}) C (1.675×10^{-20}) 3S (1.811×10^{-13})
2	PepYLCAV-BAPep-V2 (Group 1)	PepYLCIV-BA_A6 (Group 1)/PepYLCAV-BATa-10 (Group 1)	2409/2718	R (1.779×10^{-10}) G (2.383×10^{-8}) B (1.792×10^{-10}) M (4.586×10^{-12}) C (4.441×10^{-12}) S (9.132×10^{-17}) 3S (1.189×10^{-18})
3	UKM (Group 1)	Bara (Group 1)/Sava01 (Group 1)	2631/3718	R (2.368×10^{-14}) G (1.227×10^{-11}) B (3.360×10^{-7}) M (1.689×10^{-9}) C (7.153×10^{-6}) S (7.799×10^{-16}) 3S (1.649×10^{-11})
4	A39 (Group 2)	WF-SPN-Pep2015 (Group 2)/Sava01 (Group 2)	2618/2660	R (1.033×10^{-14}) G (5.949×10^{-12}) B (9.388×10^{-6}) M (9.913×10^{-7}) C (3.815×10^{-4}) S (4.489×10^{-4}) 3S (5.388×10^{-8})
5	NC_008284 (Group 1)	F8 012328-6-2-1-1-3-1 (Group 1)/Red Habanero (Group 1)	1/619	B (5.302×10^{-3}) M (6.315×10^{-7}) C (2.582×10^{-3}) S (9.794×10^{-31}) 3S (2.808×10^{-12})
6	PepYLCAV-BATo-50 (Group 1)	PepYLCAV-BATa-10 (Group 1)/PepYLCIV-BA_A6 (Group 1)	828/2492	R (3.475×10^{-3}) B (2.470×10^{-3}) M (1.525×10^{-7}) C (8.644×10^{-7}) S (2.244×10^{-18}) 3S (6.199×10^{-10})
7	PepYLCIV-[IN:Bali:Tom:19] (Group 1)	Bara (Group 1)/Tomato1 (Group 1)	1/42 and 2279/2721	R (1.534×10^{-3}) B (2.466×10^{-3}) M (8.415×10^{-9}) C (6.016×10^{-7}) S (4.533×10^{-4})

Supplementary Table S4. Continued

No.	Recombinant isolate	Major/Minor parents	Beginning/Ending breakpoint[1]	RDP implemented method (*p*-value)
				3S (6.386×10^{-8})

Note: [1]Position in alignment. [2]R = RDP; G = GENECONV; B = BootScan; M = MaxChi; C = Chimera; S = SiScan; 3S = 3Seq