



Infectious clone–based dissection of delayed pathogenicity and evolutionary dynamics of *Papaya leaf curl Guangdong virus* (PaLCuGdV)

Renshu Huang · Muhammad Arif ·
Amna Saeed · Cheng Song · Omer Beyhan ·
Muhammed Said Yolcu · Islem Abid

Received: 27 November 2025 / Accepted: 12 February 2026 / Published online: 20 February 2026
© The Author(s) 2026

Abstract *Papaya leaf curl Guangdong virus* (PaLCuGdV) is an economically significant begomovirus, although its pathogenicity and evolutionary dynamics are poorly understood. This study involved the construction of a full infectious clone of PaLCuGdV, which was agroinoculated into *Nicotiana benthamiana* (*N. benthamiana*) to assess the infectivity and symptomatology. The infectious clone elicited typical begomoviral symptoms just at 28 days post-inoculation, signifying delayed pathogenicity. Infection efficiency varied with strain, with successful transmission achieved using *Agrobacterium* GV301, but not with EHA105 alone. Southern blot analysis confirmed active viral DNA replication solely in diseased plants. Comparative sequencing and recombination analysis identified numerous recombination events among worldwide PaLCuGdV isolates, with the newly defined strain serving as a primary

recombinant progenitor. These findings indicate that PaLCuGdV displays delayed symptom expression, strain-specific infectivity, and evolutionary diversity driven by recombination, underscoring the value of infectious clone systems for functional and evolutionary studies of begomoviruses.

Keywords Infectious clone construction · Delayed pathogenicity · Strain-dependent infectivity · And recombination analysis

Background

The begomoviruses, which are members of the largest family of plant viruses, family: *Geminiviridae*, are transmitted to plants by the haplotype insect vector whitefly (*Bemisia tabaci*) and contain ssDNA

R. Huang · C. Song (✉)
College of Life and Health, West Anhui University,
Luan 237012, China
e-mail: lanniao812329218@163.com

M. Arif (✉)
Department of Plant Protection, Faculty of Agriculture,
Sakarya University of Applied Sciences, Arifiye 54580,
Sakarya, Türkiye
e-mail: muhammadarif@subu.edu.tr

A. Saeed
Department of Plant Protection, Faculty of Agriculture,
Harran University, Şanlıurfa 63050, Türkiye

O. Beyhan
Department of Horticulture, Faculty of Agriculture,
Sakarya University of Applied Sciences, Arifiye 54580,
Sakarya, Türkiye

M. S. Yolcu
Field Crops Division, Faculty of Agriculture, Sakarya
University of Applied Sciences, Arifiye 54580, Sakarya,
Türkiye

I. Abid
Center of Excellence in Biotechnology Research (CEBR),
DSR, King Saud University, P.O 2455, 11495 Riyadh,
Saudi Arabia

molecules enclosed in twinned particles. These viruses are classified into two types: monopartite begomoviruses (DNA-A) and bipartite begomoviruses (DNA A and DNA B) (Arif et al., 2020, 2022; Ban et al., 2021; Naveed et al., 2023). DNA B is essential for nuclear localization, host range determination, symptom expression, and systemic infection. On the other hand, DNA A is required for the virion production and replication of bipartite begomoviruses (Amin et al., 2021; Breves et al., 2023). Both components have a noncoding region that is approximately 200 base pairs long. This section contains the sequence patterns necessary for determining gene expression and replication (Guerrero et al., 2020; Ouattara et al., 2022).

The whole genome of begomoviruses is often cloned directly from separated dsDNA (Makesh Kumar et al., 2021), from a whole-genome PCR amplicon (Arif et al., 2018) or from a genome component produced by RCA (Mehta et al., 2020; Singh et al., 2020). Intergenic recombination may occur when viruses from two different genera of *geminiviridae* are present at the same time. Genetic recombination integrates the historical diversity of the viral genome into the host genome, enhancing its richness and facilitating novel pathways for virus evolution and the coevolution of begomoviruses. This approach offers novel avenues for examining the intricacies of begomoviruses (Pandey et al., 2021; Scholthof, 2023). Currently, it is common for begomoviruses and dicot-infecting master viruses to coinfect the same host; thus far, three cases have been documented (Arif, 2024; Crespo-Bellido et al., 2021). In each of these situations, the host is distinct and comprises of diverse plant groups. Interactions among co-infecting begomoviruses can result in the emergence of complicated diseases or the manifestation of more virulent virus strains or novel begomovirus species through recombination events (Naveen et al., 2025). The coinfection of (*chickpea chlorotic dwarf virus*; CPCDV genus mastervirus) with begomoviruses infecting hosts from distinct families (*Asteraceae*, *Malvaceae*, and *Cucurbitaceae*) in weed, cotton, and squash has been reported previously (Azeem et al., 2022; Fahmy et al., 2015; Mosharaf et al., 2020).

Papaya leaf curl disease and various other distinct species within this group are transmitted by cryptic whitefly species. This disease complex can have a substantial detrimental effect on the overall health

of papaya plants and the productivity of their crops. The disease is distinguished by the curling and deformation of the leaves, limited growth, and diminished fruit production (Soni et al., 2022; Varun et al., 2024). The PaLCuGdV is a monopartite begomovirus classified within the family *Geminiviridae* and the genus *Begomovirus*. It first emerged in Guangdong Province, China, and transmitted by the whitefly (*Bemisia tabaci*). The virus infects papaya (*Carica papaya*), resulting in distinct symptoms including pronounced leaf curling, vein thickening, leaf distortion, and slowed growth, potentially causing substantial yield losses. PaLCuGdV contains a circular single-stranded DNA genome (~2.7 kb) that encodes proteins associated with replication, encapsidation, motility, and the inhibition of host defensive mechanisms. Similar to other begomoviruses, it exhibits genetic diversity and may be associated with betasatellites, which might exacerbate symptom severity. Owing to its effective vector transmission and adaptability, PaLCuGdV poses an escalating threat to papaya cultivation in tropical and subtropical areas, underscoring the necessity for molecular surveillance and integrated disease management strategies (Huang et al., 2020; Jing et al., 2026). PaLCuGdV has caused substantial reductions in crop yields, ranging from 40 to 100% (Alcalá-Briseño et al., 2020; Soni et al., 2022; Srivastava et al., 2022).

Infectious clones offer a reliable experimental framework for examining virus–host interactions, satisfying Koch’s postulates, and analysing viral pathogenicity under controlled conditions (Jailani et al., 2025). Furthermore, comparative analyses utilizing infectious clones provide a direct connection between biological characteristics such as symptom severity or latency and the fundamental evolutionary mechanisms, including recombination and sequence divergence (Jyotika et al., 2024). To generate a standard infectious clone, the process normally includes cloning a complete genome. This cloned genome is then subcloned as a tandem, consisting of one and a half or two genome components, into a suitable vector for agroinoculation. The process of building genomic components that consist of two replication sources is laborious and time-consuming (Souza et al., 2022). In certain cases, when a single copy of a virus genome is cloned and inserted into DNA of a specific length, it can function as an infectious clone for certain viruses (Möller & Maruthi, 2025; Nair et al., 2020). Previous

studies have shown that most infectious clones are composed of tandem repeat structures of geminivirus/begomovirus genomes, which contribute to their increased pathogenicity. Consequently, this characteristic complicates the cloning procedure (Shakir et al., 2023; Sharma & Ruhel, 2019). Begomoviruses' infection clones are constructed via a multimeric approach that begins with the careful design and selection of cloning sites, followed by the assembly of viral genomic segments that are produced via the use of restriction endonucleases. This is becoming a very time-consuming procedure (Neubauer et al., 2025; Wang et al., 2024).

In addition, selecting clones with tandem-repeat viral genomes requires careful inspection of the insert quality. A novel method called rolling circle amplification (RCA) has recently been developed to amplify certain DNA or betasatellites in a linear or symmetrical manner (Oliveira et al., 2021). Multiple infectious clones of various geminiviruses have been produced with the assistance of RCA (Fondong, 2025; Quadros & Zerbini, 2025). Although molecular detection and genome sequencing of PaLCuGdV have been reported, its pathogenic characteristics, infection efficacy, and evolutionary dynamics remain unverified in experimental infectious clone systems. Specifically, it is uncertain if PaLCuGdV demonstrates quick or delayed symptom onset, whether infectivity is affected by the compatibility of *Agrobacterium* strains, and the role of recombination in its genetic diversity. The indexing, identification, and classification of papaya diseases are challenging because of the lack of knowledge regarding pests and diseases related to papaya. The primary hypothesis of this study posits that PaLCuGdV demonstrates delayed pathogenicity and strain-dependent infectivity in *Nicotiana benthamiana*, with its evolutionary diversification driven by frequent recombination events. To evaluate this hypothesis, the aims of this study were to: (i) develop and biologically validate a complete infectious clone of PaLCuGdV; (ii) assess symptomatology and infection efficacy post-agroinoculation with various *Agrobacterium* strains; and (iii) examine the evolutionary relationships and recombination dynamics of PaLCuGdV relative to global isolates. This study seeks to elucidate the pathogenic behaviour and adaptive potential of PaLCuGdV by integrating functional infectivity testing with evolutionary analysis.

Methods

Viral source collection and extraction of DNA

This study utilized a begomovirus PaLCuGdV, which was obtained from various areas within the Guangdong region of China. The prevalence of disease was substantial, characterized by a large population of cryptic whiteflies (*B. tabaci*) on host plants that were affected. The isolation of DNA from leaves exhibiting symptoms of virus infection was conducted through a meticulous protocol as described by (Lavanya & Arun, 2019). This protocol involved the utilization of cetyl trimethyl ammonium bromide (CTAB) for the isolation of viral DNA.

Cloning of PCR products and sequence analysis

The infected plants yielded full-length DNA A and B. Whole PaLCuGdV DNA A and DNA B were acquired from the RCA/PCR amplification products using the primer pair PaLCuGdV-F (5'-CGTTTG GTACTCCCCAAGA-3') and PaLCuGdV-R (5'-CAACAGCAAAGCATTCTCAGT-3'), which were subsequently cloned and subjected to sequencing. PCR reactions were performed in a final volume of 25 µL containing template DNA, 1×PCR buffer, MgCl₂, dNTPs, forward and reverse primers, and Taq DNA polymerase. The thermal cycling conditions consisted of an initial denaturation at 94 °C, followed by 35 cycles of denaturation, annealing, and extension, with a final extension step at 72 °C. After purification via a gel extraction kit following the manufacturer's recommendations, the PCR products were cloned and inserted into the pTZ57R/T vector (Fermentas). The PCR products, both cloned and un-cloned products, were sequenced from Macrogen, Korea. The DNASTAR Inc. program Lasergene was used to analyze the sequences (Chen et al., 2024). The already submitted sequences in the database (<http://www.ncbi.nlm.nih.gov/BLAST/>) were compared with the obtained sequences to determine sequence similarity. The Open Reading Frames (ORFs) finder tool, accessible at <http://www.ncbi.nlm.nih.gov/gorf/gorf.html>, was utilized to identify the ORFs of the obtained sequences.

Infectious clone construction and infiltration of *Agrobacterium*

Agrobacterium strains (EHA105, GV301) carrying a suitable plasmid were cultured in liquid broth medium (LB) enriched with the antibiotics kanamycin and rifampicin at an optical density of 0.8 at 28 °C to infect *N. benthamiana* with begomovirus (PaLCuGdV). After centrifugation at 6000–12000 RPM for 1 min, the cultures were resuspended in induction medium supplemented with 10 mM 2-(N-morpholino) ethane sulfonic acid (MES) at pH 5.6, 10 mM MgCl₂, and 150 µM acetosyringone. To create an infectious begomovirus clone, the SalI enzyme was used to partially break down the RCA product. The SalI enzyme in the pCAM-BIA1301 binary vector was used to clone head-to-tail tandem repeat dimers. The reliability of this infectious clone was verified through sequencing. The acquired sequence confirmed the authenticity of the infectious clone of PaLCuGdV. *Agrobacterium* strain GV301 was individually transformed with tandem repeat-containing recombinant pCAM-BIA1301. The amended *Agrobacterium* was grown to 1.0 at 600 nm. The cultures were incubated at room temperature for 3 h in induction buffer.

Prior to agro-infection, the *Agrobacterium* cultures containing the virus and satellite were combined in equal proportions (1:1). The culture mixture was introduced into the lower surface of the cotyledon leaves of 2-week-old seedlings. After *Agrobacterium* inoculation, *N. benthamiana* plants were maintained in an incubator at 25 °C and 80% relative humidity. After 20 days of *Agrobacterium* penetration, an infection clone of PaLCuGdV was injected into the plants following the methods of (Zhang et al., 2024). Young *N. benthamiana* leaves were injected into the lower lamina via a superfluous syringe. The agroinoculated plants were maintained at 25 °C in a controlled environment (Udavatha et al., 2022). After viral inoculation, *N. benthamiana* plants were cultivated in a traditional greenhouse at 22–24 °C with 16 h of daylight. After 28 days of *Agrobacterium* infiltration, the DNA and RNA from newly formed leaves of infected plants were extracted. Using both DNA and RNA samples, the expression levels of the target genes were evaluated.

Southern blot analysis for validation of infection

A total of 10 µg of genomic DNA extracted from PaLCuGdV, which was found in *Agrobacterium*-mediated *N. benthamiana*, was subjected to digestion with EcoRI. The digested DNA was then electrophoresed on an agarose gel and allowed to run for the entire night. After being treated with a solution containing 0.5 M NaOH and 1.5 M NaCl, the DNA was transferred to a nylon membrane via the capillary transfer technique. Following UV crosslinking, the DNA underwent 16 h of temperature-controlled hybridization with a DIG-labeled DNA probe at 42 °C. After the membrane was assembled with the aid of the CPSD substrate, it was examined via an anti-DIG antibody (Diggins & Hancock, 2023; Ramesh et al., 2021).

Comparative evolutionary divergence, history, phylogenetic, and recombination analyses

The NCBI isolates of begomoviral isolates and their satellites were used to understand their lineage. Using PaLCuGdV-associated viral isolates, an ancestor tree was created for this virus. This phylogenetic tree was built via maximum likelihood in MEGA11. The maximum composite likelihood model was used to analyze and quantify the degree of evolutionary divergence between the derived sequences of this plant virus. The Sequence Demarcation Tool (SDT) with SimPlot was used to compare both new and old PaLCuGdV isolates. The sequence alignment was performed via ClustalW. Recombination analysis was conducted utilizing RDP4 software on aligned full-length PaLCuGdV genome sequences retrieved in the present study, alongside representative isolates obtained from the NCBI database. The analyses utilized various recombination detection techniques inside RDP4, including RDP, BootScan, MaxChi, Chimaera, SiScan, and 3Seq, employing default parameters and a Bonferroni-adjusted significance threshold of $p < 0.05$. Only recombination events validated by a minimum of four separate methods were deemed reliable and incorporated into subsequent analysis (Bornancini et al., 2020; Silva et al., 2022; Tamura et al., 2023).

Results

The effective creation of the PaLCuGdV infectious clone was validated via restriction digestion and sequencing, confirming the integrity of the tandem-repeat viral genome. Subsequent to agroinoculation, *N. benthamiana* plants were observed weekly to evaluate infection efficacy and symptom development. In contrast to other begomoviruses that manifest symptoms within two weeks, PaLCuGdV-inoculated plants exhibited no symptoms during the initial three weeks post-inoculation. Distinct leaf curling, vein banding, and growth retardation manifested only after 28 days, signifying a delayed onset of pathogenicity. After four weeks of agroinoculation with EHA105, a mixed infection with the desired begomovirus was detected in *N. benthamiana*, although the onset of symptoms varied, as shown in Table 1. These symptoms confirmed the ability of the infectious clone to infect host plants. At 28 dpi, visible symptoms continued to develop, resembling typical begomovirus infection patterns.

A distinct disparity in infectivity was noted across *Agrobacterium* strains. Plants infected with GV301 exhibited distinct begomoviral symptoms and tested positive for viral DNA, while those inoculated solely with EHA105 remained asymptomatic throughout the trial duration. The results indicate that the infection efficacy of PaLCuGdV is significantly affected by the *Agrobacterium* strain utilized for delivery, underscoring a crucial experimental variable frequently neglected in infectious clone research.

Co-inoculation of *N. benthamiana* with EHA105 did not result in any symptoms. Plants that remained asymptomatic after 28 days of post inoculation were found to be in a similar state, indicating that their health status was effectively unchanged. Typical signs of PaLCuGdV infection became apparent after 28 days. No symptoms were observed in *N. benthamiana* plants following inoculation with EHA105. The results revealed that EHA105, which carries begomovirus, did not satisfy Koch's postulates and was unable to co-infect other hosts, as depicted in Table 1. All EHA105 assays were conducted exclusively in *N. benthamiana*; therefore, conclusions regarding host range or coinfection potential are restricted to this host.

Southern blot research revealed direct evidence of active virus replication in infected plants. Viral DNA signals were consistently identified in diseased plants, but asymptomatic plants exhibited no detectable hybridization signals. The stringent correlation between symptom manifestation and viral DNA accumulation verifies that the identified disease phenotype arose from effective systemic infection rather than ephemeral expression or mechanical injury during agroinoculation. The PaLCuGdV infectious clone caused developmental retardation, vein clearing, and mottling in the majority of inoculated plants, which presented characteristic begomovirus symptoms. Three out of five plants inoculated with GV301 developed symptoms, whereas two plants remained symptom free. Following agroinoculation, the symptoms of the *N. benthamiana* plants were similar to those observed under field conditions. Plants that did

Table 1 Insertion of infectious clones with designated strains via agroinoculation in *N. benthamiana*

Strain/Plant host	Number of inoculated plants	Incubation time	Signs/symptoms	Detection positivity via southern blot
<i>N. benthamiana</i> GV301	3/5	1*–35 α	C, D, RLM, IR, DR, LL	All positive
<i>N. benthamiana</i> , GV301 + EHA105	3/4, 4/4	1*–35 α	LL, Y, CL, Chs, VB, B	All positive
<i>N. benthamiana</i> EHA105	4/4	1*–35 α	healthy	All negative

*Days for first appearance of symptoms

α Days at which leaves were collected for detection

Symptoms: C=curling, D=distortion, RLM=rolling leaf margins, IR=inward rolling, DR=downward rolling, LL=leathery leaves, Y=yellowing, B=Brittling, VB=vein banding, CL=curled leaves, Chs=Chlorosis

not show symptoms yielded negative results in the Southern blot, whereas all symptomatic plants tested positive.

Through phylogenetic and sequence demarcation analyses, the genetic links between several isolates of PaLCuGdV were ascertained to understand its variety and track its evolutionary history. The ancestral history of this begomoviral species was established in MEGA11, as shown in Figs. 1 and 2. The node number represents the bootstrap value, which was determined by 1000 repeats. The sequences of the agro-infected plants collected in this study are indicated by circles. The EHA105 and GV301 strains were used to establish the ancestral tree. Further research should explore the evolutionary factors that

lead to PaLCuGdV diversification or the possibility of cross-species transmission, among other suggested concerns.

The overall sequence pairs and average base substitutions per site are shown in Table 2. The bootstrap process (1000 repeats) yielded the standard error estimate(s) displayed in the second column. The maximum composite likelihood method was used for the analyses. For every sequence pair (pairwise deletion option), all uncertain locations were eliminated.

During evaluation, the relative values of instantaneous r were considered. To simplify, the sum of the r values is set to 100. For A, T/U, C, and G, the nucleotide frequencies are 25.83%, 30.35%, 20.80%, and 23.03%, respectively. A tree topology was

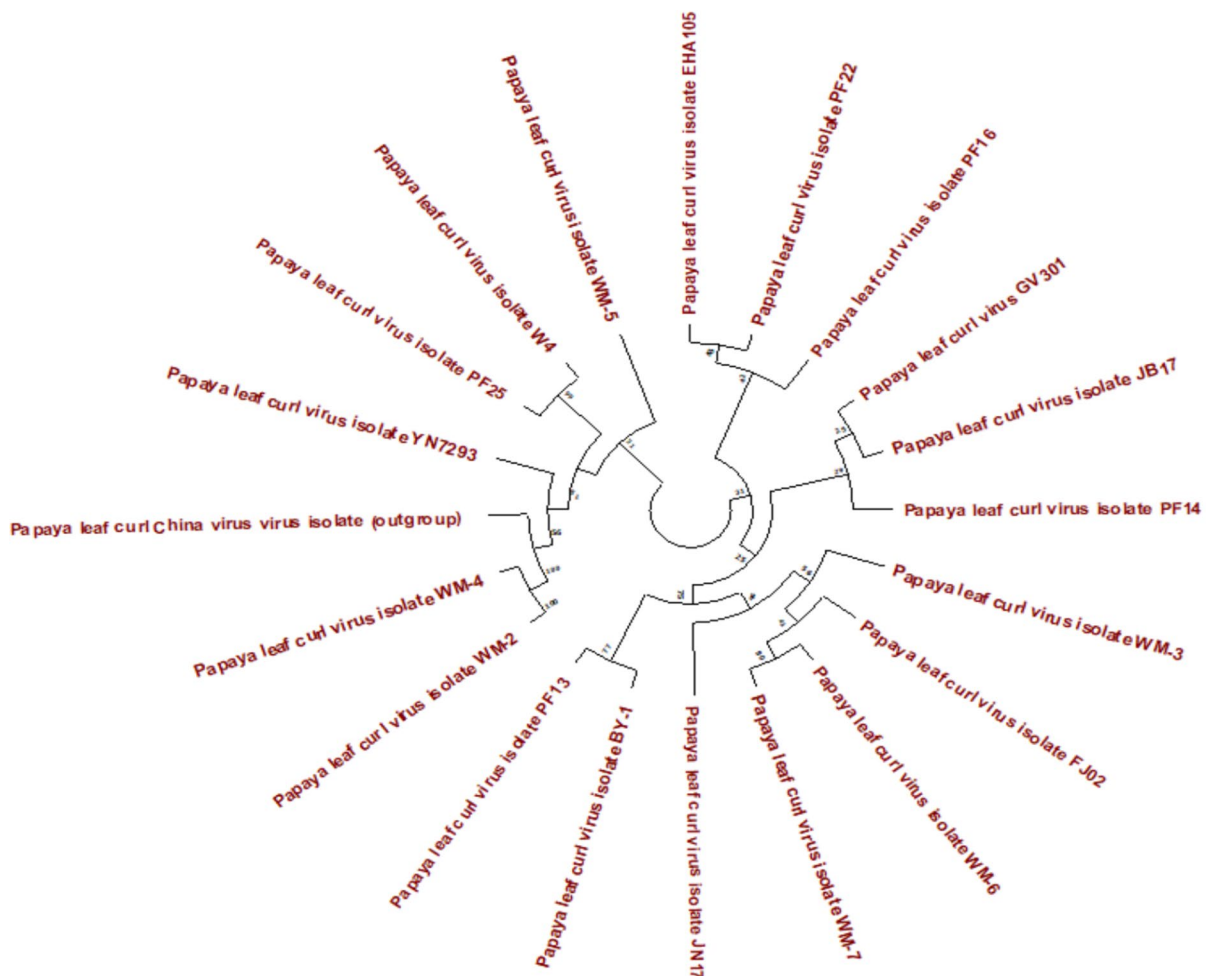


Fig. 1 A phylogenetic tree was constructed in MEGA11 for new isolates and isolates previously submitted to the NCBI database. The ancestral history of these species was monitored after agroinoculation in *N. benthamiana*

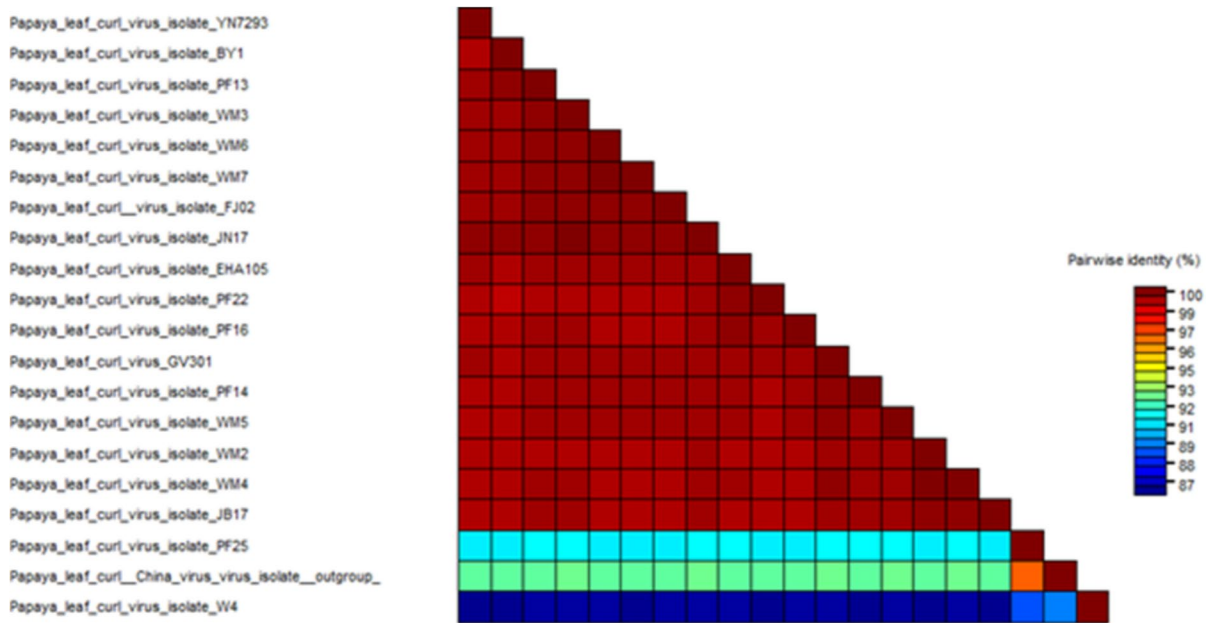


Fig. 2 A sequence demarcation tool was used to investigate the close relationship between the old and new sequences of this begomovirus. The pairwise identity represents the rela-

tionship between our isolates obtained after agroinoculation with the infectious clone and GV301 or EHA105 in *N. benthamiana*

Table 2 Estimation of substitutions per site during maximum likelihood

	A	T/U	C	G
A	-	<i>9.01</i>	<i>6.18</i>	8.80
T/U	<i>7.67</i>	-	8.91	<i>6.84</i>
C	<i>7.67</i>	13.01	-	<i>6.84</i>
G	9.87	<i>9.01</i>	<i>6.18</i>	-

The probability of substitution (*r*) from one base (row) to another base (column) is represented by each entry. The estimation of substitution patterns and rates was conducted via the Tamura–Nei model. The italicized text indicates the rates of conversion substitutions, whereas the bold text indicates the rates of other transitional substitutions

automatically constructed for the purpose of calculating ML values. The value of $-12,511.189$ was the maximum log likelihood for this calculation. By analyzing the nucleotide frequencies in the PaLCuGdV sequences, it is possible to identify genomic areas that are both conserved and varied. Understanding the genetic diversity and evolutionary trends of the virus is improved by this investigation. These findings are crucial for the development of effective diagnostic instruments, accurate treatments, and robust crop

varieties. The average was determined by calculating the sequence length of each isolate (both new and old) together with their corresponding codon regions.

ML estimates of transition/transversion bias were evaluated on the basis of new and old isolates of this begomovirus. The bias (*R*) for transition/transversion is calculated to be 0.67. The Kimura model was used to estimate the substitution pattern and rates. There are four similar nucleotide frequencies: A=25.00%, T/U=25.00%, C=25.00%, and G=25.00%. To estimate the ML values, an automated process was used to compute the tree topology. During this computation, the highest log likelihood was $-12,577.046$. The maximum likelihood estimate of the gamma parameter for site rates was determined via the Tamura–Nei model with the addition of the +G option. The form parameter for the discrete gamma distribution is predicted to be 4.6069. An evolutionary rate differential among sites was modeled via a discrete gamma distribution with 5 categories and a positive G parameter. The average evolutionary rates in these groups were 0.45, 0.71, 0.93, 1.19, and 1.72 substitutions per site, respectively. The frequencies of the nucleotides are as follows: A=25.83%, T/U=30.35%, C=20.80%, and G=23.03%. To estimate the ML values, a tree

topology was automatically generated. The computed log likelihood reached a maximum value of $-12,495.260$.

Recombination analysis performed using RDP5 identified a total of fourteen recombination events among three full-length PaLCuGdV genome sequences. Each event was supported by at least four independent detection methods implemented in RDP5, including RDP, BootScan, MaxChi, and Chimaera (Table 2; Fig. 3). In this context, the major parent is defined as the sequence contributing the larger proportion of the recombinant genome, whereas the minor parent represents the sequence donating the smaller recombinant fragment. Based on this definition, PaLCuGdV-EHA105 and PaLCuGdV WM-3 were identified as major parental contributors, while PaLCuGdV WM-2 acted as the minor parental contributor in specific recombinant regions. Nucleotide positions 1–1564 were inferred to be derived predominantly from major parents (EHA105 or WM-3), whereas sequence regions downstream of position 1564 showed contribution from the minor parent WM-2. Color-coded representations in Fig. 3 illustrate the distribution of major and minor parental

regions across the recombinant genomes. The recently reported isolate PaLCuGdV-EHA105 was recognized as a prominent recombinant across many events, with substantial corroboration from RDP, BootScan, MaxChi, and Chimaera studies. Conversely, isolates like PaLCuGdV-WM-2 often functioned as minor progenitors, supplying abbreviated genomic segments. A notable recombination breakpoint was revealed at nucleotide position 1564, delineating regions originating from the major parent (EHA105 or WM-3) and the minor parent (WM-2), as reliably observed by multiple detection techniques. Phylogenetic analysis identified the newly generated PaLCuGdV strain inside a robust clade of Asian PaLCuGdV isolates, thereby affirming its taxonomic identity. Despite significant overall sequence similarity, comparative analysis indicated considerable genetic heterogeneity among isolates, implying continuous diversification.

Recombination examination revealed fourteen notable recombination events corroborated by various independent detection techniques. The newly identified isolate often served as a principal recombinant parent, supplying extensive genomic areas to recombinant sequences, while other isolates

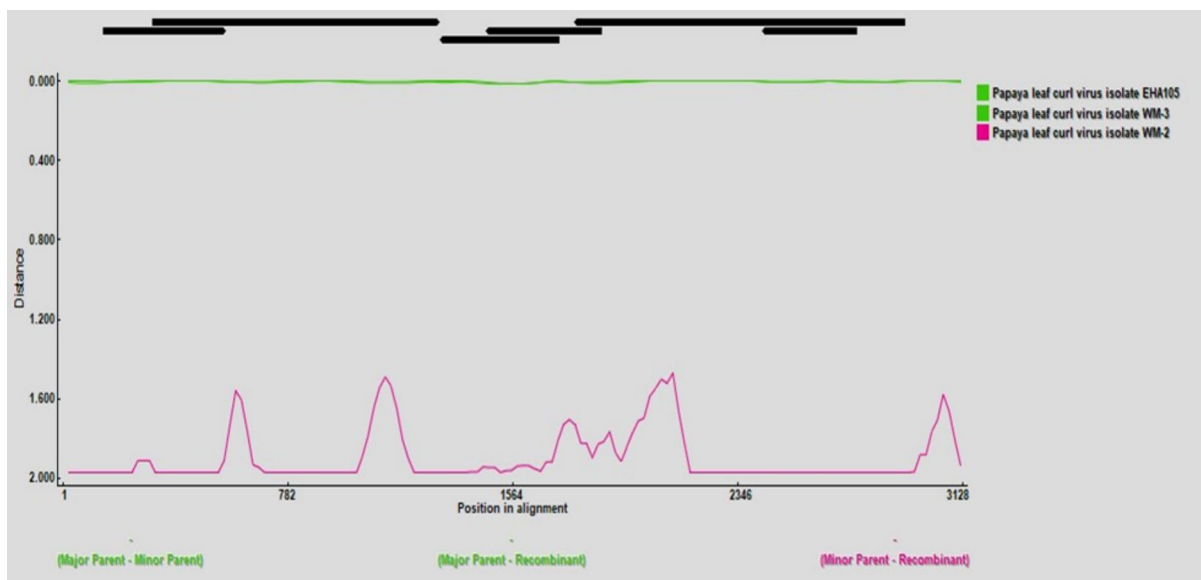


Fig. 3 Recombination analysis of full-length PaLCuGdV genomes was conducted utilizing RDP4. Only events corroborated by a minimum of four different methodologies (RDP, BootScan, MaxChi, Chimaera, SiScan, and/or 3Seq; $p < 0.05$) are presented. Major parents denote sequences that contrib-

ute significantly to the recombinant's broader genomic area, whereas minor parents provide lesser portions. Breakpoint locations are marked throughout the genome, with color coding differentiating parental contributions

provided smaller pieces as secondary parents. A significant recombination breakpoint was identified near nucleotide position 1564, delineating genomic areas with differing parental origins. These findings suggest that recombination is pivotal in determining the genomic architecture of PaLCuGdV and may enable fast adaptation to novel hosts or environmental conditions.

The PaLCuGdV also differentiates primary parents utilized to create recombinants. The data show microevolution in PaLCuGdV populations after agro-inoculation. Additionally, genetically recombined PaLCuGdV isolates have an ecological advantage over their original viral forms. DNA viruses, which are among the most basic types of pathogens, are excellent model systems for studying the relationships between cSSR and recombination processes. An ancestry tree revealed (Fig. 4) that new isolates serve as potential major recombinants, whereas some other isolates are potential minor parents. Some isolates have unknown recombination events.

Discussion

This PaLCuGdV was identified in papaya and tomato plants across various regions of Guangdong Province, where it has a wide host range. The virus prevalence was significantly higher than that previously reported. A key finding of this study is the postponed emergence of pathogenic symptoms after the agroinoculation of the PaLCuGdV infectious clone in *N. benthamiana*. Although numerous begomoviruses, including *Tomato yellow leaf curl virus* and *Cotton leaf curl Kokhran virus*, manifest discernible symptoms within 7–14 days post-inoculation (Raza et al., 2022), PaLCuGdV-infected plants displayed symptoms only after approximately 28 days. The prolonged asymptomatic duration indicates that PaLCuGdV may necessitate an extended adaption phase for systemic dissemination and replication. Comparable delayed viral symptom manifestation has been observed in certain recombinant begomoviruses in field conditions, where latent phases facilitate covert dissemination (Naveed et al., 2023). These findings collectively suggest that PaLCuGdV demonstrates unusually

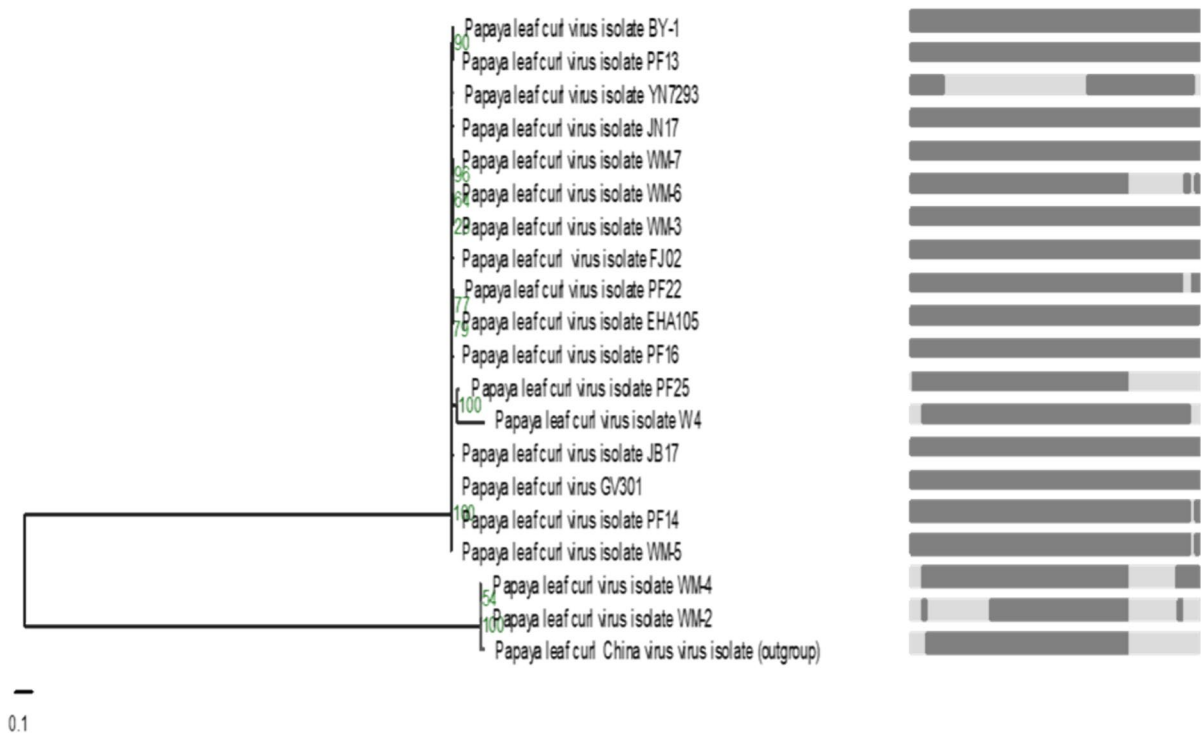


Fig. 4 The ancestry tree with new and old isolates of PaLCuGdV in which genetic recombinant regions were removed

slow pathogenic progression among begomoviruses, potentially impacting epidemiological strategies for early diagnosis and illness treatment.

To develop an infectious clone of this begomovirus, viral RCA products were partially digested following protocols to validate findings from earlier research (Jain et al., 2023; Vargas-Hernandez et al., 2022). The infectious clone was identified through restriction analysis, digestion, and sequencing. DNA and RNA were extracted from the infected upper leaves of *N. benthamiana*, and PCR/RT–PCR confirmed coinfection with begomovirus. In this study, agroinoculation was used to evaluate the biological activity and stability of an infectious clone of PaLCuGdV, resulting in reproducible symptom development in *Nicotiana benthamiana*, following the method described by (Hasan et al., 2015; Manivannan et al., 2019). The successful induction of disease symptoms confirms that the cloned viral genome is biologically functional and capable of systemic infection under experimental conditions. In contrast, EHA105 alone did not induce symptoms, but symptom expression was restored following co-inoculation with GV301, indicating a dependence on compatible helper components for pathogenicity (Guo & Yang, 2023; Jailani et al., 2023).

Although agroinoculation is a well-established technique for evaluating begomovirus infectivity, its application here specifically demonstrates the infectivity of PaLCuGdV Guangdong and highlights differential biological behavior among related isolates. This finding is consistent with previous reports showing that infectious clone-based inoculation provides greater reliability and reproducibility than direct RCA or particle bombardment approaches, particularly when evaluating recombinant or satellite-associated viruses (Abdel-Salam et al., 2024; Parmar et al., 2021).

The detection of PaLCuGdV in Guangdong Province and the observation of coinfection-related effects further support the capacity of begomoviruses to engage in complex interactions with non-cognate viral components. While coinfection between viruses from distinct genera is uncommon, such interactions have been documented under experimental conditions and may contribute to viral persistence, host adaptation, and evolutionary diversification (Kumari et al., 2024; Roumagnac et al., 2022). (Macedo et al., 2018; Souza et al., 2022). The observed delayed symptom

onset, strain-specific infectivity, and numerous recombination events indicate a virus that maintains a balance between restricted aggressiveness and significant evolutionary potential (Baebler et al., 2020). These traits may promote long-term survival in mixed cropping systems and allow PaLCuGdV to acclimate to new hosts or environmental circumstances. These findings enhance our comprehension of PaLCuGdV biology and underscore the significance of infectious clone systems for analysing host–virus interactions and viral evolution in begomoviruses.

Prior studies have indicated that the majority of infectious clones incorporate tandem repeat constructs of begomovirus and geminivirus genomes to increase their pathogenicity. This, in turn, complicates the cloning procedure (Dai et al., 2022). Begomovirus multimeric constructs are made by carefully choosing and designing cloning sites and then assembling the viral genomic fragments with restriction endonucleases (Hu et al., 2020; Zhao et al., 2022). Furthermore, selecting clones with tandem-repeat viral genomes requires careful inspection of insert quality. A novel method, RCA, has recently been developed to amplify specific DNA or betasatellite sequences in a linear or symmetrical manner (Fondong, 2025). The use of RCA facilitated the production of multimeric infectious clones for many geminiviruses. Microprojector bombardment was used to test CoYVV and CoGMV pathogenicity in tobacco culture. Gum and phenolic substances in several agricultural plants prevent the mechanical transmission of yellow mosaic disease (Makeshkumar & Sankar, 2022; Reddy et al., 2024).

The examination of nucleotide diversity among the various nations indicated that there was very little distinction between our isolated group and those from other Asian countries. However, nucleotide diversity could not be calculated for two hosts because of the limited availability of sequences (Saeed et al., 2024; Song et al., 2021). We estimated substitution patterns and rates. Notably, in comparison with eukaryotes such as yeast and humans, the frequency of recombination events between cSSR repeats and recombination is anticipated to be lower in geminiviruses. For example, geminiviruses have well-characterized gene locations and expression levels, which allows for correlations among transcription, recombination, and microsatellites. In naturally occurring ssDNA viral genomes, the detectable recombination breakpoints are not randomly distributed. Instead, they are

concentrated in certain recombination hotspots or in recombination cold spots, with the latter occurring less frequently (George et al., 2015; Mishra et al., 2020; Renukadevi et al., 2024). In our study, some isolates were potential major recombinants, whereas some old isolates were minor parents. Some of them have unknown regions. These results are totally aligned with multiple previous studies.

Conclusion

In this study, an infectious clone of the recently identified PaLCuGdV Guangdong was successfully constructed and evaluated through agroinoculation in *Nicotiana benthamiana*. The PaLCuGdV clone induced visible disease symptoms under the experimental conditions employed, whereas the EHA105 clone did not produce detectable symptoms when inoculated alone. However, symptom development was observed following co-inoculation of EHA105 with GV301, indicating a dependence on a compatible helper virus. Because symptom development was assessed qualitatively and experiments were restricted to a single host species, conclusions regarding pathogenicity strength or broader host interactions cannot be quantitatively inferred. Nevertheless, these findings suggest that begomoviruses from distinct geographical regions may interact with non-cognate betasatellites and alphasatellite, although such interactions appear to be biologically constrained. Consistent with previous studies, frequent recombination events were detected, and the non-random distribution of recombination breakpoints indicates the combined influence of selective and mechanistic processes in begomovirus evolution.

Contributions

Conceptualization, Renshu Huang, Muhammad Arif, Amna Saeed, Cheng Song, Omer Beyhan, and Islem Abid; Data curation, Renshu Huang, Muhammad Arif, Cheng Song; Formal analysis, Renshu Huang, Muhammad Arif, Cheng Song, Muhammed Said Yolcu, Islem Abid; Funding acquisition, Renshu Huang, Cheng Song, and Islem Abid; Investigation, Renshu Huang, Muhammad Arif and Cheng

Song; Methodology, Renshu Huang, Muhammad Arif, and Cheng Song; Project administration, Muhammad Arif, Cheng Song, Omer Beyhan and Islem Abid; Resources, Renshu Huang, Song Cheng; Software, Muhammad Arif, Amna Saeed, Omer BEYHAN and Muhammed Said YOLCU; Supervision, Renshu Huang, Muhammad Arif and Cheng Song; Validation, Muhammad Arif, Amna Saeed, Cheng Song,; Writing – original draft, Muhammad Arif, Amna Saeed, Cheng Song, Omer Beyhan and Islem Abid; Writing – review & editing, Muhammad Arif, Cheng Song, Omer BEYHAN and Muhammed Said YOLCU.

Acknowledgements The authors are grateful to Sakarya University of Applied Sciences, West Anhui University, for assisting with and providing laboratory facilities and equipment. This work was funded by the Quality Engineering Project of West Anhui University (wxy2024011), Quality Engineering Project of Anhui Province (2024zybj032). The Authors Extend their appreciation to the ongoing research funding program (ORF-Ctr-2026-6) King Saud University, Riyadh, Saudi Arabia.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This work was funded by Quality Engineering Project of West Anhui University (wxy2024011), Quality Engineering Project of Anhui Province (2024zybj032). The Authors Extend their appreciation to the ongoing research funding program (ORF-Ctr-2026-6) King Saud University, Riyadh, Saudi Arabia.

Data availability The datasets generated and analyzed during the current study are available in the Figshare and Genome sequence archive (GSA) repository with persistent weblink. The current data can be obtained from this link <https://doi.org/10.6084/m9.figshare.28033724>. Further data will be available upon request.

Declarations

Studies in humans and animals This work does not involve the study of humans or animals.

Consent for publication Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process In the context of preparing this work, the author(s) utilized AI-assisted technologies to enhance the text's grammar. Subsequent to utilizing these tools/services, the author(s) evaluated and revised the text as necessary and acknowledge full responsibility for the publication's content.

Conflict of interest The authors declare that they have no financial or competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abdel-Salam, A. M., Mujaddad-ur-Rehman, M., & Soliman, D. Z. (2024). Molecular detection and phylogeny of the bipartite begomovirus Pepper golden mosaic virus associated with Okra leaf curl betasatellite in the *Iresine herbstii* ornamental plant in Egypt. *Egyptian Academic Journal of Biological Sciences. c, Physiology and Molecular Biology*, 16(2), 297–316.
- Alcalá-Briseño, R. I., Casarrubias-Castillo, K., López-Ley, D., Garrett, K.A., Silva-Rosales, L. (2020) Network analysis of the papaya orchard virome from two agroecological regions of Chiapas, Mexico. *Msystems*, 5(1), <https://doi.org/10.1128/msystems.00423-00419>.
- Amin, I., Ahmed, N., Kamal, H., & Mansoor, S. (2021). Geminiviruses and their interaction with host proteins. *Plant Virus-Host Interaction* (pp. 191–229). Elsevier.
- Arif, M. (2024). Cap-snatching mechanism-mediated unveiling of the transcriptional initiation sites of two distinct begomoviruses. *Journal of Plant Diseases and Protection*. <https://doi.org/10.1007/s41348-024-00941-x>
- Arif, M., Lin, W., Lin, L., Islam, W., Jie, Z., He, Z., Du, Z., & Wu, Z. (2018). Cotton leaf curl Multan virus infecting *Hibiscus sabdariffa* in China. *Canadian Journal of Plant Pathology*, 40(1), 128–131.
- Arif, M., Atta, S., Bashir, M. A., Hussain, A., Khan, M. I., Farooq, S., Hannan, A., ul Islam, S., ud din Umar, U., & Khan, M. (2020). Molecular characterization and RSV co-infection of *Nicotiana benthamiana* with three distinct begomoviruses. *Methods*, 183, 43–49.
- Arif, M., Farooq, S., Alasmari, A., Alshehri, M. A., Hashem, M., Alamri, S., Hemeg, H. A., & Alabdallah, N. M. (2022). Molecular study of geminiviruses: Complex biology, host-vector interactions, and increasing diversity. *Journal of King Saud University - Science*, 34(4), Article 102051.
- Azeem, H., Perveen, R., Tahir, M. N., Umar, U.-U.-D., Ölmez, F., & Ali, A. (2022). Prevalence, transmission and molecular characterization of Cotton leaf curl Multan virus infecting hollyhock plants in Pakistan. *Molecular Biology Reports*, 49(6), 5635–5644.
- Baebler, Š, Coll, A., & Gruden, K. (2020). Plant molecular responses to Potato Virus Y: A continuum of outcomes from sensitivity and tolerance to resistance. *Viruses*, 12(2), 217.
- Ban, F., Zhong, Y., Pan, L., Mao, L., Wang, X., & Liu, Y. (2021). Coinfection by two begomoviruses aggravates plant symptoms but does not influence the performance and preference of insect vector *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Economic Entomology*. <https://doi.org/10.1093/jeet/toaa326>
- Bornancini, V. A., Irazoqui, J. M., Flores, C. R., Vaghi Medina, C. G., Amadio, A. F., & López Lambertini, P. M. (2020). Reconstruction and characterization of full-length begomovirus and alphasatellite genomes infecting pepper through metagenomics. *Viruses*, 12(2), Article 202.
- Breves, S. S., Silva, F. A., Euclides, N. C., Saia, T. F., Jean-Baptiste, J., Andrade Neto, E. R., & Fontes, E. P. (2023). Begomovirus–host interactions: Viral proteins orchestrating intra and intercellular transport of viral DNA while suppressing host defense mechanisms. *Viruses*, 15(7), Article 1593.
- Chen, N., Zou, C., Pan, L.-L., Du, H., Yang, J.-J., Liu, S.-S., & Wang, X.-W. (2024). Cotton leaf curl Multan virus subverts the processing of hydroxyproline-rich systemin to suppress tobacco defenses against insect vectors. *Journal of Experimental Botany*, 75(18), 5819–5838.
- Crespo-Bellido, A., Hoyer, J.S., Dubey, D., Jeannot, R.B., Duffy, S., 2021. Interspecies recombination has driven the macroevolution of cassava mosaic begomoviruses. *Journal of Virology*. 95(17), <https://doi.org/10.1128/jvi.00541-00521>.
- Dai, K.-W., Tsai, Y.-T., Wu, C.-Y., Lai, Y.-C., Lin, N.-S., & Hu, C.-C. (2022). Identification of crucial amino acids in begomovirus C4 proteins involved in the modulation of the severity of leaf curling symptoms. *Viruses*, 14(3), Article 499.
- Diggins, N. L., Hancock, M. H. (2023). Viral miRNA regulation of host gene expression, *Seminars in cell & developmental biology*. Elsevier, pp. 2–19.
- Fahmy, I. F., Taha, O., & El-Ashry, A. N. (2015). First genome analysis and molecular characterization of Chickpea chlorotic dwarf virus Egyptian isolate infecting squash. *Virus-disease*, 26(1–2), 33–41.
- Fondong, V. N. (2025). DNA satellites impact begomovirus diseases in a virus-specific manner. *International Journal of Molecular Sciences*, 26(12), Article 5814.
- George, B., Alam, C. M., Kumar, R. V., Gnanasekaran, P., & Chakraborty, S. (2015). Potential linkage between compound microsatellites and recombination in geminiviruses: Evidence from comparative analysis. *Virology*, 482, 41–50.
- Guerrero, J., Regedanz, E., Lu, L., Ruan, J., Bisaro, D. M., & Sunter, G. (2020). Manipulation of the plant host by the Geminivirus AC2/C2 protein, a central player in the infection cycle. *Frontiers in Plant Science*. <https://doi.org/10.3389/fpls.2020.00591>
- Guo, L., Yang, G. (2023). Pioneering DNA assembling techniques and their applications in eukaryotic microalgae. *Biotechnology Advances*. 108301.
- Hasan, M., Meah, M., Ali, M., Okazaki, K., Sano, Y. (2015). Characterization and confirmation of corchorus golden mosaic virus associated with jute in Bangladesh. *Journal of Plant Pathology & Microbiology*. 6(1).

- Hu, T., Song, Y., Wang, Y., & Zhou, X. (2020). Functional analysis of a novel β V1 gene identified in a geminivirus betasatellite. *Science China. Life Sciences*, 63(5), 688–696.
- Huang, A., Ding, M., Cao, M., Zhang, S., Zhong, L., & Wang, Y. (2020). First report of papaya leaf curl China virus on passion fruit in China. *Plant Disease*, 104(4), 1265.
- Jailani, A. A. K., Chattopadhyay, A., Kumar, P., Singh, O. W., Mukherjee, S. K., Roy, A., Sanan-Mishra, N., & Mandal, B. (2023). Accelerated long-fragment circular PCR for genetic manipulation of plant viruses in unveiling functional genomics. *Viruses*, 15(12), Article 2332.
- Jailani, A. A. K., Chattopadhyay, A., Roy, A., & Mandal, B. (2025). Plant-based production of monoclonal antibodies for rapid detection of Papaya ringspot virus. *Physiology and Molecular Biology of Plants*. <https://doi.org/10.1007/s12298-025-01563-9>
- Jain, H., Chahal, S., Singh, I., Sain, S. K., & Siwach, P. (2023). The rising threat of geminiviruses: Molecular insights into the disease mechanism and mitigation strategies. *Molecular Biology Reports*, 50(4), 3835–3848.
- Jing, C., Luo, L., Li, K., Li, S., Zhao, Y., & Feng, X. (2026). First report of papaya leaf curl China virus infecting *Phaseolus vulgaris* in China. *Journal of Plant Diseases and Protection*, 133(1), Article 4.
- Jyotika, R., Harish, S., Karthikeyan, G., Kumar, K., Murugan, M., Jayakanthan, M., & Chen, T.-C. (2024). Molecular approaches for the management of papaya ringspot virus infecting papaya: A comprehensive review. *Molecular Biology Reports*, 51(1), 981.
- Kumari, N., Aski, M. S., Mishra, G. P., Roy, A., Dikshit, H. K., Saxena, S., Kohli, M., Mandal, B., Sinha, S. K., & Mishra, D. C. (2024). Development of infectious clones of mungbean yellow mosaic India virus (MYMIV, Begomovirus vignaradiataindiaense) infecting mungbean [*Vigna radiata* (L.) R. Wilczek] and evaluation of a RIL population for MYMIV resistance. *PLoS ONE*, 19(10), Article e0310003.
- Lavanya, R., & Arun, V. (2019). Standardization of DNA extraction protocol and screening of begomovirus in chilli and tomato plants. *Journal of Plant Diseases and Protection*, 126(4), 343–349.
- Macedo, M., Albuquerque, L., Maliano, M., Souza, J., Rojas, M., Inoue-Nagata, A., & Gilbertson, R. (2018). Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. *Archives of Virology*, 163, 737–743.
- Makeshkumar, T., Sankar, M. S. A. (2022). Recent developments in the diagnosis of geminiviruses. Geminivirus: Detection, Diagnosis and Management. 33–42.
- Makeshkumar, T., Divya, K., & Asha, S. (2021). Transgenic Technology for Disease Resistance in Crop Plants. *Emerging Trends in Plant Pathology* (pp. 499–560). Springer.
- Manivannan, K., Renukadevi, P., Malathi, V. G., Karthikeyan, G., & Balakrishnan, N. (2019). A new seed-transmissible begomovirus in bitter melon (*Momordica charantia* L.). *Microbial Pathogenesis*, 128, 82–89.
- Mehta, D., Cornet, L., Hirsch-Hoffmann, M., Zaidi, S.S.-e-A., & Vanderschuren, H. (2020). Full-length sequencing of circular DNA viruses and extrachromosomal circular DNA using CIDER-Seq. *Nature Protocols*, 15(5), 1673–1689.
- Mishra, M., Verma, R. K., Marwal, A., Sharma, P., & Gaur, R. (2020). Biology and interaction of the natural occurrence of distinct monopartite begomoviruses associated with satellites in *Capsicum annum* from India. *Frontiers in Microbiology*, 11, Article 512957.
- Möller, S., & Maruthi, M. (2025). A review of factors affecting the success of geminivirus infectious clones. *Plant Cell Reports*, 44(8), 189.
- Mosharaf, N., Tabein, S., Behjatnia, S. A. A., & Accotto, G. P. (2020). Identification of Cotton leaf curl Multan virus, a new threatening Begomovirus in Iran. *Iranian Journal of Plant Pathology*, 56(2), 217–218.
- Nair, A., Chatterjee, K. S., Jha, V., Das, R., & Shivaprasad, P. (2020). Stability of Begomoviral pathogenicity determinant β C1 is modulated by mutually antagonistic SUMOylation and SIM interactions. *BMC Biology*, 18(1), 1–24.
- Naveed, H., Islam, W., Jafir, M., Andoh, V., Chen, L., & Chen, K. (2023). A review of interactions between plants and whitefly-transmitted begomoviruses. *Plants*, 12(21), 3677.
- Naveen, M., Shanmugam, P. S., Srinivasan, R., Shanthi, M., Murugan, M., Sivagnanapazham, K., Angappan, K., Oliva, R., Karthikeyan, G., & Boopathi, N. M. (2025). Recombination, phylogenetic insights and co-existence dynamics of begomoviruses and satellite DNAs: Implications for tomato viral disease management. *Molecular Biology Reports*, 52(1), 390.
- Neubauer, M., Vollen, K., Zhao, C., Ascencio-Ibáñez, J. T., Hanley-Bowdoin, L., Stepanova, A. N., & Alonso, J. M. (2025). Development of modular geminivirus-based vectors for high cargo expression and gene targeting in plants. *Plant Biotechnology Journal*. <https://doi.org/10.1111/pbi.70320>
- Oliveira, B. B., Veigas, B., & Baptista, P. V. (2021). Isothermal amplification of nucleic acids: The race for the next “gold standard.” *Frontiers in Sensors*, 2, Article 752600.
- Ouattara, A., Tiendrébéogo, F., Becker, N., Urbino, C., Thébaud, G., Hoareau, M., Allibert, A., Chiroleu, F., Vernerey, M.-S., & Traoré, E. V. (2022). Synergy between an emerging monopartite begomovirus and a DNA-B component. *Scientific Reports*, 12(1), Article 695.
- Pandey, V., Srivastava, A., & Gaur, R. (2021). Begomovirus: A curse for the agricultural crops. *Archives of Phytopathology and Plant Protection*, 54(15–16), 949–978.
- Parmar, S., Deshmukh, D. B., Kumar, R., Manohar, S. S., Joshi, P., Sharma, V., Chaudhari, S., Variath, M. T., Gangurde, S. S., & Bohar, R. (2021). Single seed-based high-throughput genotyping and rapid generation advancement for accelerated groundnut genetics and breeding research. *Agronomy*, 11(6), 1226.
- Quadros, A. F., & Zerbini, F. M. (2025). High-throughput Sequencing (HTS)-Based Diagnosis of Geminiviruses. *Geminiviruses: Methods and Protocols* (pp. 173–182). Springer.
- Ramesh, S., Yogindran, S., Gnanasekaran, P., Chakraborty, S., Winter, S., & Pappu, H. R. (2021). Virus and viroid-derived small RNAs as modulators of host gene expression: Molecular insights into pathogenesis. *Frontiers in Microbiology*, 11, Article 614231.

- Raza, A., Khare, T., Zhang, X., Rahman, M. M., Hussain, M., Gill, S. S., Chen, Z. H., Zhou, M., Hu, Z., & Varshney, R. K. (2025). Novel strategies for designing climate-smart crops to ensure sustainable agriculture and future food security. *Journal of Sustainable Agriculture and Environment*, 4(2), e70048.
- Reddy, M. K., Nagendran, K., Kumari, S., Pradeep, R. M., & Karthikeyan, G. (2024). Global scenario of begomovirus diseases in vegetable crops. *Vegetable Science*, 51, 43–53.
- Renukadevi, P., Devi, R. G., Jothika, C., Karthikeyan, G., Malathi, V., Balakrishnan, N., Rajagopal, B., Nakkeeran, S., & Abd-Allah, E. F. (2024). Genomic distinctiveness and recombination in tomato leaf curl New Delhi virus (ToLCNDV-BG) isolates infecting bitter melon. *3 Biotech*, 14(8), Article 184.
- Roumagnac, P., Lett, J.-M., Fiallo-Olivé, E., Navas-Castillo, J., Zerbini, F. M., Martin, D. P., & Varsani, A. (2022). Establishment of five new genera in the family Geminiviridae: Citlodavirus, Maldovirus, Mulcrilevirus, Opunvirus, and Topilevirus. *Archives of Virology*, 167(2), 695–710.
- Saeed, A., Arif, M., Rafiq, M., Song, C., Albaqami, M., & Abdelbacki, A. M. (2024). Molecular characterization, genetic divergence, expression of encapsidation and synergism by a bipartite begomovirus; Tomato leaf curl Palampur virus (ToLCPMV) infecting bitter melon (*Momordica charantia*). *Microbial Pathogenesis*, 196, Article 106953.
- Scholthof, K.-B. (2023). The past is present: Coevolution of viruses and host resistance within geographic centers of plant diversity. *Annual Review of Phytopathology*, 61(1), 119–136.
- Shakir, S., Zaidi, S.-e-A., Hashemi, F. S. G., Nyirakanani, C., & Vanderschuren, H. (2023). Harnessing plant viruses in the metagenomics era: From the development of infectious clones to applications. *Trends in Plant Science*, 28(3), 297–311.
- Sharma, N., & Ruhel, R. (2019). Rolling Circle Replication and Transcription Processes in Geminiviruses. *Geminiviruses* (pp. 17–38). Springer.
- Silva, J. M. F., Melo, F. L., Elena, S. F., Candresse, T., Sabanadzovic, S., Tzanetakis, I. E., Blouin, A. G., Villamor, D. E. V., Molloy, D., & Constable, F. (2022). Virus classification based on in-depth sequence analyses and development of demarcation criteria using the Betaflexiviridae as a case study. *Journal of General Virology*, 103(11), Article 001806.
- Singh, S., Nirmalkar, V. K., Awasthi, L. (2020). Recent advances in begomovirus research in India. *Applied Plant Virology*. 493–513.
- Song, X., Yang, T., Zhang, X., Yuan, Y., Yan, X., Wei, Y., Zhang, J., & Zhou, C. (2021). Comparison of the micro-satellite distribution patterns in the genomes of euarchontoglires at the taxonomic level. *Frontiers in Genetics*, 12, Article 622724.
- Soni, S. K., Mishra, M. K., Mishra, M., Kumari, S., Saxena, S., Shukla, V., Tiwari, S., & Shirke, P. (2022). Papaya leaf curl virus (PaLCuV) infection on papaya (*Carica papaya* L.) plants alters anatomical and physiological properties and reduces bioactive components. *Plants*, 11(5), Article 579.
- Souza, J. O., Melgarejo, T. A., Vu, S., Nakasu, E. Y., Chen, L.-F., Rojas, M. R., Zerbini, F. M., Inoue-Nagata, A. K., & Gilbertson, R. L. (2022). How to be a successful monopartite begomovirus in a bipartite-dominated world: Emergence and spread of tomato mottle leaf curl virus in Brazil. *Journal of Virology*, 96(18), Article e00725-00722.
- Srivastava, A., Pandey, V., Sahu, A. K., Yadav, D., Al-Sadi, A. M., Shahid, M. S., & Gaur, R. (2022). Evolutionary dynamics of begomoviruses and its satellites infecting papaya in India. *Frontiers in Microbiology*, 13, Article 879413.
- Tamura, T., Ito, J., Uriu, K., Zahradnik, J., Kida, I., Anraku, Y., Nasser, H., Shofa, M., Oda, Y., & Lytras, S. (2023). Virological characteristics of the SARS-CoV-2 XBB variant derived from recombination of two Omicron subvariants. *Nature Communications*, 14(1), 2800.
- Udavatha, P., Mesta, R. K., Basavarajappa, M. P., Venkataramanappa, V., Devappa, V., Narasimha Reddy, L. R. C., & Shankarappa, K. S. (2022). Identification of novel begomoviruses associated with leaf curl disease of papaya (*Carica papaya* L.) in India. *Agronomy*, 13(1), Article 3.
- Vargas-Hernandez, M., de Jesus Rivero-Montejo, S., Macias-Bobadilla, I., Acuña-Flores, N. L., Rivas-Ramirez, L. K., Guevara-González, R. G., Ascencio-Ibáñez, J. T., & Torres-Pacheco, I. (2022). Evaluating management strategies to control geminivirus. *Geminivirus: Detection, Diagnosis and Management* (pp. 629–654). Elsevier.
- Varun, P., Akhter, Y., & Saxena, S. (2024). Molecular evidence of novel Begomoviruses and associated Betasatellite complexes linked to Papaya Leaf Curl Disease in Indian Provinces. *Ecological Genetics and Genomics*, 33, Article 100286.
- Wang, D., Yin, Y., Zhang, X., & Ye, J. (2024). An interspecies recombinant sida yellow mosaic China virus isolate and betasatellite cause a leaf curl disease in tobacco in Hainan, China. *Plant Disease*, 108(4), 877–886.
- Zhang, L., Wu, S., Zhao, M., Ghanem, H., Wu, G., Li, M., & Qing, L. (2024). Molecular identification and genetic diversity analysis of Papaya Leaf Curl China Virus infecting *Ageratum conyzoides*. *The Plant Pathology Journal*, 40(5), 551.
- Zhao, S., Gong, P., Ren, Y., Liu, H., Li, H., Li, F., & Zhou, X. (2022). The novel C5 protein from tomato yellow leaf curl virus is a virulence factor and suppressor of gene silencing. *Stress Biology*, 2(1), 19.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.