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Phylogeny and recombination of papaya begomoviruses in Northern and Central-East India

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Abstract. *Srivastava A, Pandey V, Verma RK, Marwal A, Gupta R, Shahid MS, Gaur RK. 2025. Phylogeny and recombination of papaya begomoviruses in Northern and Central-East India. Nusantara Bioscience 17: 298-312.* Papaya plants exhibiting characteristic leaf curl symptoms were surveyed in 2022 across Uttar Pradesh, Delhi, and Chhattisgarh, India. *Begomovirus* infection was suspected based on symptomatology and confirmed in 11 of 47 collected samples using PCR with *Begomovirus*-specific primers. This study aimed to elucidate the diversity and distribution of begomoviruses infecting papaya in these regions. Full-length viral genomes were amplified through Rolling Circle Amplification (RCA), and associated alphasatellites and betasatellites were detected using PCR with universal primers. The amplified viral genomes (~2.7 kb), betasatellites (~1.4 kb), and alphasatellites (~1.3 kb) were cloned and sequenced. Sequence analysis revealed 94.57-99.46% nucleotide identity of DNA-A with known isolates of Papaya Leaf Curl Virus (PaLCuV), Cotton Leaf Curl Virus (CLCuV), Croton Yellow Vein Mosaic Virus (CYVMV), Tomato Leaf Curl New Delhi Virus (ToLCNDV), and Cotton Leaf Curl Multan Virus (CLCuMuV). The DNA-B component exhibited 98.33% identity with ToLCNDV. Ten betasatellites shared 86.81-99.71% identity with related species, whereas two alphasatellites showed approximately 98.5% identity with PaLCuA and PaLCVSA. One betasatellite (PL36) displayed 86.81% identity and was identified as a novel Papaya Leaf Curl Raipur Betasatellite (PaLCuRPRB). Furthermore, six PaLCuV isolates showing <91% identity was classified as new PaLCuV variants.

Keywords: *Begomovirus*, diversity, papaya leaf curl disease, phylogeny, species demarcation tool

INTRODUCTION

Papaya (*Carica papaya* L.) is an important tropical fruit crop of high nutritional and economic value. However, its production is severely constrained by several biotic stresses, among which Papaya Leaf Curl Disease (PaLCD) is the most destructive. The disease manifests as interveinal chlorosis, severe leaf curling, stunted growth, and poor fruit quality, leading to significant yield losses (Udavatha et al. 2022; Varun et al. 2024). First observed in India in 1998, the causal agent was later identified as Papaya Leaf Curl Virus (PaLCV), a member of the *Begomovirus* genus transmitted by the whitefly *Bemisia tabaci* (Gennadius, 1889) (Saxena et al. 1998; Soni et al. 2022). Its efficient transmission and broad host range have contributed to the widespread occurrence of PaLCD across tropical and subtropical regions.

Begomoviruses possess circular single-stranded DNA genomes of approximately 2.7 kb, which may occur as monopartite (DNA-A) or bipartite (DNA-A and DNA-B) components. These genomes encode essential proteins required for replication, transcription, and movement within host cells. Many monopartite Begomoviruses are associated with betasatellites and alphasatellites—small DNA molecules (~1.3-1.4 kb) that rely on helper viruses for replication (Nogueira et al. 2021). The βC1 gene of betasatellites is known to induce symptom expression and suppress host defense mechanisms. Genetic recombination and mutation events are major drivers of *Begomovirus* evolution, host adaptation, and pathogenic variability (Fiallo-Olivé and Navas-Castillo 2023; Akram et al. 2025).

Viruses are subjected to diverse selective pressures resulting from the interplay between shifting fitness requirements and the conservation of essential functions (Spielman et al. 2019; Butković and González 2022). Because different crop species cultivated across diverse agro-climatic zones possess distinct genetic backgrounds and immune systems, Begomoviruses likely encounter geographically variable host immune responses. Consequently, virus sub-strains circulating in India may undergo region-specific selective pressures.

Despite the widespread incidence of PaLCD in India, comprehensive information on the molecular diversity, evolutionary dynamics, and recombination patterns of PaLCV and its associated satellites remains limited particularly in northern and central India. Understanding

this variability is crucial for identifying emerging viral strains and devising effective management strategies. Therefore, the present study investigates the genetic diversity, recombination events, and phylogenetic relationships of PaLCV and its associated satellites infecting papaya in Uttar Pradesh, Delhi, and Chhattisgarh. These findings provide new insights into the molecular epidemiology of PaLCD and offer a foundation for developing region-specific disease management approaches.

PaLCD severely impacts papaya plant health and productivity, causing leaf curling, distortion, stunted growth, and fruit yield losses ranging from 40 to 100% in severely affected regions, including the United States and Mexico (Srivastava et al. 2022a). Symptom severity varies with the host genotype, environmental factors, and virus strain. The complex genomic organization of Begomoviruses , coupled with their extensive host and vector range, makes PaLCD management particularly challenging. Integrated management strategies currently include whitefly control, the use of resistant cultivars, removal of infected plants, adoption of appropriate cultural practices, and implementation of monitoring and early detection programs (Nadeem et al. 1997; Saxena et al. 1998).

PaLCD represents a complex viral disease system caused by multiple begomoviral species, including Papaya Leaf Curl Virus (PaLCuV), Papaya Leaf Curl China Virus (PaLCuCNV), Papaya Leaf Curl Guandong Virus (PaLCuGDV), Chili Leaf Curl Virus (ChiLCV), Tomato Leaf Curl Virus (ToLCV), Pedilanthus Leaf Curl Virus (PeLCuV), and Papaya Leaf Crumple Virus (PaLCrV), often in association with betasatellites. Among these, Indian isolates of PaLCuV constitute the major component of the disease complex affecting papaya, related crops, and several weed species (Hallan et al. 1998). Although sporadic studies have reported *Begomovirus* infections in papaya, this study provides the first comprehensive account of the diversity and distribution of Begomoviruses infecting papaya in northern and central India.

MATERIALS AND METHODS

Virus isolates: Detection and characterization

Papaya plants exhibiting typical leaf curl symptoms, including wrinkled and twisted leaves with yellow veins, were observed (Figure 1). The petioles appeared short, thick, and twisted. Symptomatic plants either lacked fruits or bore small, misshapen ones. A total of forty-seven symptomatic and one non-symptomatic papaya leaf samples were collected from different locations across Chhattisgarh, Delhi, and Uttar Pradesh, India. The collected leaf samples were designated as PL-1 to PL-47 and stored at 4°C in the laboratory for further analysis (Table 1). Total genomic DNA was extracted from each sample using the CTAB method (Doyle and Doyle 1990). PCR amplification was performed using *Begomovirus*-specific primer sets for virus detection. Out of forty-seven samples, eleven tested positive and were selected for Rolling Circle Amplification (RCA) to obtain the complete viral genome. Samples were further screened for the presence of DNA-B, betasatellites, and alphasatellites using universal primer pairs. One sample was positive for DNA-B, ten for betasatellites, and two for alphasatellites (Table 2), and these were used for complete genome sequencing. RCA products were subjected to restriction digestion to release begomoviral genome components, which were subsequently cloned into the pTZ57RT vector and transformed into *Escherichia coli* DH5α cells. The cloned products were fully sequenced using an ABI automated sequencer (BioKart, Bangalore, India).

Figure 1. Papaya plants showing different symptoms of *Begomovirus* infection under field conditions: A. Vein thickening, Blistering; B. Leaf Curl, Interveinal Chlorosis; C. Vein enation, Distorted petioles, Upward/Downward Curling; D. Yellow mosaic patterns; E. Healthy stocks

Table 1. Geographical locations of symptomatic papaya leaf isolates collected from papaya and RCA representative isolates used for full length genome sequencing

Sl. No.	Location of collected isolates	State	Name assigned	PCR/RCA result	Sequenced
1.	Gorakhpur (Nakha Jangle)	Uttar Pradesh	PL 1	Positive	MZ605904 - MZ605905
2.	Gorakhpur (Transport Nagar)	Uttar Pradesh	PL 2	Negative	-
3.	Gorakhpur (Amrood mandi)	Uttar Pradesh	PL 3	Negative	-
4.	Gorakhpur (Belwar)	Uttar Pradesh	PL 4	Negative	-
5.	Gorakhpur (D.D.U. Campus)	Uttar Pradesh	PL 5	Negative	-
6.	Gorakhpur (Gida)	Uttar Pradesh	PL 6	Positive	MZ669217 - MZ606364
7.	Gorakhpur (Singhariya)	Uttar Pradesh	PL 7	Negative	-
8.	Gorakhpur (Salempur)	Uttar Pradesh	PL 8	Negative	-
9.	Gorakhpur (Kunraghat)	Uttar Pradesh	PL 9	Negative	-
10.	Bastar	Chhattisgarh	PL 10	Positive	OQ440383 - OQ440384 - OQ440385
11.	Bastar	Chhattisgarh	PL 11	Negative	-
12.	Deoria	Uttar Pradesh	PL 12	Negative	-
13.	Delhi	Delhi	PL 13	Positive	OR489166 - OQ440377
14.	Delhi	Delhi	PL 14	Negative	-
15.	Delhi	Delhi	PL 15	Negative	-
16.	Gonda	Uttar Pradesh	PL 16	Negative	-
17.	Gonda	Uttar Pradesh	PL 17	Negative	-
18.	Durg	Chhattisgarh	PL 18	Negative	-
19.	Durg	Chhattisgarh	PL 19	Negative	-
20.	Durg	Chhattisgarh	PL 20	Positive	OQ134774 - OQ134775
21.	Varanasi	Uttar Pradesh	PL 21	Negative	-
22.	Varanasi	Uttar Pradesh	PL 22	Negative	-
23.	Varanasi	Uttar Pradesh	PL 23	Negative	-
24.	Khalilabad	Uttar Pradesh	PL 24	Negative	-
25.	Khalilabad	Uttar Pradesh	PL 25	Negative	-
26.	Khalilabad	Uttar Pradesh	PL 26	Negative	-
27.	Khalilabad	Uttar Pradesh	PL 27	Positive	OQ168370 - OQ168371
28.	Maharajganj	Uttar Pradesh	PL 28	Negative	-
29.	Maharajganj	Uttar Pradesh	PL 29	Positive	OQ091756 - OQ091757
30.	Maharajganj	Uttar Pradesh	PL 30	Negative	-
31.	Nautanwa	Uttar Pradesh	PL 31	Positive	OQ290944 - OQ290945
32.	Nautanwa	Uttar Pradesh	PL 32	Negative	-
33.	Nautanwa	Uttar Pradesh	PL 33	Negative	-
34.	Nautanwa	Uttar Pradesh	PL 34	Negative	-
35.	Raipur	Chhattisgarh	PL 35	Negative	-
36.	Raipur	Chhattisgarh	PL 36	Positive	OQ290942 - OQ290943
37.	Raipur	Chhattisgarh	PL 37	Negative	-
38.	Bhilai	Chhattisgarh	PL 38	Negative	-
39.	Ambikapur	Chhattisgarh	PL 39	Negative	-
40.	Berla	Chhattisgarh	PL 40	Negative	-
41.	Raigarh	Chhattisgarh	PL 41	Negative	-
42.	Bilaspur	Chhattisgarh	PL 42	Negative	-
43.	Bilaspur	Chhattisgarh	PL 43	Positive	OQ440378 - OQ440379 - OQ440380
44.	Kurud	Chhattisgarh	PL 44	Negative	-
45.	Mahasamund	Chhattisgarh	PL 45	Positive	OQ440381 - OQ440382
46.	Mahasamund	Chhattisgarh	PL 46	Negative	-
47.	Mahasamund	Chhattisgarh	PL 47	Negative	-

Table 2. PCR primers and amplification condition used for detection and characterization of Begomoviruses infecting papaya

Primer	PCR condition							
	Initial-denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension	Remarks	Reference
Pal1v1978/PAR1c496	94°C for 3 min	35	94°C for 1 min	55°C for 2 min	72°C for 2 min	72°C for 10 min	DNA-A	Wyatt and Brown 1996
DNABLC1/DNABLV2	94°C for 3 min	35	94°C for 1 min	50°C for 1 min	72°C for 2 min	72°C for 10 min	DNA-B	Green et al. 2001
Beta01/Beta02	94°C for 3 min	35	94°C for 1 min	52°C for 1 min	72°C for 2 min	72°C for 10 min	Betasatellite	Bull et al. 2003
DNA 101/DNA102	94°C for 3 min	35	94°C for 1 min	52°C for 1 min	72°C for 2 min	72°C for 10 min	Alphasatellite	Xie et al. 2010

Sequence analysis

The complete genomes of Begomoviruses and their associated satellites were sequenced and analyzed. Sequences showing the highest BLAST alignment scores with the present isolates were retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov) for comparative analysis. Open Reading Frames (ORFs) were identified using the ORF Finder tool (https://www.ncbi.nlm.nih.gov/orffinder/). To determine pairwise sequence identities between the newly identified isolates and known sequences, the Sequence Demarcation Tool (SDT v1.2) was employed. Multiple sequence alignments were performed using the MUSCLE algorithm for both DNA-A and betasatellite datasets, independently and in combination (Muhire et al. 2014).

Phylogenetic analysis

Bayesian phylogenetic analyses were performed using BEAST v1.10 (Suchard et al. 2018) with Markov Chain Monte Carlo (MCMC) parameters. Prepared datasets comprising DNA-A, DNA-B, betasatellites, and alphasatellites were analyzed to generate Maximum Clade Credibility (MCC) trees. These analyses aimed to elucidate evolutionary relationships and potential host adaptation patterns among the Begomoviruses. The resulting trees were visualized and annotated using the Interactive Tree of Life (iTOL) platform, version 6.5 (https://itol.embl.de) (Letunic and Bork 2021).

Recombination analysis

Recombination plays a key role in the evolution and epidemiology of plant viruses. To assess the presence and frequency of recombination events within the *Begomovirus* genomes, two complementary approaches were employed. First, phylogenetic network reconstruction was performed using the Neighbor-Net method implemented in SplitsTree v4 (Huson and Bryant 2006). The Pairwise Homoplasy Index (PHI) test integrated in SplitsTree was used to detect recombination signals, with significance determined at $p \leq 0.05$. Recombination between and within genetic groups was considered significant when the PHI test yielded a corrected $p \leq 0.05$. Second, to validate recombination events and identify putative breakpoints and parental sequences, the RDP v4.1 software package was used. This package integrates seven algorithms: RDP, Bootscan, Geneconv, MaxChi, Chimaera, SiScan, and 3Seq (Martin et al. 2015). Analyses were conducted using predefined detection thresholds, with $p \geq 0.05$ set as the upper Bonferroni-corrected limit. Recombination breakpoints supported by at least three independent algorithms were considered reliable to minimize false positives.

RESULTS AND DISCUSSION

Survey and identification of *Begomovirus*

Typical *Begomovirus*-like symptoms were observed in papaya plants at forty-seven locations across Chhattisgarh, Delhi, and Uttar Pradesh, India. Field surveys indicated that outbreaks of *Begomovirus*-associated diseases in papaya were frequently linked to an increased distribution of the whitefly vector, *B. tabaci*. Infected plants exhibited diverse symptoms, including vein clearing and thickening, zigzag and enated veins, downward and upward leaf curling, leaf rolling, yellowing, leathery texture, distorted petioles, and overall leaf deformation (Figure 1). A total of forty-seven symptomatic and one non-symptomatic papaya leaf samples (designated as PL-1 to PL-47) were collected for analysis. Genomic DNA was extracted from both infected and healthy samples using the CTAB method. PCR amplification with universal *Begomovirus* primers produced the expected 1.2 kb amplicon in eleven samples (PL-1, PL-6, PL-10, PL-13, PL-20, PL-27, PL-29, PL-31, PL-36, PL-43, and PL-45). Further analysis detected betasatellites (Beta01/Beta02) in ten samples (PL-1, PL-6, PL-10, PL-13, PL-27, PL-29, PL-31, PL-36, PL-43, and PL-45) and alphasatellites (UN101/UN102) in two samples (PL-10 and PL-43). One sample (PL-20) produced a 1.2 kb amplicon with DNA-B-specific primers, confirming the presence of a bipartite *Begomovirus*. Positive samples were subjected to Rolling Circle Amplification (RCA) to obtain complete viral genomes and associated satellite components. RCA products were digested with *BamHI* (DNA-A) and *XbaI* (DNA-B) to generate linear fragments of approximately 2.8 kb and 1.3 kb, respectively. The digested products, along with amplified satellite molecules, were purified and cloned into the pTZ57RT plasmid as described by Sahu et al. (2018) (Figure 2). Recombinant plasmids were transformed into *E. coli* DH5α cells, and positive clones were confirmed through PCR and restriction analysis. The confirmed clones were sequenced bidirectionally, and consensus sequences were assembled into circular contigs using standard bioinformatics tools. All sequences were submitted to the NCBI GenBank database (Table 3).

Sequence datasets and alignment

Query sequences from each isolate were compared with available *Begomovirus* sequences in the GenBank database using the BLASTn algorithm, and those showing the highest nucleotide identity were retrieved for further analysis. Open Reading Frames (ORFs) in each viral genome were identified using the ORF Finder tool (Table 4). Four datasets DNA-A, DNA-B, alphasatellites, and betasatellites were aligned using the ClustalW algorithm implemented in MEGA X (Kumar et al. 2018). Pairwise nucleotide sequence identities were determined using the Sequence Demarcation Tool (SDT), which corroborated the BLASTn results. The SDT analysis provided detailed percentage identity values among isolates from different geographical regions, confirming close relationships among several sequences (Figure 3; Table 5).

Relationship between papaya-infecting present *Begomovirus* isolate with known Begomoviruses

Complete genome sequences of the eleven *Begomovirus* isolates were compared with representative sequences of known Begomoviruses. Based on species demarcation and strain classification criteria, six isolates (PL-1, PL-6, PL-13, PL-20, PL-36, and PL-43) shared >93.5% nucleotide identity with Papaya Leaf Curl Virus (PaLCuV) isolates infecting various crops, and were thus classified as new variants of PaLCuV. Two isolates (PL-31 and PL-45) shared 97% nucleotide identity with Tomato Leaf Curl New Delhi Virus (ToLCNDV), while isolates PL-10 and PL-29 exhibited >96% identity with Cotton Leaf Curl Multan Virus (CLCuMuV) and Cotton Leaf Curl Virus (CLCuV), respectively. Another isolate (PL-29) showed >96% identity with Croton Yellow Vein Virus (CYVV) infecting *Croton glandulosus* L.. In accordance with the current species demarcation criteria, six isolates were classified as PaLCuV strains, two as ToLCNDV strains, two as CLCuMuV and CLCuV strains, and one as a CYVV strain. All isolates and their associated subviral components exhibited >91% nucleotide identity with previously reported Begomoviruses , except for the betasatellite of isolate PL-36, which showed only 86.81% identity with Papaya Leaf Curl Betasatellite (PaLCuB/IN:ND:03, AY244706). This distinct sequence was identified as a novel subviral component and designated Papaya Leaf Curl Raipur Betasatellite (PaLCuRPRB). Among the six PaLCuV isolates, pairwise identity analysis revealed that isolate PL-6 shared 77.10%, PL-13 shared 80.10%, PL-20 shared 78.30%, PL-36 shared 78.60%, and PL-43 shared 81% nucleotide identity with PL-1. Although these isolates shared >91% identity with other reported PaLCuV isolates, the relatively lower identity among themselves (<91%) suggests that they represent novel PaLCuV variants, a conclusion supported by SDT analysis.

Phylogenetic analysis

Phylogenetic analyses of DNA-A, DNA-B, betasatellite, and alphasatellite sequences were conducted using Markov Chain Monte Carlo (MCMC) methods with 1,000 bootstrap replicates. The resulting phylogenetic trees revealed that all eleven *Begomovirus* isolates from Uttar Pradesh, Delhi, and Chhattisgarh formed distinct clusters. As expected from the maximum-likelihood and Bayesian analyses, the newly characterized sequences grouped with known Begomoviruses infecting similar hosts (Figure 4). Notably, six papaya leaf curl isolates (PL-1, PL-6, PL-13, PL-20, PL-36, and PL-43) clustered closely with previously reported monopartite Begomoviruses from different regions of India. The Maximum Clade Credibility (MCC) dendrogram further indicated clear segregation of isolates based on host specificity, suggesting co-adaptation of associated betasatellites with their respective helper viruses (Saleem et al. 2016).

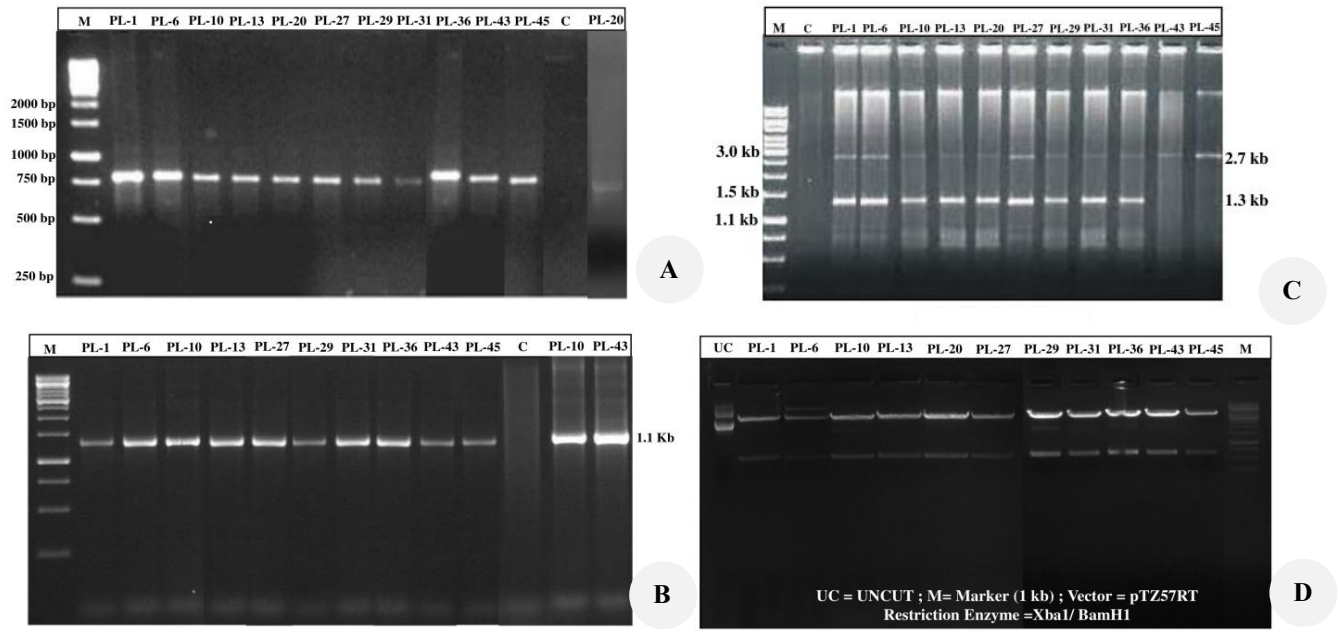

Figure 2. Agarose gel detection of papaya leaf curl disease in papaya leaf samples: A. PCR positive sample (DNA-A; DNA-B); B. PCR positive sample (Betasatellite; Alphasatellite); C. RCA positive sample; D. Restriction Digestion using Xba1/ BamH1

Recombination analysis

Neighbor-Net phylogenetic network analysis revealed that the eleven *Begomovirus* isolates were grouped into distinct lineages corresponding to their recombination patterns. PHI tests conducted on multiple datasets indicated statistically significant recombination signals ($p \leq 0.05$), confirming the occurrence of recombination among and within genetic groups. For all genomic datasets, recombination was deemed significant ($p = 0.00$), suggesting that the viral genomes may have originated from parental Begomoviruses infecting diverse crops (Figure 5). To further validate these findings, recombination events were analyzed using the RDP v4.1 software package, which integrates seven detection algorithms. The analyses identified multiple putative recombination breakpoints and parental sequences, providing evidence of past recombination events (Figure 6; Table 6). Each isolate exhibited unique recombination profiles involving both major and minor parental sequences. These events are likely to have contributed to the adaptive evolution and diversification of papaya-infecting Begomoviruses in India.

Discussion

Papaya (*C. papaya*) is a significant tropical fruit crop valued for its nutritional and economic importance. However, its productivity is severely affected by various biotic stressors, among which Papaya Leaf Curl Disease (PaLCD) is the most devastating (Udavatha et al. 2022; Varun et al. 2024). The disease is caused by Papaya Leaf Curl Virus (PaLCuV), a member of the *Begomovirus* genus (family Geminiviridae), transmitted by the whitefly *B. tabaci*. Since its first report in India in 1998 (Saxena et al. 1998), PaLCD has emerged as a serious threat to papaya cultivation across multiple states. The present study provides a comprehensive molecular characterization of Begomoviruses infecting papaya in key papaya-growing regions of India, revealing remarkable genetic heterogeneity and evolutionary complexity underlying PaLCD. Field surveys across Chhattisgarh, Delhi, and Uttar Pradesh recorded variable disease incidence, which correlated with high whitefly abundance a well-known determinant of *Begomovirus* transmission (Srivastava et al. 2022b).

Table 3. Features and accession number of positive samples (sequenced) of *Begomovirus* isolates infecting papaya crop

Sample	Begomoviruses and associated component				Accession no.	Isolate
	DNA-A	DNA-B	Betasatellite	Alphasatellite		
PL 1	Papaya Leaf Curl Virus (PaLCuV)	_	Papaya Leaf Curl Zetasatellite (PaLCuB)	_	MZ605904 - MZ605905	Gorakhpur_av1
PL 6	Papaya Leaf Curl Virus (PaLCuV)	_	Papaya Leaf Curl Betasatellite (PaLCuB)	_	MZ669217 - MZ606364	Gorakhpur_av2
PL 10	Cotton Leaf Curl Multan Virus (CLCuMuV)	_	Tomato Leaf Curl Bangladesh Betasatellite (ToLCBDB)	Papaya Leaf Curl Vishakapuri Alphasatellite (PaLCVSA)	OQ440383 - OQ440384 - OQ440385	Bastar_RAV
PL 13	Papaya Leaf Curl Virus (PaLCuV)	_	Tomato Leaf Curl Bangladesh Betasatellite (ToLCBDB)	_	OR489166 - OQ440377	Delhi_RAV
PL 20	Papaya Leaf Curl Virus (PaLCuV)	Tomato Leaf Curl New Delhi Virus (ToLCNDV)	_	_	OQ134774 - OQ134775	Durg_RAV
PL 27	Croton Yellow Vein Mosaic Virus (CYVMV)	_	Papaya Leaf Curl Betasatellite (PaLCuB)	_	OQ168370 - OQ168371	Kahlilabad_RAV
PL 29	Cotton Leaf Curl Virus (CLCuV)	_	Cotton Leaf Curl Betasatellite (CLCuB)	_	OQ091756 - OQ091757	Maharajganj_RAV
PL 31	Tomato Leaf Curl New Delhi Virus (ToLCNDV)	_	Tomato Leaf Curl Betasatellite (ToLCB)	_	OQ290944 - OQ290945	Nautanwa_RAV
PL 36	Papaya Leaf Curl Virus (PaLCuV)	_	Papaya Leaf Curl Betasatellite (PaLCuB)	_	OQ290942 - OQ290943	Raipur_RAV
PL 43	Papaya Leaf Curl Virus (PaLCuV)	_	Papaya Leaf Curl Betasatellite (PaLCuB)	Papaya Leaf Curl Alphasatellite (PaLCuA)	OQ440378 - OQ440379 - OQ440380	Bilaspur_RAV
PL 45	Tomato Leaf Curl New Delhi Virus (ToLCNDV)	_	Cotton Leaf Curl Betasatellite (CLCuB)	_	OQ440381 - OQ440382	Mahasamund_RAV

Table 4. ORFs details of sequenced *Begomovirus* isolates from diseased papaya crops

ORFs	PL 1	PL6	PL 10	PL 13	PL 20	PL 27	PL 29	PL 31	PL 36	PL 43	PL 45
DNA-A											
CP (AV1)	307-1077	310-1080	280-1047	290-1060	307-1077	307-1077	293-1063	281-1060	304-1059	310-1080	287-1057
pre-CP (AV2)	147-503	150-506	120-485	130-486	147-503	147-503	133-480	121-459	144-500	150-506	127-465
Rep (AC1)	1526-2611	1529-2614	1496-2581	1558-2616	1531-2616	1526-2611	1521-2591	1516-2586	1529-2614	1529-2533	1506-2597
TrAP (AC2)	1219-1623	1222-1626	1147-1599	1368-1628	1224-1628	1219-1623	1205-1615	1194-1613	1222-1626	1222-1626	1199-1603
REn (AC3)	1200-1478	1077-1481	1050-1454	1057-1461	1079-1483	1074-1478	1060-1464	1064-1474	1077-1481	1077-1481	1054-1464
C4 (AC4)	2161-2460	2200-2457	2128-2430	2202-2459	2202-2459	2197-2454	2138-2446	2253-2429	2200-2457	2200-2457	2264-2440
Betasatellite											
βC1	189-557	201-557	202-558	202-558	_	221-577	194-550	192-572	201-557	200-556	195-551
DNA-B											
BC1	_	_	_	_	1303-2148	_	_	_	_	_	_
BV1	_	_	_	_	440-1246	_	_	_	_	_	_
Alphasatellite											
REP	_	_	65-1012	_	_	_	_	_	_	89-1036	_

Table 5. Results of nucleotide BLAST (BLASTn), and Sequence Demarcation Tool (SDT) of sequenced *Begomovirus* isolates from diseased papaya crop estimating homology score with other *Begomovirus* isolate

Sample	Virus isolate	Accession number	Maximum nucleotide % identity with existing isolate BLAST	Maximum whole genome nucleotide % identity with existing isolate SDT	Name assigned based on nucleotide sequenced identity among each other <91%
PL 1	PaLCuV/av1_GKP	MZ605904	KY800906_98.63 PaLCuV/IN: ND:17	KY800906_99.40 PaLCV/IN: ND:17	Papaya Leaf Curl Gorakhpur 1 Virus (PaLCuGKP1V)
	PaLCuB/av1	MZ605905	JX987089_98.83 PaLCuB/IN: NBRI:21	JX987089_99.30 PaLCuB/IN: NBRI:21	
PL 6	PaLCuV/av2_GKP	MZ669217	JN807765_98.91 PaLCuV/IN: Lkw:13	JN807765_98.90 PaLCV/IN: Lkw:13	Papaya Leaf Curl Gorakhpur 6 Virus (PaLCuGKP6V)
	PaLCuB/av2	MZ606364	JX050199_97.82 PaLCuB/IN: IYV: Del:12	JX050199_99.30 PaLCuB/IN: IYV: Del:12	
PL 10	CLCuMuV/Bastar_RAV	OQ440383	JN558358_96.85 CLCuMuV/IN:10	JN558358_97.50 CLCuMuV/IN:10	
	ToLCBDB/BSTR_RAV	OQ440384	KX302716_94.17 ToLCBDB/IN:GU2B3:11	KX302716_93.40 ToLCBDB/IN:GU2B3:11	
	PaLCVSA/BSTR_RAV	OQ440385	ON054962_98.16 PaLCVSA/IN: PVS2:18	ON054962_99.80 PaLCVSA/IN: PVS2:18	
PL 13	PaLCuV/DL_RAV	OR489166	MN839534_94.57 PaLCuV/PAK: WA1:18	MN839534_94.50 PaLCuV/PAK: WA1:18	Papaya Leaf Curl Delhi Virus (PaLCuDLV)
	ToLCBDB/DL_RAV	OQ440377	KX302716_91.57 ToLCBDB/IN:GU2B3:11	KX302716_94.57 ToLCBDB/IN:GU2B3:11	
PL 20	PaLCuV/Durg_RAV	OQ134774	FJ593629_98.95 PaLCuV/IN: Pt:08	FJ593629_99.30 PaLCuV/IN: Pt:08	Papaya Leaf Curl Durg Virus (PaLCuDGV)
	ToLCNDV/Durg_RAV	OQ134775 (DNA-B)	MT161675_98.33 ToLCNDV/BD: PapND71:16	MT161675_99.40 ToLCNDV/BD: PapND71:16	
PL 27	CYVV/KLD_RAV	OQ168370	FN543112_97.88 CrYVMV/PAK:06	FN543112_99.71 CrYVMV/PAK:06	
	PaLCuB/KLD_RAV	OQ168371	MH359169_99.71 PaLCuB/IN:18	MH359169_99.70 PaLCuB/IN:18	
PL 29	CLCuV/ MHJ_RAV	OQ091756	GU440580_98.44 CLCuV/IN: Lko: 2:06	GU440580_99.20 CLCuV/IN: Lko: 2:06	
	CLCuB/MHJ_RAV	OQ091757	GQ369731_98.53 CLCuB/IN: LKO:08	GQ369731_98.50 CLCuB/IN: LKO:08	
PL 31	ToLCNDV/NTW_RAV	OQ290944	MH520664_97.84 ToLCNDV/PAK: ARG:12	MH520664_99.00 ToLCNDV/PAK: ARG:12	
	ToLCuB/NTW_RAV	OQ290945	MT385292_99.33 ToLCuB/IN: GKP04:20	MT385292_99.60 ToLCuB/IN: GKP04:20	
PL 36	PaLCuV/RPR_RAV	OQ290942	KY926899_99.46 PaLCuV/PAK:RM428:16	KY926899_99.60 PaLCuV/PAK:RM428:16	Papaya Leaf Curl Raipur Virus (PaLCuRPRV)
	PaLCuB/RPR_RAV	OQ290943	AY244706_86.81 PaLCuB/IN: ND:03	AY244706_88.00 PaLCuB/IN: ND:03	Papaya Leaf Curl Raipur Betasatellite (PaLCuRPRB)
PL 43	PaLCuV/BLS_RAV	OQ440378	Y15934_98.37 PaLCuV/IN:97	Y15934_98.30 PaLCuV/IN:97	Papaya Leaf Curl Bilaspur Virus (PaLCuBLSV)
	PaLCuB/BLS_RAV	OQ440379	GU370715_97.47 PaLCuB/IN: PRM:05	GU370715_98.47 PaLCuB/IN: PRM:05	
	PaLCuA/BLS_RAV	OQ440380	JQ322970_98.49 PaLCuA/IN: CLCuV:10	JQ322970_99.20 PaLCuA/IN: CLCuV:10	
PL 45	ToLCNDV/MAHA_RAV	OQ440381	DQ989325_98.89 ToLCNDV/IN: PD:06	DQ989325_98.10 ToLCNDV/IN: PD:06	
	CLCuB/MAHA_RAV	OQ440382	JX217745_97.10 CLCuB/IN:10	JX217745_95.10 CLCuB/IN:10	

Table 6. Recombination analysis of breakpoint and its putative parental sequences listed based on best method having minimum p-Value for each recombinant isolate of Begomoviruses isolated from papaya

Present isolates				**Breakpoint and its putative parental sequences listed based on best method having minimum *p*-Value for each recombinant isolate**					
***Carica papaya* (sample)**	***Begomovirus***	**Events (Total)**	**Recombinant (Yes/No)**	**Breakpoints**		**Parent**		**Method**	***p*-value**
				Begin	**End**	**Minor**	**Major**		
PL 1	MZ605904 PaLCV/IN:GKP:av1:21	5	YES	2076	1159	MF574143 ChiLCINV/IN: Meerut:17	HM140364 ChiLCV/IN: DU:09	***R***, G, **M**, S, ***3S***	4.42E-07
	MZ605905 PaLCuB/IN: GKP: av1:21	7	YES	189	495	MN839536 PaLCuB/Pak: WA22:19	MF374787 PaLCuB/IN: betaRad07:17	***R***, G, M, **S**, ***3S***	3.02E-20
PL6	MZ669217 PaLCV/IN:GKP:av2:21	32	YES	1548	1900	KU376493 PaLCV/IN:CN2:15	KY026598 PaLCV/IN: Rad07:15	***R***, G, M, S, *3S*	4.25E-08
	MZ606364 PaLCuB/IN: GKP: av2:21	3	YES	622	801	MG686555 PaLCuB/IN: kang:17	LT600718 PaLCuB/Pak: IH51:16	*R*, G, M, S, ***3S***	1,03E+01
PL 10	OQ440383 CLCuMuV/IN: Bstr: RAV:21	18	YES	1517	2581	AJ002459 CLCuMuV/UK: olc311:97	AJ002447 CLCuMuV/PAK: clc62:97	*R*, G, M, S, ***3S***	1.38E-19
	OQ440384 ToLCBDB/IN: Bstr: RAV:21	3	YES	1264	601	MZ151263 ToLCBDB/IN: CHB8:21	MZ172977 ToLCBDB/IN:ZN:KOD:19	*R*, G, M, S, ***3S***	3.52E-14
	OQ440385 PaLCVSA/Bstr: RAV:21	6	YES	1310	488	OQ4403377 ToLCBDB/IN: DL: RAV	MN417113 ToLCBDB/IN: CHL44:14	*R*, G, M, S, ***3S***	4,38E-11
PL 13	OR489166 PaLCuV/IN: DL: RAV:21	19	YES	2742	1499	MN839534 PaLCuV/PAK: WA1:18	LT009395 PaLCuV/PAK: MNT: D18:12	***R***, G, M, **S**, ***3S***	2.56E-34
	OQ440377 ToLCBDB/IN: DL: RAV:21	4	YES	1169	633	MZ172977 ToLCBDB/IN:ZN:KOD:19	MN417113 ToLCBDB/IN: CHL44:14	***R***, G, **M**, S, ***3S***	4.27E-06
PL 20	OQ134774 PaLCV/IN: Durg: RAV:21	11	YES	2600	2692	Unknown (LN886525 CYVMV/PAK:17RS60:15)	JN663850 CYVMV/IN: Bhu:09	***R***, **G**, M, S, ***3S***	1.49E-13
	OQ134775 ToLCNDV/IN: Durg: RAV:21	8	YES	2576	2655	Unknown (MG373560 ToLCGuV/IN: ND:16)	MT161675 ToLCNDV/BD: PapND71:16	***R***, **G**, M, S, ***3S***	4.67E-08
PL 27	OQ168370 CYVV/IN: KLD: RAV:21	14	YES	2117	2405	FN678906 CYVMV/PAK: HY:06	Y15934 PaLCV/IN:97	*R*, G, M, S, ***3S***	6.19E-05
	OQ168371- PaLCuB/IN: KLD: RAV:21	9	YES	1248	642	KY825245 PaLCuB/PAK: 2491:17	Unknown (LT600709 CroYVMB/PAK: bhainsa: 21:16)	*R*, G, M, S, ***3S***	1.28E-16
PL 29	OQ091756 CLCuV/IN: MHJ: RAV:21	14	YES	2551	2567	Unknown (AF195782 ToLCLAV/LA:99)	GU253915 ToLCPatV/IN: Tb:09	***R***, **G**, M, S, ***3S***	3.26E-06
	OQ091757 CLCuB/IN: MHJ: RAV:21	3	YES	1145	1297	EU126825 PaLCuB/IN:07	OQ440382 CLCuB/IN: Maha: RAV:21	*R*, G, M, S, ***3S***	1,52E-19

PL 31	OQ290944 ToLCNDV/IN: NTW: RAV:21	6	YES	1613	2582	KC914896 ToLCNDV/PAK: Mn: 05:12	MT316388 ToLCNDV/IN: Matar:19	***R***, G, M, S, *3S*	1.42E-18
	OQ290945 ToLCuB/IN: NTW: RAV:21	***NOT DETECTED AS RECOMBINANT***							
PL 36	OQ290942 PaLCV/IN: RPR: RAV:21	11	YES	1028	1088	Unknown (HG937524 CYVMV/IN: ZI73:12)	KY926899 PaLCV/PAK:RM428:16	***R***, **G**, M, S, ***3S***	4.19E-11
	OQ290943 PaLCuB/IN: RPR: RAV:21	7	YES	361	714	AY244706 PaLCuB/IN: ND:03	JN663849 PaLCuB/IN: Bengaluru:08	***R***, G, M, S, *3S*	6.55E-70
PL 43	OQ440378 PaLCuV/IN: BLS: RAV:21	32	YES	2569	2700	Unknown (JQ954859 PaLCV/IN: ASLko:11)	Y15934 PaLCV/IN:97	***R***, G, **B**, M, S, ***3S***	6.14E-27
	OQ440379 PaLCuB/IN: BLS: RAV:21	5	YES	1080	1158	JN663874 PaLCuB/IN: Salem:08	LN828709 ToLCuB/PAK: ND16:15	*R*, G, M, S, ***3S***	1,13E-11
	OQ440380 PaLCuA/IN: BLS: RAV:21	1	YES	316	351	MN885461 AYVINA/PAK: R43:16	JQ322970 PaLCuA/IN: CLCuV:10	***R***, **G**, M, S, ***3S***	6,33E-18
PL 45	OQ440381 ToLCNDV/IN: MAHA: RAV:21	8	YES	1412	1785	KC513822 ToLCNDV/IN:AS:12	KX827602 ToLCNDV/PAK: NJ: 56:15	*R*, G, M, S, ***3S***	3.87E-11
	OQ440382 CLCuB/IN: MAHA: RAV:21	4	YES	631	1264	AY744380 CLCuB/IN:04	KT447040 CLCuMuB/IN:1YF:11	***R***, G, M, **S**, ***3S***	3.01E-10

Note: R: RDP; G: Geneconv; B: Bootscan; M: MaxChi; C: Chimarea; S: SiScan; 3Seq: Sequence Triplets; The lowest p-value calculated for the underline and bold method are given in the column

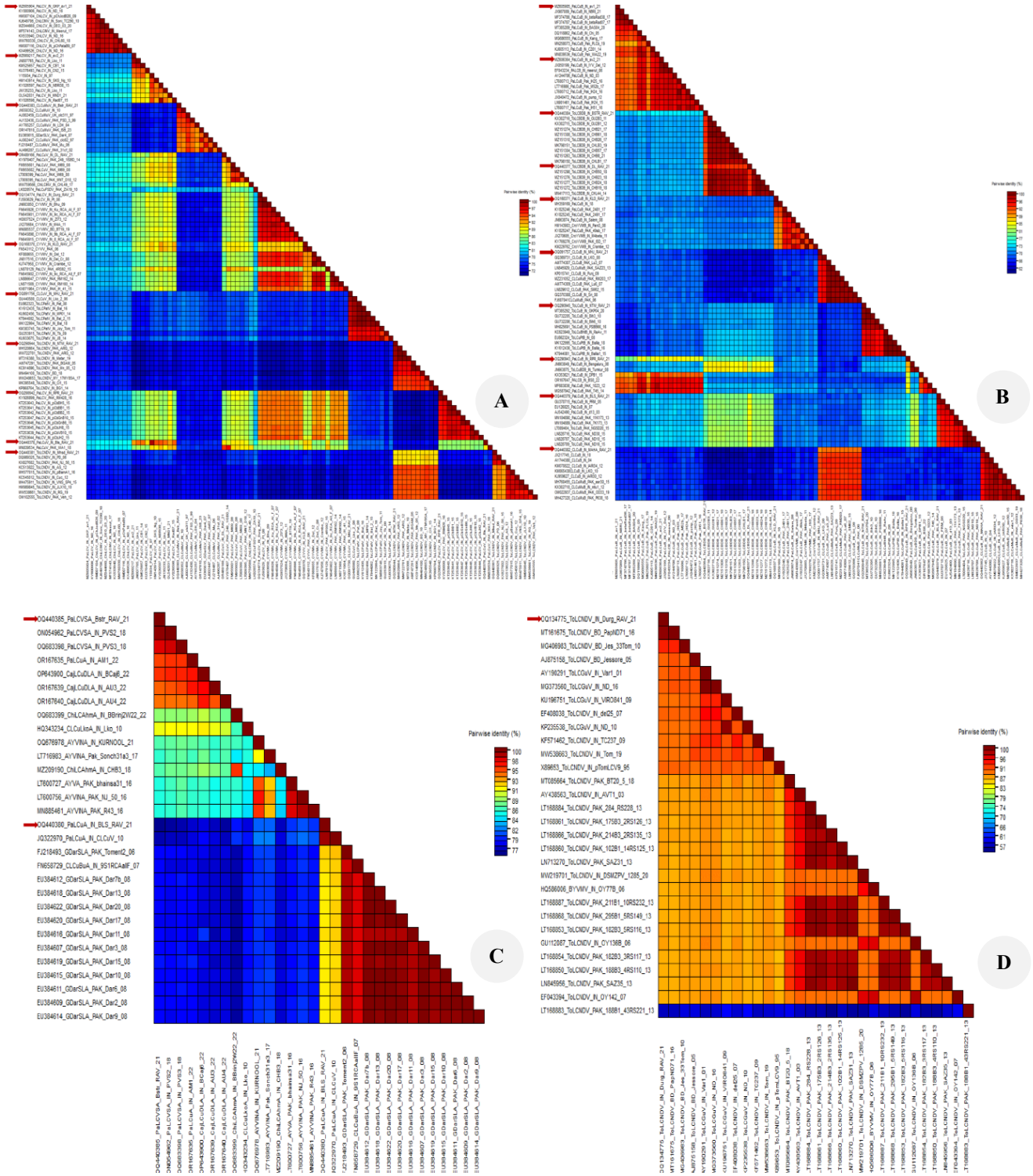

Figure 3. Color-coded matrix of percent pair wise nucleotide identity plot and genome score of full-length genomic component of *Begomovirus* isolated from diseased papaya leaves (Red Bars) using the SDT v1.2. A. DNA-A; B. Betasatellite; C. Alphasatellite; D. DNA-B

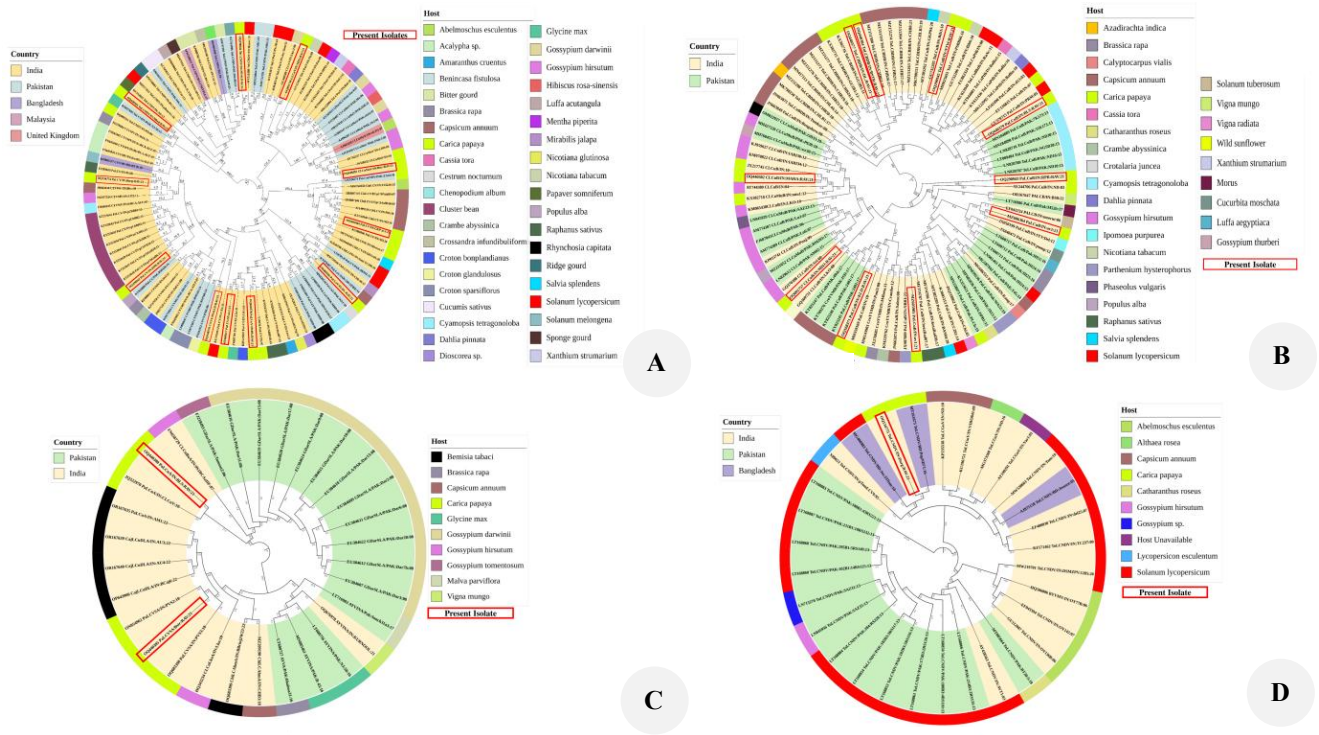

Figure 4. Maximum Clade Credibility Phylogenetic tree illustrating the relationship of isolate av1 and av2 within and among Begomoviruses: A. DNA-A; B. Betasatellites; C. Alphasatellite; D. DNA-B. The tree was constructed using Bayesian technique and Tree annotator tool with BEAST v.1.10 package. Bootstrap values are shown on branches. The inner ring represents the origin and outer ring represents the host of isolates

Genetic characterization and species demarcation

Based on sequence identity and ICTV species demarcation criteria (Brown et al. 2015), eleven *Begomovirus* isolates were identified. Among them, five isolates showed >91% nucleotide sequence identity with known Begomoviruses , classifying them as variants of existing species specifically, PL-29 and PL-43 as variants of Cotton Leaf Curl Virus (CLCuV) and Papaya Leaf Curl Virus (PaLCuV), and PL-10, PL-13, and PL-45 as variants of Cotton Leaf Curl Multan Virus (CLCuMuV) and Croton Yellow Vein Mosaic Virus (CYVMV), respectively. In contrast, six isolates (PL-1, PL-6, PL-20, PL-27, PL-31, and PL-36) exhibited <91% nucleotide identity in their DNA-A component, fulfilling the ICTV threshold for novel species classification. Accordingly, we propose these as new *Begomovirus* species: papaya leaf curl Gorakhpur 1 virus, papaya leaf curl Gorakhpur 6 virus, papaya leaf curl Delhi virus, papaya leaf curl Durg virus, papaya leaf curl Raipur virus, and papaya leaf curl Bilaspur virus. The discovery of these six novel Begomoviruses significantly broadens the known genetic diversity of papaya-infecting Begomoviruses and underscores the dynamic evolution occurring within Indian agroecosystems. These findings provide the first molecular evidence of emergent *Begomovirus* species in papaya and highlight their potential epidemiological importance. The molecular tools applied here PCR-based detection, RCA amplification, and sequence-based classification offer valuable frameworks for monitoring viral evolution and predicting potential outbreaks.

Recombination and viral evolution

Recombination analysis demonstrated that genome reshuffling plays a pivotal role in *Begomovirus* evolution (Mishra et al. 2022). Statistically significant recombination events were identified through PHI tests, indicating both intra- and interspecific exchange among isolates. Isolates PL-6 and PL-43 displayed the highest number of recombination breakpoints, followed by PL-13 and PL-10, suggesting frequent genetic exchange among sympatric virus populations. In contrast, isolate PL-31 (PaLCuB) exhibited no evidence of recombination, implying strong purifying selection that may constrain recombinant persistence. These results support the hypothesis that *Begomovirus* evolution is driven by a dynamic balance between adaptive selection and recombination-mediated diversification. The observed mosaic genome structures, derived from mixed parental lineages, likely facilitate enhanced host adaptability and vector transmission efficiency. Consistent with previous findings (Kumar et al. 2015; Quadros et al. 2023; Khan and Dijkstra 2024), our data reinforce the central role of homologous recombination, pseudo-recombination, and mixed infections as key mechanisms underlying *Begomovirus* diversification and host-range expansion. Such processes accelerate viral speciation and may ultimately contribute to the emergence of epidemic outbreaks (Sattar et al. 2025).

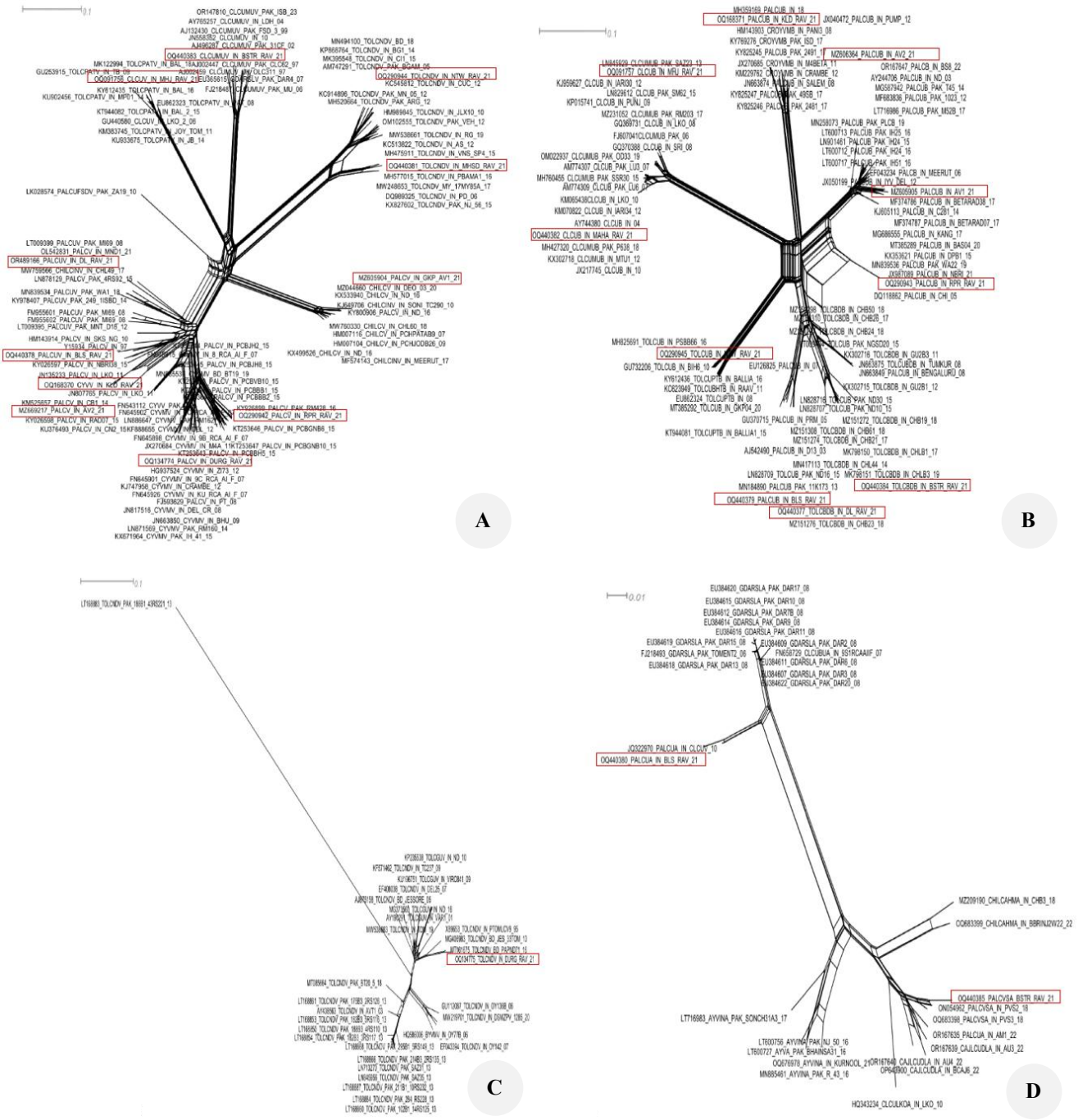

Figure 5. A Neighbour-net phylogenetic network generated for sequence-based data set comprising of papaya-infecting isolates and other Begomoviruses performed using Splits Tree v.4. A. DNA-A; B. Betasatellite; C. DNA-B; D. Alphasatellite

Evolutionary and epidemiological implications

The emergence of novel *Begomovirus* species in papaya reflects ongoing evolutionary pressures driven by ecological, agronomic, and vector-related factors. Agricultural intensification, overlapping crop cycles, and climatic variations that favor *B. tabaci* proliferation may create ecological niches conducive to viral recombination and reassortment. Moreover, intercropping practices involving *Begomovirus*-susceptible hosts (e.g., cotton, tomato, and okra) further increase opportunities for co-infection and cross-species transmission. Understanding these evolutionary dynamics is critical for designing sustainable management strategies. Continuous molecular surveillance and vector population monitoring are essential to detect emergent variants before large-scale outbreaks occur. Integrated disease management approaches combining resistant cultivars, vector control, and molecular diagnostics should be prioritized to mitigate the threat posed by rapidly evolving Begomoviruses in tropical fruit systems.

Figure 6. Recombination analysis of breakpoints and its putative parental sequences showing distribution of recombination frequency across the genome of isolated Begomoviruses from papaya. A. DNA-A; B. Betasatellite; C. Alphasatellite; D. DNA-B

This study provides the first comprehensive molecular characterization of Begomoviruses associated with Papaya Leaf Curl Disease (PaLCD) in the major papaya-growing regions of Uttar Pradesh, Delhi, and Chhattisgarh, India. Using an integrated approach involving PCR detection, Rolling Circle Amplification (RCA), molecular cloning, and full-length genome sequencing, we confirmed the widespread occurrence of multiple Begomoviruses , including Papaya Leaf Curl Virus (PaLCuV), Tomato Leaf Curl New Delhi Virus (ToLCNDV), and their associated satellite molecules. Notably, six PaLCuV isolates exhibiting <91% nucleotide identity was identified as novel species, along with a newly characterized betasatellite, Papaya Leaf Curl Raipur Betasatellite (PaLCuRPRB), underscoring the extensive genetic plasticity within the *Begomovirus*complex. The detection of active recombination signals and the presence of geographically distinct variants indicate that region-specific selection pressures and host-virus interactions are key drivers of *Begomovirus* diversification and adaptation in Indian papaya ecosystems. Overall, these findings substantially enhance our understanding of the molecular diversity, evolutionary mechanisms, and epidemiology of PaLCD. The identification of new *Begomovirus* species and satellite molecules provides valuable genomic resources for refining phylogenetic frameworks and guiding the development of virus-resistant cultivars through molecular breeding and biotechnology. Continued surveillance and evolutionary monitoring will be critical for predicting future outbreaks and implementing region-specific, sustainable disease management strategies to safeguard papaya production in India.

The expanding diversity of Begomoviruses infecting papaya underscores the need for continuous molecular surveillance and integrated disease-management strategies. The emergence of novel Papaya Leaf Curl Virus (PaLCuV) variants and new satellite molecules, as revealed in this study, highlights the dynamic evolutionary potential of these pathogens under changing agro-ecological and climatic conditions. Future research should therefore focus on establishing a nationwide genomic monitoring network to track spatiotemporal variations in *Begomovirus* populations and their whitefly vectors (*B. tabaci*). Such real-time monitoring would facilitate early detection of virulent strains and inform region-specific control strategies. Advances in high-throughput sequencing and metagenomics now provide powerful tools for dissecting the complex viromes associated with papaya and its neighboring host species. Comprehensive virome mapping will be crucial for uncovering hidden virus-virus and virus-vector interactions that drive recombination and host adaptation. Integrating genomic data with ecological and epidemiological modeling could enable predictive frameworks for PaLCD outbreak risk assessment under future climate scenarios. From a management perspective, the development of durable resistance remains a top priority. Emerging molecular breeding technologies such as marker-assisted selection, RNA interference (RNAi), and CRISPR/Cas-based genome editing offer unprecedented opportunities to engineer broad-spectrum and sustainable resistance against Begomoviruses and their satellites.

Simultaneously, functional characterization of key viral and satellite proteins, including βC1 and Rep, could uncover molecular targets for antiviral gene silencing or chemical inhibition. Lastly, effective control of PaLCD will require ecosystem-based management, integrating vector control, crop diversification, and deployment of resistant cultivars. Strengthening collaborations among plant virologists, molecular biologists, breeders, and data scientists will be vital to translate genomic insights into applied solutions for the papaya industry. Together, these multidisciplinary efforts will pave the way toward resilient papaya production systems and contribute to safeguarding tropical fruit agriculture against rapidly evolving viral threats.

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