



## Original Article

Biocomputational Recombination, Evolution, and Distribution Patterns Analysis of *Begomovirus* Beta-satellites in Chilli Crop Affected by Leaf Curl Disease in PakistanMuhammad Atif<sup>1</sup>, Uzma Bashir<sup>1</sup>, Muhammad Tariq Manzoor<sup>1</sup>, Qandeel Ishfaq<sup>1</sup> and Madiha Zahoor<sup>1</sup><sup>1</sup>Department of Plant Pathology, University of the Punjab, Lahore, Pakistan

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## ABSTRACT

*Geminiviridae* is one of the largest families of single-stranded DNA (ssDNA) plant viruses. Among 15 genera, *begomoviruses* are the most important, comprising more than 464 species. Vegetable crops are primary hosts of *begomoviruses*, and whiteflies are insect vectors that persistently transmit them. Beta-satellites are subviral particles and are actively associated with leaf curl diseases of solanaceous crops caused by *begomoviruses*. **Objectives:** To classify *begomoviruses* beta-satellites into species and variants and to check the recombination in isolated molecules. **Methods:** 59 full-length sequences of Chilli leaf curl beta-satellite were downloaded from the National Center for Biotechnology Information and were analyzed using different bioinformatics tools such as MEGA, SDT, and RDP. Our phylogenetic analysis suggested that chilli leaf curl beta-satellites associated with chilli crops having accession number OQ076340, isolated from Pakistan, appear at the bottom of the phylogenetic tree. **Results:** The current study recombination tests (BootScan, SiScan, Chimaera, MaxChi, RDP, GENCONV, and 3Seq) showed recombination in 21 satellite molecules, 11 sequences missing their major and minor parents, and 27 sequences found to be unique. **Conclusions:** Betasatellites show huge diversity in nature. Recombination with other viruses helps betasatellites produce a more complex structure and greater diversity. This complex nature makes them difficult to control.

## INTRODUCTION

Chilli leaf curl disease (ChiLCD) complex caused by the complex of *begomoviruses* is the major threat to chilli production across India and Pakistan. Chilli leaf curl virus (ChiLCV) and its associated chilli leaf curl beta-satellite (ChiLCB) are the major players in ChiLCD. Beta-satellite molecules are single-stranded DNA (SSDNA) molecules with a satellite conserved region which play key role in replication. The length of the genome is almost 1400bp [1]. ChiLCV is persistently transmitted by whiteflies. Recent studies suggest that *begomoviruses* also use a recombination-dependent replication mechanism (RDR) [2]. According to numerous studies, ChiLCV has unique

genetic diversity among other *begomoviruses*. The coat protein (V1 or AV1) of ChiLCV performs a crucial role in transmission. Replication-associated protein (Rep; C1) is involved in helicase activities [3]. Pre-coat protein (pre-cp V2) of ChiLCV is involved in cell-to-cell movement [4]. The transmission of ChiLCV depends greatly on the specific biotype of whitefly. *Begomoviruses* are able to evolve very rapidly in a very short period of time to adapt to new cropping systems. For example, the number of whiteflies on flat-leaved cotton varies with hairy leaves, and such things lead to the selection of a particular biotype [5]. Experiments have shown that two different types of



whiteflies, one from cassava (Q-Biotype) and the other from sweet potato (*Bemisia tabaci*), have dramatically changed the existence of strains that are well established on these crops [6]. As quickly as *Begomoviruses* evolve and change their genome in a particular region, their existence and prevalence depend upon crops grown in that region. For example, it has been found that tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato yellow leaf curl virus (TYLCV) are more dangerous than their ancestors [7]. In case of mixed infection, their pathogenicity depends upon the host in which they occur. It has been shown that *begomoviruses* of the New World lack V2 genes but have a similar genome organization to old-world *begomoviruses* [8]. Such types of variations occur during replication due to some errors during replication, which can be recovered in the next generations [8]. From old world origin, *begomoviruses* have been categorized into different classification like Indian, African-Mediterranean, Legume infecting and Asian [9]. *Begomoviruses* have seven major subdivisions, which have further 34 sub-populations [10]. The epidemiological factors are missing, which are of great importance in virus dissemination. The main problems are the collection of virus vectors and the identification of viruliferous vectors. This is because the virus genome integrated with the host genome multiplies within the host. Another problem is the software's authenticity, the sensitivity of data analysis, and the correctness of findings. This study aims to classify *begomoviruses* and beta-satellites into species and variants and to check the recombination in isolated molecules.

## METHODS

A retrospective in silico study was conducted in the Department of Plant Pathology, University of the Punjab, during 2025. Sequences were downloaded in September, and bioinformatics analyses were done in October 2025. Sequences that were submitted to NCBI during the last twenty years are only considered for analysis. A bio-computational research design was used to interpret the molecular data. Sequences of beta-satellites were obtained from the taxonomy section of the National Center of Biotechnology Information (NCBI) in FASTA format. A gene bank file was also downloaded to prepare a complete dataset of beta-satellites. 59 full-length (1300bp) sequence molecules of beta-satellites were collected only from Pakistani regions from different crops. Partial sequences or clones were not collected for analysis. Phylogeny was determined by the minimum evolution method and given in the form of a complete phylogenetic tree. A dataset of all 59 molecules was also given. MEGA6 bio software was used to align beta-satellite sequences. Sequences were aligned in the muscle alignment section by eliminating gaps between nucleotides, and a tree was constructed by the minimum evolution method for differences [11]. Gamma Parameter

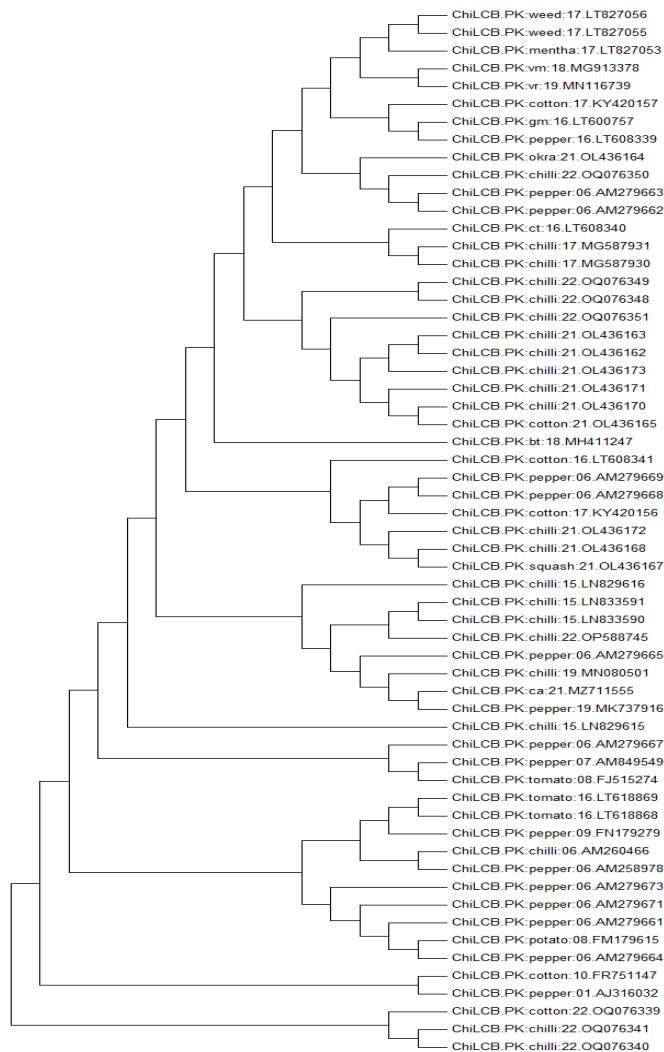
for Site Rates was also performed in the MEGA6 Program by the maximum likelihood method using the GTR (General time reversible) model [12]. Substitution Matrix Estimation was also prepared in the form of a table by using the maximum likelihood method with the shape parameter of the gamma distribution. The Neutrality Test of Tajima was also conducted to check nucleotide diversity and the number of segregating sites [12]. The sequence demarcation tool software (SDT) was used for matrix construction of beta-satellites [13]. Pairwise identity was also determined by using this Program. The SDT plot was given [14]. Recombination among chilli leaf curl beta-satellite sequences was checked by the RDP program (version 4). Different indicators were used to check recombination, including BootScan, SiScan, Chimaera, MaxChi, RDP, GENCONV, and 3Seq [15].

The p-value was set at 0.05, and the test was repeated three times, with a large step size (20+ nucleotides) used for confirmation. The RDP data set was also constructed to highlight the recombination and major and minor parents of a particular sequence.

## RESULTS

A total of 59 beta-satellite sequences associated with ChiLCD were retrieved from the database of NCBI. These sequences were reported from fifteen (15) different crops, including tomato, chilli, potato, weeds, mint, cotton, Cyamopsis, squash, okra, cannabis, mung bean, mash bean, pepper, and *Glycin max* (gene bank file). Twenty-two (22) betasatellite molecules were found in chilli crop, sixteen (16) from pepper, and three (3) from tomato crop, five (5) from cannabis, eleven (11) from okra, one (1) from mung bean, and one (1) from mash bean. Beta-satellite molecules were also found in *Glycin max*, weeds, and insects. A total of 59 satellite molecule sequences were aligned in MUSCLE alignment format, and a tree was constructed by the minimum evolution method. Bootstrap value was 90% with the Standard Non-parametric bootstrap method. Eighteen (18) clades were present in the phylogenetic tree. ChiLCB was found to infect chilli crop in 5 clades and pepper crop in 2 clades. An ideal tree with a total branch length of 0.96010526 was produced by estimating the evolutionary history using the Minimum Evolution (ME) approach. Using the Maximum Composite Likelihood approach, evolutionary distances were computed as base substitutions per site. At search level 1, the Close-Neighbor-Interchange (CNI) method was used to refine the ME tree. The Neighbor-Joining approach was used to build the first tree. 59 nucleotide sequences covering noncoding regions and codon positions 1–3 were included in the analysis. A final dataset of 1,193 positions was obtained by removing any positions with gaps or missing data. MEGA6 software was used for all evolutionary analyses. Beta satellite having accession number

OQ076340 isolated from chilli crop appears at the bottom of the phylogenetic tree. The phylogeny tree is showing that chilli and pepper are the major hosts due to 38 isolated sequences. Other molecules are also infecting medicinal plants and vegetable plants (Figure 1).



**Figure 1:** Dendrogramic Tree of Chilli Leaf Curl Beta-Satellite Isolated from Different Crops

The likelihood of a base (row) being substituted with another base (column) is represented by each entry (r). This model was used to estimate the substitution rates and patterns. Whereas transversal substitution rates are italicized, transitional substitution rates are in bold. These were kept under a single table. When assessing instantaneous r, relative values should be taken into account. In order to keep things simple, the nucleotide frequencies are as follows: T/U = 25.56%, A = 35.74%, G = 19.25%, and C = 19.45%. A hierarchy of trees was generated by software for the purpose of predicting ML values. For this calculation, the greatest Log likelihood was -8653.552. There were 59 nucleotide sequences in the analysis. Codon positions 1st+2nd+3rd+Noncoding were included.

Positions with gaps and incomplete data were all removed. The complete database contained 1193 locations altogether. Evolutionary studies were carried out in MEGA6. Negative result of 'D' indicates that sequences undergo recent mutations with low-frequency polymorphism (Table 1).

**Table 1:** Substitution of ChiLCB among Sequences

Variables	A	T/U	C	G
A	–	6.52	4.96	7.10
T/U	9.12	–	12.40	4.91
C	9.12	16.30	–	4.91
G	13.18	6.52	4.96	–

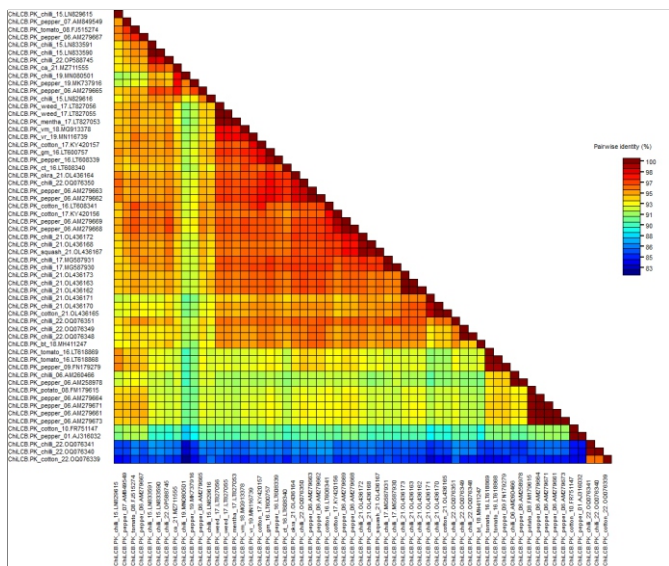
The analysis includes 59 nucleotide sequences from 1193 sites, spanning all codon and noncoding areas (excluding incomplete data). MEGA6 was used to carry out evolutionary analysis. Negative result of 'D' indicates that sequences undergo recent mutations with low-frequency polymorphism. This has happened due to two main reasons: one is population expansion, in which the population expands with low mutation without losing genetic drift. The other reason is purifying selection, in which the virus undergoes a clean-out phase to remove harmful mutations (Table 2).

**Table 2:** Results from Tajima's Neutrality Test

m	S	$P_s$	$\theta$	$\pi$	D
59	569	0.476949	0.102652	0.072049	-1.066996

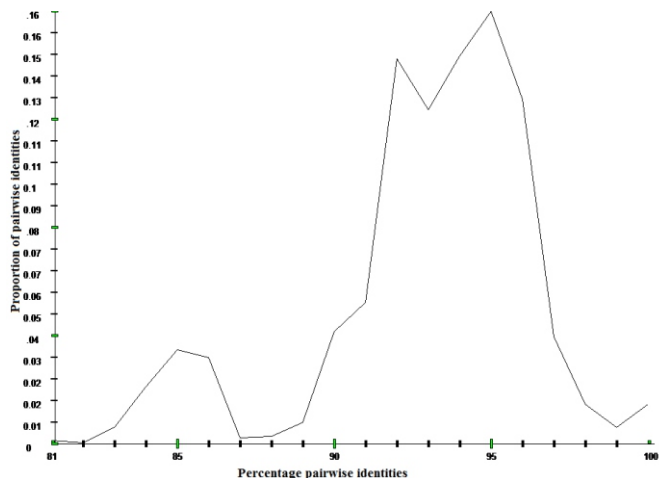
**Abbreviations:** D is the Tajima test statistic, n = total number of sites,  $\pi$  = nucleotide diversity, S = Number of segregating sites, m = number of sequences,  $\theta = p_s/a_1$ , and  $p_s = S/n$

ChiLCB isolated from chilli crop having accession number LN829615 was showing 100% resemblance to ChiLCB infecting cotton crop. Transmission would happen through trade between the materials of these two crops. Blue colour indicated the least resemblance of sequences among each other. The extreme red colour was showing 100% resemblance. The value of scale was kept between 94-82 to check homology and resemblance with each other (Figure 2).



**Figure 2:** Matrix of ChiLCB Infecting Different Crops

The plot of the SDT analysis shows the percentage of nucleotide diversity. Maximum pairwise identity was calculated at 96 when the proportion of pairwise reached the maximum value of 0.16 (Figure 3).



**Figure 3:** Percentage Pairwise Identities

All 59 sequences of beta-satellites were tested against the RDP Program to check for recombination in sequences. Thirty-eight (38) sequences were found to be unique and do not show any recombination. Recombination would happen due to the exchange or mixing of one viral DNA with another viral DNA. Beta-satellite sequence isolated from chilli crop base of the phylogenetic tree was found recombined. The

sequence isolated from Pakistan from the tomato was the major parent, and the minor parent was okra (RDP test). A complete overview of the RDP of selected tests was given. Results were confirmed three times by using different methods. Parents of some sequences were unknown. Parents of some sequences were found in weed crops (Table 3).

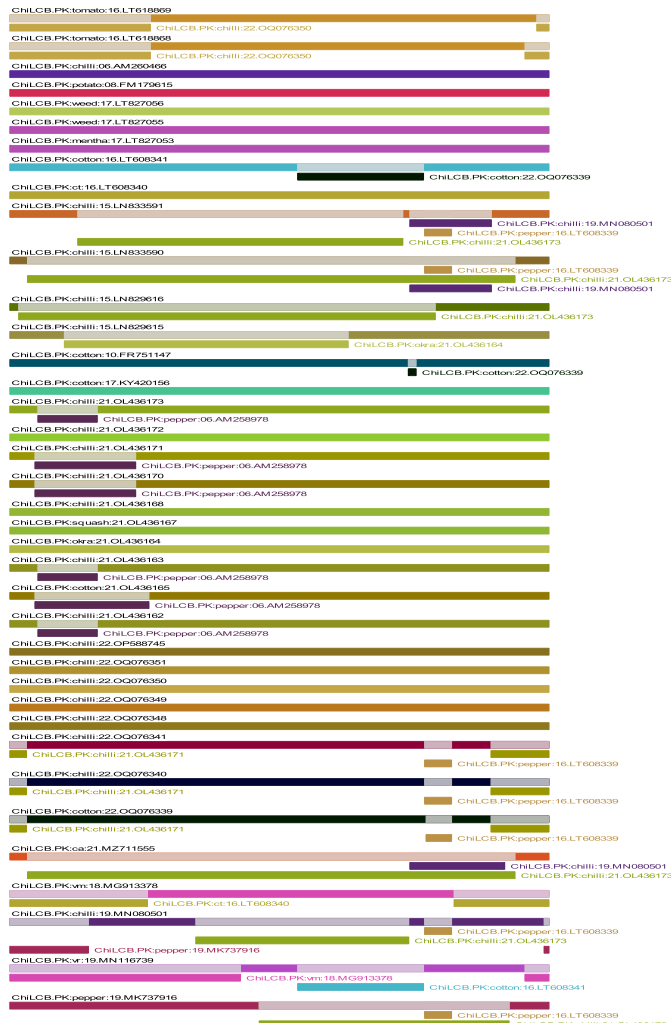
**Table 3:** Complete Overview of RDP Program

Event no	Found in	Recombinant	Major Parent	Minor Parent	Detection Methods						
					R	G	B	M	C	S	T
1	1	ChiLCB.PK: pepper	ChiLCB.PK:ca	Unknown	-	+	+	+	-	+	+
2	1	ChiLCB.PK:vr	ChiLCB.PK: cotton	ChiLCB.PK:vm	-	-	+	+	-	+	+
3	7	ChiLCB.PK: chilli	Unknown	ChiLCB.PK: chilli	+	+	+	+	+	+	+
4	3	ChiLCB.PK: pepper	ChiLCB.PK: chilli	ChiLCB.PK: potato	-	+	+	+	-	+	-
5	1	ChiLCB.PK: chilli	ChiLCB.PK: tomato	ChiLCB.PK:okra	-	-	-	+	-	+	+
6	2	ChiLCB.PK: chilli	ChiLCB.PK: chilli	ChiLCB.PK: chilli	+	+	+	+	+	+	+
7	1	ChiLCB.PK:bt	ChiLCB.PK: okra	Unknown	+	+	+	-	-	+	+
8	4	ChiLCB.PK:gm	ChiLCB.PK:ct	Unknown	-	-	-	+	+	-	+
9	6	ChiLCB.PK: pepper	ChiLCB.PK: gm	ChiLCB.PK: tomato	-	-	-	-	-	+	-
10	2	ChiLCB.PK: pepper	Unknown	ChiLCB.PK: chilli	-	+	+	+	-	+	-
11	3	ChiLCB.PK: tomato	Unknown	ChiLCB.PK: chilli	-	-	-	+	+	+	+
12	6	ChiLCB.PK:bt	ChiLCB.PK: chilli	ChiLCB.PK: chilli	-	-	-	-	-	+	-
13	3	ChiLCB.PK: cotton	Unknown	ChiLCB.PK: chilli	-	+	+	-	-	-	-
14	7	ChiLCB.PK: chilli	Unknown	ChiLCB.PK: pepper	+	+	-	+	-	+	+
15	1	ChiLCB.PK: chilli	Unknown	ChiLCB.PK: pepper	-	-	-	+	+	+	+
16	4	ChiLCB.PK: chilli	ChiLCB.PK: chilli	Unknown	-	-	-	-	-	+	-
17	6	ChiLCB.PK: chilli	ChiLCB.PK: chilli	ChiLCB.PK: pepper	-	-	-	+	+	-	+
18	1	ChiLCB.PK:	ChiLCB.PK: cotton	ChiLCB.PK: pepper	-	-	-	-	-	+	-
19	1	ChiLCB.PK: chilli	ChiLCB.PK: chilli	Unknown	-	-	-	-	-	-	+
20	2	ChiLCB.PK:vr	ChiLCB.PK: menthol	ChiLCB.PK: cotton	-	-	-	-	-	+	-
21	2	ChiLCB.PK: cotton	ChiLCB.PK:vr	ChiLCB.PK: cotton	-	-	-	-	-	+	-

T=3seq, S=SiScan, C=Chimaera, M=MAXchi, B=Boot-Scan, G=GENCOV, R=RDP

Twenty-one (21) sequences were found recombined with a frequency of 35.6%. In *begomoviruses*, this value indicates that

recombination is a primary source of genetic diversity. Major parents indicate a large proportion of fragments contributing towards viral replication and integrity. Minor parents make a portion of some important genes, like coat protein, which makes the virus more virulent and makes it adaptable to new species (Figure 4).



**Figure 4:** RDP Test Showing Major and Minor Parents of the Sequences

## DISCUSSION

Gradual change in DNA is a continuous process in nature. It helps viruses to evolve with the passage of time and adapt to new crop species [16, 18]. The history of the evolution of viruses is much older than we think. However, their evolution records are not available properly; therefore, scientists are relying on present viruses to determine their history, their ancestors, and the mechanisms by which they evolve, what factors are affecting their evolution and emergence, etc. Through mutation and recombination, *geminiviruses* evolved gradually, and many natural factors, including chemical, biological and physical were also assisting this mechanism. According to some studies,

plant viruses were present in nature about 450 million years ago. With the passage of time, these viruses have been discovered with different features and properties. Some *begomoviruses* isolated from India and Vietnam showed unique characteristics of old viruses. So, it can be said that these are descendants of old viruses. Phylogenetic studies have shown that old world *begomoviruses* (OW) are forefathers of new world *begomoviruses* (NW). Besides a gradual change in DNA, sudden changes in DNA also play an important role in virus evolution and the arrival of new species. In recent times, in Pakistan, a new strain of cotton leaf curl virus proved very devastating to the cotton crop. Experiments have been conducted on Tobacco etch virus (TEV) to check its effectiveness when it evolves. These experiments tell the virulence of TEV after evolution. Experiments also reveal that there is an increase in biotypes after evolution [19]. Artificial mutations were induced in TEV and tested against different host plants [20]. Results have revealed that the virulence of TEV increases as the number of mutations increases, but adaptability to new crops and environments decreases. A previous study has shown that a mutated strain of TEV was virulent against pepper but not against tobacco [21]. When this infected plant sap is inoculated into tobacco plants, the tobacco plants show equal symptoms. This phenomenon is known as a pleiotropic effect [22]. Studies have shown that recombination frequently occurs in the *Bromoviridae* family. RNA viruses in the *Bromoviridae* family evolve more quickly than DNA viruses [23, 24]. In the cucumber mosaic virus (CMV), recombination occurs at a faster rate. Different strains with recombination showed different degrees of virulence [25]. Greater mutations do not necessarily mean a higher degree of virulence, but if virulence increases due to mutation, adaptability to new plants and environments decreases [26]. Gene bank analysis of Chilli ring spot virus (ChiRSV) revealed that it has been isolated from three different countries so far. Phylogenetic analysis of ChiRSV revealed that the sequence with accession number KT633930, isolated in China, serves as the basis and supports further evolution and transmission of this virus [27]. Our phylogenetic analysis suggested that OLCuA isolated from Oman was an ancestral sequence and provided a platform for further evolution and distribution [28]. *Begomoviruses* are the most notorious viruses regarding replication, evolution, and transmission. Their plasmid-like replication makes them more virulent to infect more crop species. Pepper leaf curl Lahore virus (PepLCLV) is showing great diversity in chilli and pepper crops across the subcontinent. SDT analysis of PepLCLV indicated that some sequences showing 100% homology with each other, while they are isolated from different countries [29]. In this article, our SDT analysis is also showing 100% similarity in some

sequences. *Geminiviruses* are thought to be very diverse among plant viruses. Chickpea chlorotic dwarf virus (CpCDV), a mastrevirus belonging to the *Geminiviridae* family, infects diverse plant species. Data analysis revealed that it not only infects the *Fabaceae* family but also the *Malvaceae* family [30].

Despite the knowledge acquired, this study has a number of drawbacks. First, because data from some places is underrepresented in comparison to others, the dependence on sequences found in gene banks introduces a geographical bias. This could distort evolutionary interpretations of ancestral origins, like those proposed for OLCuA and ChiRSV. Second, there is no wet-lab validation to verify the biological effects of certain mutations or recombination events on host-pathogen interactions, and the evolutionary inferences derived from sequence homology and phylogenetic analysis are solely computational. Lastly, despite the limitations of molecular clock analysis, it is challenging to definitively recreate the timeline of viral development due to the fragmented nature of historical viral data. To address these limitations and advance the understanding of viral evolution, future research should prioritize the following areas, which include Comprehensive Scrutiny to create a more comprehensive worldwide picture of viral diversity. Functional authentication to confirm the biological importance of detected recombination events and mutations. environmental factor analysis to better predict emergence patterns, future research should look into the particular biological and environmental stressors (such as vector population dynamics and climate change variables) that cause the high recombination rates seen in *Bromoviridae* and *Geminiviridae*.

## CONCLUSIONS

Satellite molecules were downloaded from the database. Different evolutionary tests were conducted on them. A phylogenetic tree and SDT matrix were created using different software. Recombination was detected by using the RDP Program. Eighteen (18) clades were present; five (5) clades were found only in the chilli crop. In the SDT plot, the resemblance value was kept between 82 and 94, and the sequences showed a maximum proportion of homology at 0.02. In the RDP test, 21 sequences were found to be recombined with major and minor parents. This means that viruses also get some proportion of their genomes from other viruses. Seven different types of selected tests were applied in the RDP program.

## Authors' Contribution

Conceptualization: MA

Methodology: MA, UB, QI

Formal analysis: UB, MTM

Writing and Drafting: UB

Review and Editing: MA, UB, MTM, QI, MZ

All authors approved the final manuscript and take responsibility for the integrity of the work.

## Conflicts of Interest

All the authors declare no conflict of interest.

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