



# Molecular Insights into the Adaptability and Pathogenicity of Tomato Leaf Curl New Delhi Virus

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Tomato Leaf Curl New Delhi Virus (ToLCNDV), a highly destructive begomovirus, poses a significant threat to vegetable crops, particularly in the Indian subcontinent. First identified in tomato, ToLCNDV has expanded its host range to include cucurbits and other economically important crops. Transmitted by the whitefly vector *Bemisia tabaci*, the virus follows a persistent transmission mode, allowing its rapid spread across diverse agro-ecosystems. Despite efforts to control ToLCNDV, its adaptability and broad host range have made effective management

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challenging. This study explores the molecular mechanisms underlying ToLCNDV's transmission and host adaptability, focusing on specific viral genetic features, such as the 36-nucleotide duplication in the DNA-B component, which may enhance its ability to manipulate host defenses. The research also highlights the virus's interactions with satellite molecules like betasatellites, which suppress host gene silencing, further contributing to its virulence. Through comparative genome analysis using BLAST and phylogenetic methods, this study examines genetic diversity among ToLCNDV isolates. Findings demonstrate evolutionary adaptations that have facilitated the virus's spread and persistence across different crops and geographical regions. The study underscores the importance of developing integrated pest management strategies, breeding virus-resistant plant varieties, and identifying genetic markers associated with host resistance. By understanding ToLCNDV's genomic features and transmission dynamics, this research aims to inform strategies for mitigating its impact on global agricultural systems. Our research demonstrated that ToLCNDV infection disrupts key physiological processes in tomatoes, leading to stunted growth, leaf curling, and yield reduction. Furthermore, we observed that whiteflies retained the virus for up to two weeks, supporting its high transmission efficiency. These findings underscore the need for more targeted control strategies to mitigate the virus's impact on global agriculture.

**Keywords:** Tomato leaf; pathogenicity; plant diseases; agro-ecosystems; plant tissues.

## 1. INTRODUCTION

Plant viral diseases pose significant threats to economically important crops, often leading to devastating outcomes and substantial losses worldwide (Navas-Castillo et al., 2011). Among these, the Tomato Leaf Curl New Delhi Virus (ToLCNDV) has emerged as a particularly destructive Begomovirus, severely impacting vegetable crops in the Indian subcontinent. First identified in tomato in India [1]. ToLCNDV has since expanded its host range to include a variety of cucurbit species and other important crops, intensifying its agricultural significance Pratap et al., 2011; Moriones & Navas-Castillo, [2].

ToLCNDV is primarily transmitted by the whitefly vector *Bemisia tabaci*, which plays a critical role in its rapid spread across diverse agro-ecosystems [3,4]. Viral transmission by insect vectors can occur through nonpersistent, semipersistent, or persistent modes, depending on the duration of virion acquisition and retention within the vector [5,6]. ToLCNDV follows a persistent transmission mode, in which the virus must move from the foregut to the salivary glands of the whitefly before being released into plant tissues during feeding [7]. This efficient transmission mechanism allows the virus to infect a broad range of plant species, making it increasingly difficult to manage in field conditions [8].

Despite ongoing efforts to control ToLCNDV, its rapid spread and adaptability have raised

significant challenges for plant health and crop management [9]. A deeper understanding of the molecular mechanisms governing ToLCNDV transmission and pathogenicity is crucial for developing effective control strategies. To this end, this study hypothesizes that specific genetic features of ToLCNDV, such as the 36-nucleotide duplication in the DNA-B component, enhance the virus's adaptability and ability to manipulate host plant defenses, leading to its efficient transmission by *B. tabaci* [10,11].

By exploring the genetic features of ToLCNDV that contribute to its persistence and host range expansion, this study aims to provide insights into novel control measures that could mitigate its impact on vegetable crops. These insights will inform the development of integrated pest management strategies and the breeding of virus-resistant plant varieties [12].

### 1.1 Vector and Begomovirus

Whiteflies are small insects, ranging from 1 to 3 mm in length, named for their powdery white wings and bodies. They thrive in tropical and subtropical regions but can also be found in greenhouses in temperate areas due to the controlled conditions that favor their survival. Whiteflies cause direct damage by feeding on plant phloem, leading to reduced plant vigor and growth. Additionally, their excretion of honeydew facilitates the development of sooty mold, which can interfere with photosynthesis and reduce crop quality [13]. However, the most significant threat posed by whiteflies is their ability to

transmit plant viruses, especially those from the Geminiviridae family, which includes Tomato Leaf Curl New Delhi Virus (ToLCNDV).

Among the approximately 1,500 species of whiteflies [14] only a few are recognized as vectors of plant viruses. The *Bemisia tabaci* species complex, in particular, is notorious for its capacity to spread begomoviruses like ToLCNDV. The *Bemisia tabaci* complex is not a single species but a group of cryptic species that are morphologically similar but genetically distinct, and their global distribution and behavior vary significantly [15]. These species are found across tropical and subtropical regions and have spread globally due to international trade and the movement of infected plant material [16].

### 1.2 Virulence Mechanism

Three classes of circular single-stranded DNA satellites-betasatellites, alpha satellites, and delta satellites-are associated with begomoviruses Zhou, 2013; Lozano et al., [17]. Alpha satellites require the helper begomovirus for movement and transmission by *B. tabaci*, while betasatellites and deltasatellites rely on these viruses for replication. Betasatellites often modulate virulence by suppressing host gene silencing, whereas the role of alpha satellites remains unclear. Deltasatellites, which do not encode any proteins, can affect viral DNA accumulation and symptomatology [18].

Begomoviruses exhibit a clear subdivision into four phylogenetic groups: Old World, New World, legumoviruses, and sweepoviruses [19]. Trenado et al., 2011). The Old World begomoviruses can

be mono- or bipartite and contain an AV2/V2 ORF, while the New World viruses are mostly bipartite and lack this ORF. The specific interactions between ToLCNDV and these satellites, particularly betasatellites, have allowed the virus to adapt to various hosts, expanding its host range and contributing to its persistence in different crops.

### 1.3 Host Range and Disease Symptoms in Cucurbits

ToLCNDV affects a wide range of cucurbit hosts, including melon, cucumber, watermelon, zucchini, and various gourds [20] all of which share several common traits that may contribute to their susceptibility. Cucurbits typically have a similar vascular system structure, which could facilitate the systemic movement of ToLCNDV once transmitted by whiteflies. Additionally, these plants often grow in similar agro-ecological environments, providing overlapping conditions that may enhance viral transmission. Many cucurbits are also cultivated intensively and on a large scale, increasing opportunities for whitefly infestation and virus spread. The virus may also exploit common defense pathways in cucurbits, such as RNA silencing, which ToLCNDV is known to suppress, making these crops particularly vulnerable to infection. Symptoms in these crops include severe yellowing, mosaic discoloration, leaf curling, vein swelling, and short internodes. Infected fruits often exhibit skin roughness and cracking, which reduces their market value [21]. Lopez et al., 2015). The virus's ability to infect such a broad range of hosts highlights its adaptability and the challenges it poses to crop management.

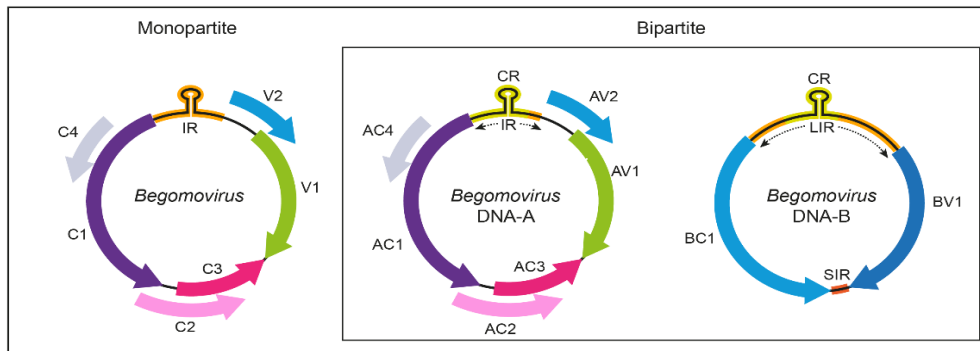


Fig. 1. A

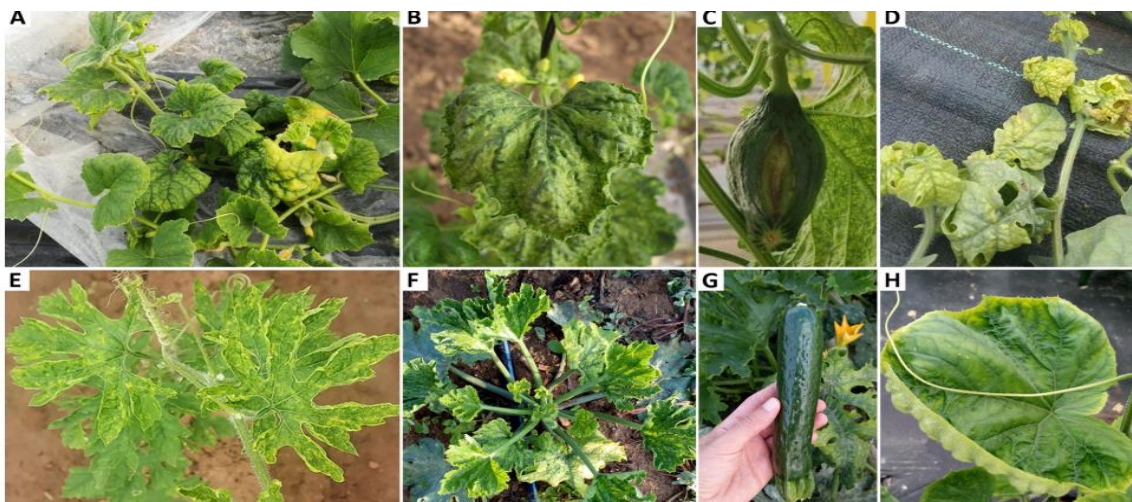
Fig 1. B

Fig. 1. C

Fig. 1. A, B and C shows an infestation of whiteflies (*Bemisia tabaci*) on the underside of the plant leaves. These pictures taken at College of Horticulture Bangalore, Karnataka, India by authors on Zucchini plants



**Fig. 2.** The genomic organization of begomoviruses, such as Tomato Leaf Curl New Delhi Virus (ToLCNDV), consists of two DNA components, DNA-A and DNA-B, each encoding several essential proteins. DNA-A contains ORFs for proteins like the coat protein (CP), replication-associated protein (Rep), and transcriptional activator protein (TrAP), while DNA-B encodes movement protein (MP) and nuclear shuttle protein (NSP). The common region (CR), shared by both components, contains a conserved stem-loop structure critical for replication initiation. These proteins facilitate virus replication, movement, and interaction with the host plant Padidam et al., [1]



**Fig. 3.** Symptoms of Tomato leaf curl New Delhi virus in Cucurbits: Yellowing and curling of melon leaves (A, B) and fruit cracking (C); watermelon yellowing and downward curling (D); bitter melon mosaic mottling and leaf curling (E); zucchini short internodes, vein swelling, mosaic symptoms (F), and reduced fruit quality (G); cucumber yellowing and upward leaf curling (H). Images A, F, G, H from EPPO (European and Mediterranean Plant Protection Organization); B, C from Siskos et al. [22] D from Venkatara vanappa et al. (2020); E from Kiran et al. [23]

#### 1.4 Extending Host Range in Other Crops

ToLCNDV was first observed in tomatoes, but it has since been reported in various other plant families, including Cucurbitaceae (e.g., cucumber, melon, and watermelon), Caricaceae (e.g., papaya), and Malvaceae (e.g., hibiscus) [20,13]. This adaptability underscores the importance of ongoing research aimed at

understanding the molecular mechanisms behind ToLCNDV's transmission, host range expansion, and pathogenicity. The study seeks to investigate specific genetic features of the virus, such as unique open reading frames (ORFs) and their roles in viral replication and interaction with host plants. By elucidating these mechanisms, the research aims to develop targeted control strategies, including resistant plant varieties and

effective management practices, to mitigate the virus's impact on agricultural systems.

## 2. METHODS

The study utilized BLAST (Basic Local Alignment Search Tool) to compare nine ToLCNDV sequences against a reference sequence, specifically the "Tomato leaf curl New Delhi virus DNA A" (Accession: NC\_004611.1) [1]. The BLAST search aimed to identify regions of similarity, conserved sequences, and potential mutations among the isolates. The sequences were selected based on their geographical diversity and previous reports of ToLCNDV infections [24]. Detailed alignment parameters, such as the e-value threshold and scoring matrix, were specified to optimize the search results.

### 2.1 Progressive MAUVE Genome

The first report of ToLCNDV infecting tomatoes in Spain marked the virus's introduction to Europe [25]. Genetic sequencing of the virus has provided insights into its structure and evolutionary relationships. Sequencing revealed typical bipartite Old World begomovirus characteristics, with some unique features, such as a 36-nucleotide duplication in the DNA-B component, whose significance is still unknown [26,27]. Understanding these genetic variations is essential for tracking the virus's spread and developing effective management strategies.

- 1. Conserved Regions:** Continuous lines in the alignment indicate conserved sequences across different isolates, suggesting essential functional elements that are critical for the virus's replication and pathogenicity.
- 2. Mutations:** Symbols such as doors or specific markers on the left of the alignment represent areas where mutations occur, providing insights into genetic diversity and potential adaptations of the virus to different hosts or environments.
- 3. Evolutionary Relationships:** By analyzing the alignment, researchers can infer evolutionary relationships among the isolates, identifying patterns of transmission and evolution that may inform control strategies.

### 2.2 Summary of Key BLAST Matches for ToLCNDV Sequences

The query sequence refers to the specific DNA sequence being analyzed or compared in the

context of the study, which in this case aligns perfectly with the "Tomato leaf curl New Delhi virus DNA A, complete sequence" (Accession: NC\_004611.1). This sequence is likely derived from a sample collected from infected plants, confirming that it belongs to the ToLCNDV strain. The sequences referred to include:

**1."Tomato leaf curl New Delhi virus DNA A, complete sequence" (Accession: NC\_004611.1):** This is the reference sequence of ToLCNDV, originally characterized and deposited in genomic databases. It serves as a standard for comparison against other isolates [1].

**2."Tomato leaf curl New Delhi virus isolate Zhejiang segment DNA-A" (Accession: OP356207.1):** This sequence comes from an isolate collected from the Zhejiang province in China. It represents a regional variant of ToLCNDV that may exhibit genetic differences from the reference strain [24].

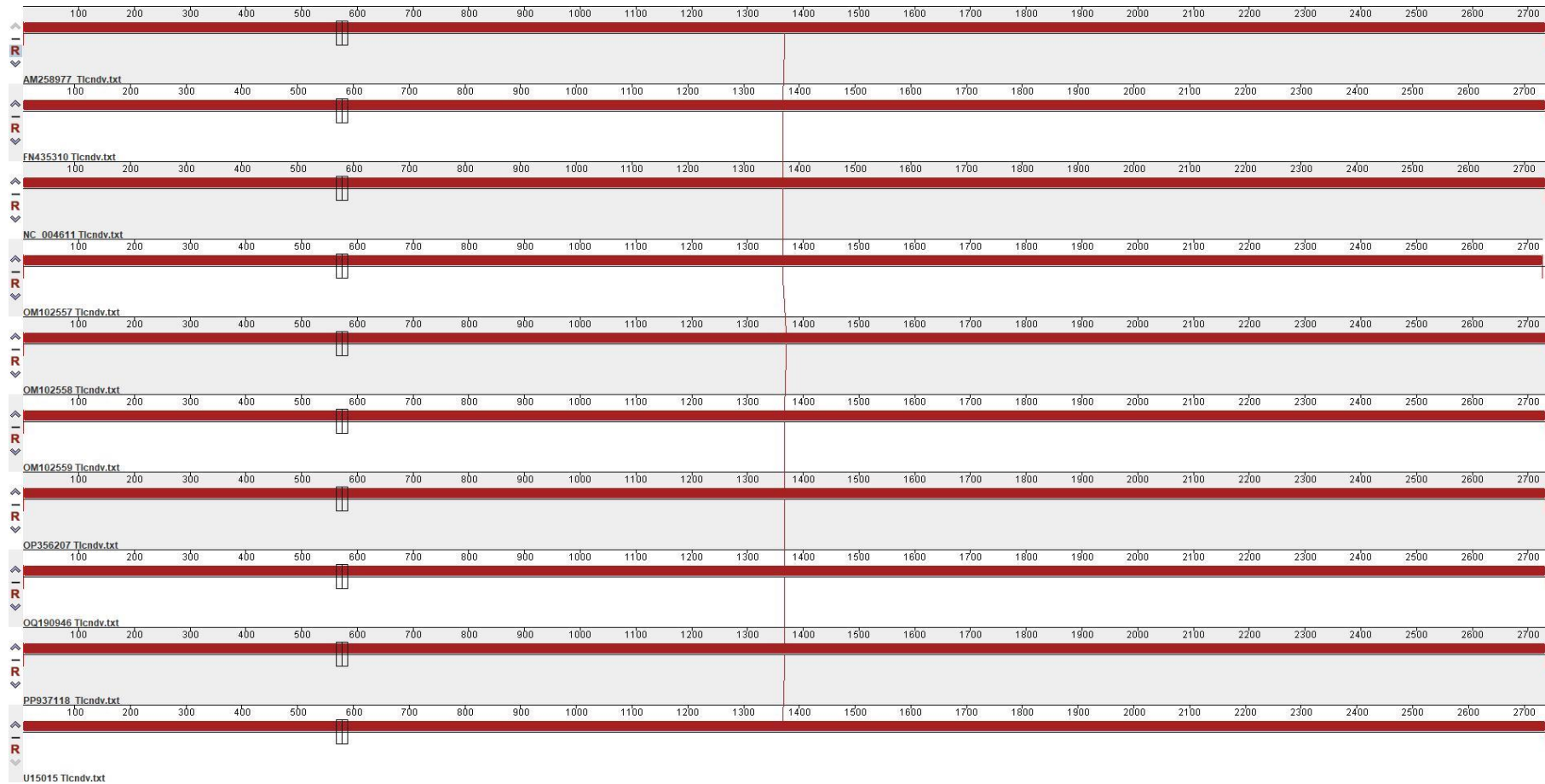
These sequences are obtained from genomic databases like GenBank, where researchers submit viral sequences collected from various locations and host plants. This geographic and genetic diversity is crucial for studying the evolutionary dynamics of the virus and its potential impact on agricultural practices. Understanding where these sequences come from helps researchers track the spread of ToLCNDV and assess risks associated with its various isolates.

The relevance of this analysis lies in the ability to identify genetic variations between different isolates of ToLCNDV. Understanding these differences can provide insights into the virus's evolution, host adaptation, and potential impacts on crop management strategies. Identifying strains with minor nucleotide differences helps researchers assess the risk of emerging variants and develop targeted control measures for affected crops.

Bootstrap analysis was performed with 1,000 iterations to assess the confidence of tree branches, with values above 70% indicating strong support for relationships among isolates. The resulting tree illustrates distinct clusters based on geographic origins or host plants, highlighting ToLCNDV's genetic diversity and adaptation. This analysis provides insights into the virus's evolutionary dynamics, crucial for understanding its spread and informing management strategies.



### Comparative Alignment of Tomato Leaf Curl New Delhi Virus (ToLCNDV) Genome Sequences



**Fig. 4.** This figure presents a comparative alignment of ToLCNDV genome sequences from various isolates, each labeled with its respective accession number (e.g., NC\_004611.1, OM102559.1). It highlights regions of interest, including conserved regions and potential mutations. The alignment is significant for several reasons

**Table 1. BLAST Analysis Results for ToLCNDV Sequences**

Description	Scientific Name	Query Cover	Per. Ident	Accession
Tomato leaf curl New Delhi virus DNA A, complete sequence	Tomato leaf curl New Delhi virus	100%	100.00%	NC_004611.1
Tomato leaf curl New Delhi Virus-Severe segment A, complete sequence	Tomato leaf curl New Delhi virus	100%	100.00%	U15015.2
Tomato leaf curl New Delhi Virus (Pakistan: Solanum:2009) complete segment DNA-A, clone MS4	Tomato leaf curl New Delhi virus	100%	99.85%	FN435310.1
Tomato leaf curl New Delhi virus isolate 68_ToLCNDV_Solanum_nigrum_Lahore_2011 segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	99.16%	OM102559.1
Tomato leaf curl New Delhi virus isolate 350_ToLCNDV_Chilli_Vehari_2012 segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	99.12%	OM102558.1
Tomato leaf curl New Delhi virus isolate 74_ToLCNDV_Cucumber_Lahore_2011 segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	99.12%	OM102557.1
Tomato leaf curl New Delhi virus, complete genome, clone ToA2	Tomato leaf curl New Delhi virus	99%	98.54%	AM258977.1
Tomato leaf curl New Delhi virus isolate HF23CB segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	97.37%	PP937118
Tomato leaf curl New Delhi virus isolate 8066 segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	97.29%	OQ190946.1
Tomato leaf curl New Delhi virus isolate Zhejiang segment DNA-A, complete sequence	Tomato leaf curl New Delhi virus	100%	97.08%	OP356207.1

### 2.3 Identification of Host Resistance

Host resistance offers a long-term solution to Tomato leaf curl New Delhi virus (ToLCNDV). Several resistant accessions have been identified in cucurbits:

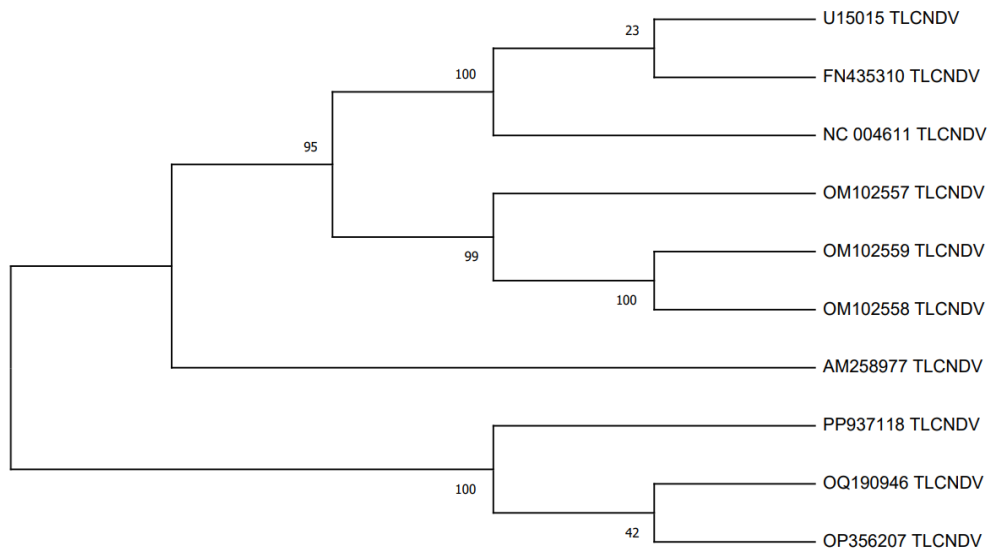
- **Sponge Gourd:** Resistant lines such as DSG-6, DSG-7, IIHR-137, IIHR-138, and IIHR-Sel-1 have been identified. Notably, the resistance in DSG-6 and DSG-7 is controlled by a single dominant gene [28].
- **Squash:** Resistant sources include Large Cheese (USA), an Indian landrace (PI 381814), and a Japanese accession

(BSUAL-252). Resistance in these varieties is conferred by specific genes located on Chromosome 8 [29].

- **Melon:** Nine resistant accessions have been identified, including Kharbuja and PI 124112. Resistance in these accessions is linked to quantitative trait loci (QTLs) on Chromosomes 11, 12, and 2. Candidate gene validation suggests that genes such as CmARP4 and CmNAC play a significant role in resistance [30].

These findings underscore the potential for breeding programs to develop ToLCNDV-resistant cultivars, enhancing crop resilience and ensuring sustainable agricultural practices.

### Phylogenetic Tree of Tomato Leaf Curl New Delhi Virus (ToLCNDV) Isolates



**Fig. 5. The phylogenetic analysis of ToLCNDV isolates was conducted through multiple sequence alignment and tree construction methods. First, genomic sequences of various isolates were retrieved from databases like GenBank and aligned using software such as MUSCLE or Clustal Omega. A phylogenetic tree was then constructed using programs like MEGA or RAxML, employing maximum likelihood (ML) or neighbor-joining (NJ) methods, with models like the Kimura 2-parameter model for nucleotide substitution**

### 3. RESULTS

The BLAST analysis revealed that all nine sequences displayed significant similarity to the reference sequence, with varying percentages of identity. Notably, the analysis identified conserved regions critical for viral replication and potential mutations that could affect pathogenicity. These findings suggest that the isolates share a common evolutionary lineage but also highlight genetic variations that may influence their adaptability to different hosts and environments [31,32].

The relevance of this method lies in its ability to provide a comprehensive overview of the genetic diversity among ToLCNDV isolates, which is essential for understanding the virus's spread and its potential impact on agricultural practices. By identifying specific mutations and conserved sequences, the study contributes valuable insights into viral behavior, informing future research directions and management strategies to combat ToLCNDV infections [33].

### 4. CONCLUSION

This study significantly advances our understanding of Tomato Leaf Curl New Delhi

Virus (ToLCNDV) and its interactions with host plants and vectors. The identification of specific genetic features, particularly the 36-nucleotide duplication in the DNA-B component, sheds light on the virus's pathogenicity and adaptability across various hosts. Molecular analyses demonstrate that ToLCNDV effectively manipulates host gene expression to suppress defenses, thereby enhancing susceptibility.

The research also highlights the role of whiteflies, specifically *Bemisia tabaci*, in the virus's efficient transmission, linking its movement within the insect to increased transmission efficacy. The evaluation of early detection methods, with a focus on next-generation sequencing (NGS), underscores its potential for revealing the genetic diversity and spread of ToLCNDV.

Overall, the findings underline the complexity of ToLCNDV infection dynamics and the need for ongoing research to explore the molecular mechanisms involved. A deeper understanding of these interactions will be crucial for developing targeted strategies to address the challenges posed by ToLCNDV in agricultural systems.



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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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