Review Article

"Exploring Gene Expression and Molecular Pathways in Tomato Leaf Curl New Delhi Virus (ToLCNDV) Infections: Understanding Host Responses & Viral Adaptation"

Abstract

Tomato leaf curl New Delhi virus (ToLCNDV), a highly pathogenic Begomovirus transmitted by whiteflies, presents a major threat to vegetable crops, particularly in the Indian subcontinent. Originally identified in tomatoes, ToLCNDV has now expanded its host range to include various cucurbits and other plants, aided by interactions with betasatellites and circular DNA satellites. The virus's persistence is due to its movement from the whitefly's foregut to the salivary glands, which enhances transmission efficiency. Molecular studies have identified distinct genetic features of ToLCNDV, such as a 36-nucleotide duplication in the DNA-B component, which may impact virus pathogenicity and adaptability. Gene expression analyses reveal how ToLCNDV and its associated satellites affect host plant responses and vector interactions. Early detection techniques, including PCR, LAMP, and next-generation sequencing (NGS), play a crucial role in monitoring and managing ToLCNDV. Effective management strategies integrate pest control with the development of resistant plant varieties. Understanding the molecular mechanisms of gene expression related to ToLCNDV infection and vector transmission is essential for creating targeted control measures and mitigating the virus's impact on agriculture. The abstract actually lacks the presence of the hypothesis the group want to test and the results they got.

Comment [t1]: Are these the methods of the study?

Introduction

Plant viral diseases pose significant threats to economically important crops, leading to devastating outcomes worldwide (Navas-Castillo et al., 2011). Among these, the Tomato leaf curl New Delhi virus (ToLCNDV), a highly infectious and emerging Begomovirus, has been particularly detrimental to vegetable crops in the Indian subcontinent. First identified in tomato in India (Padidam et al., 1995), ToLCNDV is primarily transmitted by the whitefly vector. The virus transmission by insect vectors can occur in nonpersistent, semipersistent, or persistent modes, depending on the acquisition and retention periods of the virions (Nault, 1997; Ng & Falk, 2006). Persistent viruses, like ToLCNDV, move from the foregut to the salivary glands of the vector, where they are released into plant tissues. Understanding these transmission mechanisms is critical in addressing the spread and impact of ToLCNDV.

Comment [t2]: Still unsure if what is the hypothesis they want to test and this phrase is out ot cointest here. To me introduction has to be expanded.

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 regions but can be found in greenhouses in temperate areas. Whiteflies cause direct damage by feeding on plant phloem, reducing plant vigour, and by excreting honeydew that leads to sooty mold, which interferes with photosynthesis (Fiallo-Olive et al., 2019). However, their most significant impact is their ability to transmit plant viruses. Of the approximately 1,500 species of whiteflies (Martin, 2004), only a few, including some species of the *Bemisia tabaci* complex, are known to vector plant viruses. Where do the spiecies of Bemisia tabaci are specifically? Why is this relevant? Please explain and expand

Begomoviruses are a group of plant viruses that have emerged as serious threats to vegetable, root, and fiber crop production in various regions (Navas-Castillo et al., 2011). These viruses have monopartite or bipartite genomes and are transmitted by whiteflies. The

Bemisia tabaci whitefly is a complex of cryptic species (De Barro et al., 2011), and its specificity as a vector is linked to specific amino acid sequences in the viral coat protein (Briddon et al., 1990). Begomoviruses infect dicot plants, with bipartite begomoviruses encoding seven to eight proteins and monopartite viruses encoding five or six proteins.

Fig 1. A **Fig 1. B** **Fig 1. C**

Figure 1. A, B and C shows an infestation of whiteflies (*Bemisia tabaci*) on the underside of the plant leaves (Source- College of Horticulture Bangalore, Karnataka, India).

Comment [t3]: Have these pictures been taken by the group or it is a reference?
In case they are relevant, figure 1 A, B, C need to be explained and detailed. In case it is a reference l

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., 2016). 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 (Ferro et al., 2021).

Begomoviruses exhibit a clear subdivision into four phylogenetic groups: Old World, New World, legumoviruses, and sweepoviruses (Ilyas et al., 2009; 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.

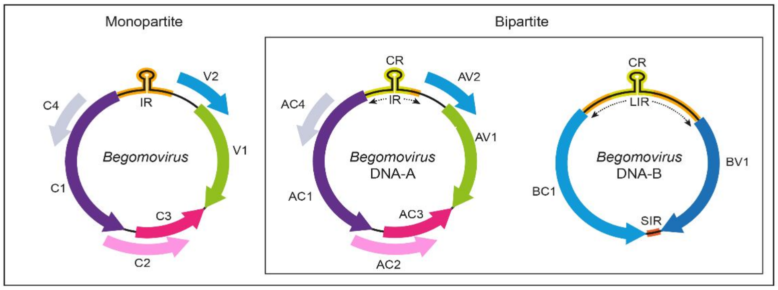

Figure 2. Genomic Organization of Begomoviruses**:** This figure shows the genomic structure of begomoviruses, highlighting ORFs on the virion-sense (V) or complementary-sense (C) strand. The AV2/V2 ORF, absent in New World begomoviruses, and the "common region" shared between bipartite components in light green within the intergenic region (IR) or long intergenic region (LIR) are depicted. The conserved stem-loop with the 5′-TAATATTAC-3′ sequence and key proteins like CP (coat protein), Rep (replication-associated protein), TrAP (transcriptional activator protein), REn (replication enhancer protein), MP (movement protein), NSP (nuclear shuttle protein), and SIR (short intergenic region) are also illustrated.

Here it is not clear to me

1) what is the reference of this picture, it has to be specified

2) I am not familiar with this virus and I cannot understand what all the C1, C2, C3, C4, V1, V2, AC1, AC2, AC3, AC4, AV1, AV2, BC1, SIR, CR, BV1 correspond to.

I guess they are ORFs in sense and antisense but there is no clue on the dimension of the orf, its function and the correspondence to the protein..

Either the explanation explains the picture or they find another picture that visually describes what they wrote.

Host range and disease symptoms in Cucurbits

ToLCNDV affects a wide range of cucurbit hosts, including melon, cucumber, watermelon, zucchini, and various gourds (Bragard et al., 2020). 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 (Juarez et al., 2019; 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.

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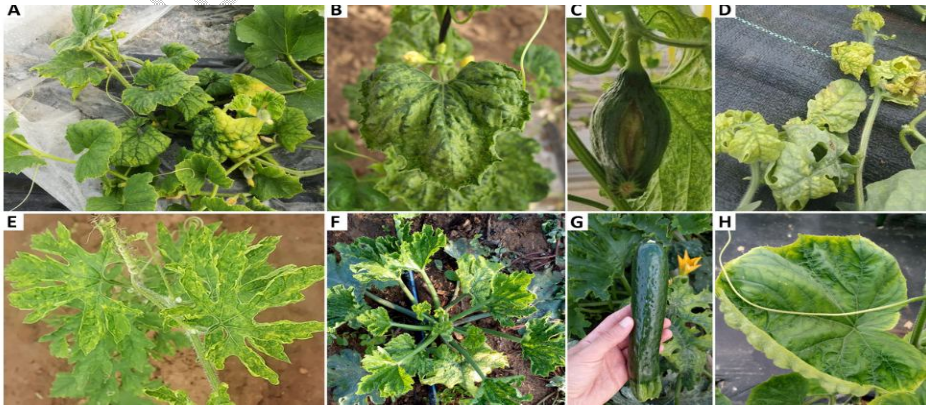

Figure 3. Symptoms of Tomato leaf curl New Delhi virus in Cucurbits: Yellowing and curling of melon leaves (A, B) and fruit cracking (C); watermelon yellowing and downward curling (D); bitter gourd mosaic mottling and leaf curling (E); zucchini short internodes, vein swelling, mosaic symptoms (F), and reduced fruit quality (G); cucumber yellowing and upward leaf curling (H). Images A, F, G, H from EPPO; B, C from Siskos et al. (2022); D from Venkatara vanappa et al. (2020); E from Kiran et al. (2021).

Comment [t5]: What is EPPO?

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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, Caricaceae, and Malvaceae. The association of ToLCNDV with betasatellites has played a key role in expanding its host range, allowing the virus to infect new hosts and break down natural resistance in crops like chili (Singh et al., 2012; Singh A K et al., 2016). This adaptability underscores the importance of ongoing research to understand and mitigate the virus's impact.

Comment [t7]: References need to be added here

Comment [t8]: Why is chili so relevant? To me already the spectrum of vegetables/fruits indicated above is pretty large already

Comment [t9]: Still I don't understand the aim of the study

Disease Scoring and Vulnerability Index Estimation

To evaluate the resistance of different plant genotypes to ToLCNDV, plants are scored based on disease symptoms using a six-point scale (Sohrab, 2005; Islam et al., 2011). The vulnerability index (VI) is then calculated to determine the degree of resistance, with categories ranging from immune to susceptible (Havey, 1996; Islam et al., 2011). This methodology is crucial for breeding programs aimed at developing resistant crop varieties.

Comment [t10]: Or vulnerability I would say.. If they truly talk about resistance, they need to explain its reason, not just describe its effect with a score

$$VI = \frac{(0n0 + 1n1 + 2n2 + 3n3 + 4n4 + 5n5) \times 100}{nt(nc - 1)}$$

Where,
$n_0, n_1, \ldots n_5$ = number of plants in each score category (0–5),
nt = total number of plants, and
nc = total number of categories
Genotypes were categorized into five groups based VI values (Havey, 1996; Islam et al., 2011)

Comment [t11]: Either they rewrite the formula into a text or it becomes a picture where this part is a didascaly

VI = 0—immune
VI = 1%–25%—resistant
VI = 26%–50%—moderately resistant
VI = 51%–75%—moderately susceptible
VI = 76%–100%—susceptible

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Comment [t12]: I would have represented it either in a graph or a chart

To me up to here we are still at the introduction

Progressive MAUVE Genome

The first report of ToLCNDV infecting tomatoes in Spain marked the virus's introduction to Europe. 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 (Wilisiani et al., 2019; Troiano & Parrella, 2023). Understanding these genetic variations is essential for tracking the virus's spread and developing effective management strategies.

Comment [t13]: Reference please

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

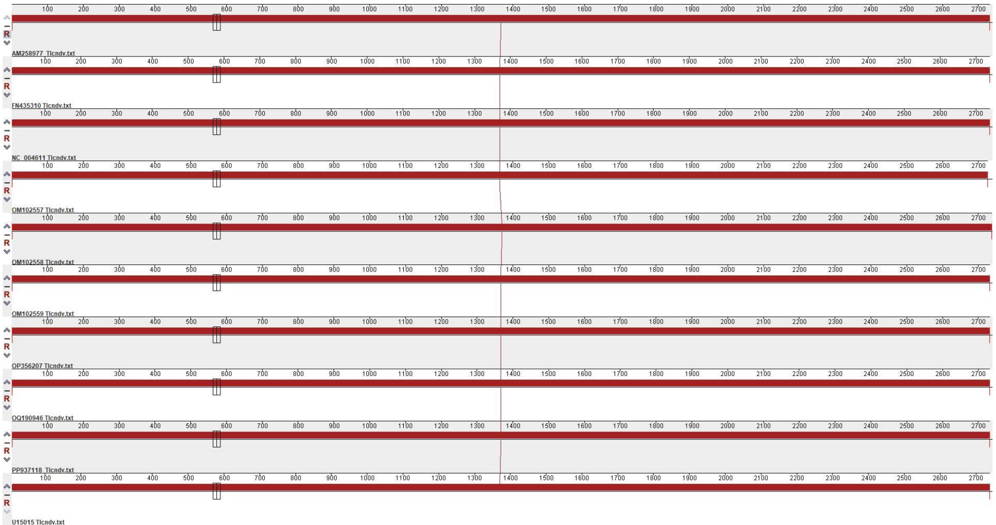

Figure 4. This figure presents a comparative alignment of ToLCNDV genome sequences from various isolates. Each sequence is labelled with its respective accession number (e.g., NC_004611.1, OM102559.1) and highlights regions of interest, including conserved regions and potential mutations. This alignment facilitates the understanding of genetic diversity and evolutionary relationships among different ToLCNDV isolates.
They have to explain the alignment significance: is the continous line they are referring to? Or the simble like a door on the left of the line?

Comment [t14]: Isolates have to be highlighte or explained, I cannot appreciate the differences neither the scale above, it is write above

Comment [t15]: Why did they choose these sequences and not others?

Comment [t16]: How? I don't understand what was facilitated

Summary of Key BLAST Matches for ToLCNDV Sequences

The query sequence aligns perfectly with the "Tomato leaf curl New Delhi virus DNA A, complete sequence" (Accession: NC_004611.1), showing 100% coverage and 100% identity, confirming it as a strain of ToLCNDV. Other sequences in the results, such as the "Tomato leaf curl New Delhi virus isolate Zhejiang segment DNA-A" (Accession: OP356207.1), display a full 100% query cover but a slightly lower identity of 97.08%, indicating minor nucleotide differences. Why is this relevant?

Comment [t17]: What is the query sequence? Where does it comes from?

Comment [t18]: Which ones are they referring to? Where do these sequences come from?

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

Comment [t19]: Are these in the same order as above?

Comment [t20]: If this is the reference sequence, it needs to be specified and also why..

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

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

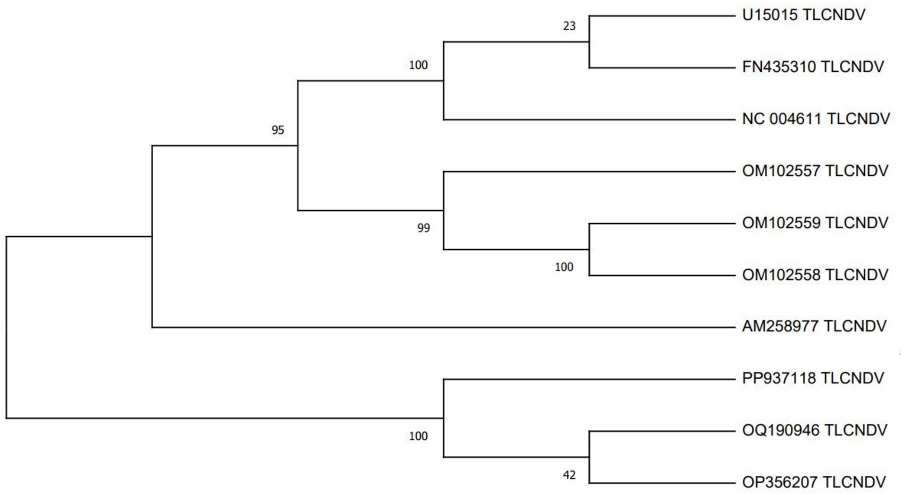

Figure 5. The phylogenetic tree shows the evolutionary relationships among ToLCNDV isolates, with bootstrap values indicating confidence levels. This analysis highlights the genetic diversity and evolutionary history of ToLCNDV, aiding in understanding its spread and adaptation across various hosts and regions. How did they perform this analysis? This lacks methods and results description

Comment [t21]: Please expand

Management Practices for Tomato Leaf Curl New Delhi Virus (ToLCNDV)

Management of ToLCNDV through the chemical control of its vector, *Bemisia tabaci*, is challenging and often ineffective. Overreliance on insecticides can lead to the emergence of resistant *B. tabaci* populations and cause significant environmental harm. A more sustainable approach includes integrating biological control methods, understanding the epidemiology of the disease, and adopting a multi-faceted strategy to minimize damage, as no single method is sufficient to contain ToLCNDV.

Comment [t22]: Please examples or references

Comment [t23]: Please reference

Comment [t24]: Please expand, what do you mean?

1. Early Detection

The cornerstone of effective ToLCNDV management is early detection. Common nucleic acid-based detection methods include Polymerase Chain Reaction (PCR) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR), which are widely used for detecting a range of viruses and virus-like pathogens. Multiplex PCR and RT-PCR assays have been developed for the simultaneous detection of multiple viruses. Loop-mediated isothermal amplification (LAMP) is a newer, simpler detection method now applied in India. Next-generation sequencing (NGS), which allows massive parallel sequencing followed by bioinformatics analysis, has revolutionized novel virus discovery and is increasingly used in India. Did you perform any experiment?

Comment [t25]: Is this used only in Inda or also abroad? Please add a reference for eachof these methods

2. Cultural Practices

Cultural control strategies are essential for preventing the spread of ToLCNDV, particularly in regions free from the disease. This includes controlling the commercial trade of plants and planting materials, reinforcing quarantine measures, and adopting cultural practices to reduce virus sources and disease pressure. Strategies include:

- **Crop Timing and Placement:** Avoid periods of high *B. tabaci* populations by adjusting crop placement in time and space.

Comment [t26]: Reference please

Comment [t27]: By whom and how?

Comment [t28]: What are these periods?

- **Virus-Free Planting Material:** Use certified virus-free seeds and seedlings.
- **Physical Barriers:** Implement barriers between crops and vectors, particularly in protected crops.
- **Discontinuous Cropping:** Break the disease cycle by rotating crops and implementing fallow periods.

Comment [t29]: Is there a way to certify virus free seeds and seedlings? Please add reference

Comment [t30]: How? Please add reference

Comment [t31]: By whom and how?

Comment [t32]: As above, more details are reqiored

3. Integrated Pest Management (IPM)

IPM is an ecosystem-based strategy that integrates biological control, habitat manipulation, cultural practices, and resistant varieties to manage pests effectively. Key approaches include:

- **Biological Control:** The predatory mite, *Amblyseius swirskii*, feeds on *B. tabaci* eggs and nymphs, significantly reducing ToLCNDV transmission in zucchini crops.

- **Cultural Controls:** Implementing fallow periods, early or late planting, and intercropping can help manage whitefly populations and associated viral diseases.

- **Mechanical and Physical Controls:** Use traps, mulches, steam sterilization, and screens to manage pests and prevent the spread of ToLCNDV.

- **Chemical Control:** While insecticides have been the primary method for controlling *B. tabaci*, their overuse can lead to resistant populations. Natural plant bio-stimulants offer an environmentally friendly alternative.

4. Identification of Host Resistance

Comment [t33]: As above, references required

Host resistance offers a long-term solution to 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, with resistance in DSG-6 and DSG-7 controlled by a single dominant gene.

- **Squash:** Resistant sources include Large Cheese (USA), an Indian landrace (PI 381814), and a Japanese accession (BSUAL-252), with resistance conferred by specific genes located on Chromosome 8.

- **Melon:** Nine resistant accessions have been identified, including Kharbuja and PI 124112, with resistance linked to QTLs on Chromosomes 11, 12, and 2. Candidate gene validation suggests that genes such as *CmARP4* and *CmNAC* play a role in resistance.

Methods

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Results

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Sorry but I cannot understand the work? They blasted 9 sequences to a reference sequence and propose a method with no reference and no clear explanation , not showing even in a small scale why the proposed method should be relevant or might have an impact or at least show some results.

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Conclusion

The Tomato Leaf Curl New Delhi Virus (ToLCNDV) represents a significant threat to vegetable crops globally, particularly in regions like the Indian subcontinent. Understanding the molecular mechanisms underlying the virus's infection process, host range expansion, and transmission dynamics is crucial for developing effective management strategies. From early detection methods to integrated pest management (IPM) practices and the identification of resistant crop varieties, a multifaceted approach is necessary to mitigate the impact of ToLCNDV on agriculture.

Ongoing research into the molecular interactions between ToLCNDV, its satellite components, and host plants will provide further insights into the virus's adaptability and virulence mechanisms. By integrating these findings with traditional and modern pest management strategies, it is possible to develop more sustainable and effective solutions to control ToLCNDV and safeguard crop production.

Comment [t34]: Please see my comment above

References

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