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Molecular Tools to Control Tomato Leaf Curl New Delhi Virus (ToLCNDV) in Cucurbits

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Abstract
Tomato Leaf Curl New Delhi Virus (ToLCNDV), a bipartite begomovirus belonging to the Geminiviridae family, poses a severe threat to cucurbit production worldwide, resulting in significant economic losses. Its ability to infect a broad range of host plants and rapidly adapt to changing environments has exacerbated the problem. The advent of molecular tools has provided promising strategies for managing ToLCNDV infections. CRISPR/Cas systems have emerged as powerful genome-editing tools, enabling precise targeting of viral genes, such as the replication-associated protein (Rep) and coat protein (CP), to disrupt viral replication. Variants like CRISPR/Cas12a and Cas13 offer additional flexibility by targeting multiple genes or RNA intermediates. RNA interference (RNAi) has also shown success, with transgenic plants expressing virus-specific double-stranded RNA (dsRNA) or artificial microRNAs (amiRNAs) displaying strong resistance. The development of topical RNAi sprays provides a non-transgenic, environmentally friendly alternative. Concurrently, marker-assisted selection (MAS) and genome-editing technologies have facilitated the development of resistant cultivars carrying resistance genes such as Ty- and Cy- loci. Additionally, biotechnological approaches, including virus-induced gene silencing (VIGS) and synthetic biology, hold promise for disrupting host-virus interactions. Integrating these molecular tools with traditional breeding and omics technologies offers a robust and sustainable strategy to combat ToLCNDV and ensure global food security.

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I. Introduction

Tomato Leaf Curl New Delhi Virus (ToLCNDV), a member of the genus *Begomovirus* and family *Geminiviridae*, has emerged as one of the most challenging plant pathogens in global agriculture. First reported in India, the virus has spread across continents, affecting crops in Asia, Europe, and Africa (Varma & Malathi, 2003; Zaidi et al., 2017). Its capacity to infect a wide range of hosts, particularly cucurbits such as cucumbers, melons, and squashes, makes it a significant agricultural threat. The virus is transmitted by the whitefly, *Bemisia tabaci*, a highly efficient vector capable of spreading ToLCNDV rapidly across fields and regions (Morales & Anderson, 2001; Rojas et al., 2005).

The economic implications of ToLCNDV outbreaks are profound, leading to substantial yield losses and threatening the livelihoods of farmers (Pratap et al., 2011). Traditional control methods, such as chemical insecticides targeting whiteflies and cultural practices like crop rotation, have shown limited success in curbing the virus's spread due to its adaptability and the inefficiency of targeting only the vector (Navas-Castillo et al., 2011; Singh et al., 2012). Moreover, the virus's ability to undergo genetic recombination and pseudorecombination has resulted in the emergence of more virulent strains, further complicating control efforts (Hernandez-Zepeda et al., 2010; Patil et al., 2011).

Recent advancements in molecular biology and biotechnology have opened new avenues for managing ToLCNDV. Techniques such as CRISPR/Cas genome editing, RNA interference (RNAi), and the development of resistant plant varieties have shown promise in mitigating the impact of this pathogen (Ali et al., 2016; Zaidi et al., 2019). Additionally, the integration of omics technologies, including genomics, transcriptomics, and proteomics, has provided deeper insights into the host-virus interaction, paving the way for more targeted and effective management strategies (Sharma et al., 2020; Singh et al., 2015). This review delves into the molecular tools available for combating ToLCNDV, highlighting their mechanisms, potential, and challenges in implementation.

II. Molecular Biology Of ToLCNDV

ToLCNDV's genome is bipartite, consisting of two circular single-stranded DNA molecules: DNA-A and DNA-B. DNA-A encodes proteins essential for viral replication, encapsidation, and suppression of host defense mechanisms. It includes the replication-associated protein (Rep), the coat protein (CP), and other regulatory elements crucial for initiating and sustaining infection (Padidam et al., 1996; Briddon et al., 2003).

DNA-B encodes movement proteins that facilitate the virus's spread within the host plant, such as the nuclear shuttle protein (NSP) and movement protein (MP) (Rojas et al., 2005; Morilla et al., 2005).

One of ToLCNDV's key virulence factors is its ability to suppress RNA silencing, a primary antiviral defense mechanism in plants. The virus achieves this through the expression of silencing suppressor proteins, such as AC2 (TrAP) and AC4, which inhibit the host's ability to degrade viral RNA (Vanitharani et al., 2004; Sharma et al., 2019). Furthermore, ToLCNDV's interaction with alphasatellites and betasatellites enhances its adaptability and pathogenicity, making it a formidable pathogen in agricultural systems (Briddon & Stanley, 2006; Jyothsna et al., 2013).

The virus's genetic diversity is driven by recombination events, enabling it to evade host resistance and adapt to new environments (Sanjaya et al., 2008; Briddon et al., 2010). Recombination among different begomovirus species has led to the emergence of highly virulent strains of ToLCNDV, particularly in regions with mixed infections (Fiallo-Olivé et al., 2016). This genetic plasticity underscores the need for dynamic and robust management strategies that can keep pace with the virus's evolution (Brown et al., 2015; Zaidi et al., 2017).

III. Molecular Approaches For ToLCNDV Management

CRISPR/Cas Systems

CRISPR/Cas technology has revolutionized plant virology by enabling precise genome editing to disrupt viral replication and infection. In the context of ToLCNDV, CRISPR/Cas9 systems have been employed to target the Rep gene, a critical component for viral replication. Studies have demonstrated that plants expressing CRISPR/Cas9 constructs targeting Rep exhibit reduced viral loads and milder symptoms (Ali et al., 2016; Sahu et al., 2020).

CRISPR/Cas9-mediated genome editing involves the design of guide RNAs (gRNAs) that bind to the viral DNA at specific sequences, leading to double-stranded breaks (DSBs) that inhibit viral replication. Ali et al. (2016) successfully demonstrated CRISPR/Cas9 targeting of ToLCNDV Rep and CP genes in *Nicotiana benthamiana*, resulting in significant resistance.

Beyond CRISPR/Cas9, other variants like CRISPR/Cas12a and CRISPR/Cas13 have shown potential in antiviral defense. Cas12a, for instance, can process multiple guide RNAs, enabling simultaneous targeting of different viral genes (Abudayyeh et al., 2017; Aman et al., 2018). Cas13, on the other hand, targets RNA, making it suitable for viruses like ToLCNDV that rely on RNA intermediates during replication (Aman et al., 2018). These advancements, coupled with innovations in delivery mechanisms such as nanoparticle-based systems and plant virus vectors, have expanded the applicability of CRISPR technologies in managing ToLCNDV (Zaidi et al., 2019; Kumar et al., 2021).

RNA Interference (RNAi)

RNAi is a gene silencing mechanism that plants naturally employ to defend against viral infections. By engineering plants to express double-stranded RNA (dsRNA) corresponding to viral genes, researchers have achieved significant reductions in ToLCNDV infection. Constructs expressing short interfering RNAs (siRNAs) or artificial microRNAs (amiRNAs) have been particularly effective in silencing viral transcripts, thereby curbing replication and spread (López et al., 2021; Ramesh et al., 2017).

Advancements in RNAi technology include the development of topical RNAi sprays, which eliminate the need for genetic modification. These sprays deliver dsRNA molecules directly onto plant surfaces, triggering RNAi responses without altering the plant's genome (Dalakouras et al., 2020). This approach offers an environmentally friendly alternative, aligning with regulatory and consumer preferences (Ghosh et al., 2018).

Field trials have demonstrated the efficacy of RNAi in suppressing ToLCNDV infection in cucurbits and tomatoes. For instance, transgenic lines expressing RNAi constructs targeting Rep and CP genes exhibited significant resistance under controlled conditions (Kumar et al., 2016; Singh et al., 2015).

Development of Resistant Varieties

Breeding for resistance has been a cornerstone of ToLCNDV management. Resistance genes such as Ty-1, Ty-2, and Ty-3 in tomatoes and Cy-1 in cucurbits have been identified and utilized to develop resistant cultivars (Verlaan et al., 2013; Yamamoto et al., 2022). Marker-assisted selection (MAS) has accelerated the breeding process by enabling the identification of resistance-linked markers (Hanson et al., 2016).

The advent of genome editing tools like CRISPR/Cas has further enhanced the development of resistant varieties. By editing susceptibility genes (S-genes) or introducing resistance alleles, researchers can create plants that are inherently resistant to ToLCNDV (Ji et al., 2020; Kumar et al., 2021). Transcriptomic studies have also revealed key pathways involved in host defense, providing new targets for breeding and genetic engineering (Singh et al., 2015).

Biotechnological Interventions

Biotechnological approaches complement traditional breeding efforts by providing precise tools to directly interfere with viral life cycles. One such approach involves transgenic plants engineered to express antisense RNA or viral suppressor proteins that inhibit viral replication and movement (Chakraborty & Praveen, 2017; Pratap et al., 2011). For example, plants expressing antisense RNA targeting viral coat protein genes have shown considerable resistance to ToLCNDV under laboratory and field conditions (Sharma et al., 2020).

Virus-induced gene silencing (VIGS) is another critical tool for identifying host factors essential for ToLCNDV infection. VIGS allows for functional genomics studies, leading to the identification of genes that could be silenced to provide resistance. Recent studies using *Nicotiana benthamiana* have demonstrated that silencing host susceptibility genes reduces viral replication and symptom expression (Senthil-Kumar & Mysore, 2011).

Additionally, synthetic biology innovations such as engineered peptides and artificial transcription factors hold promise for disrupting virus-host interactions. For example, synthetic proteins designed to bind viral DNA sequences can inhibit replication or gene expression, thereby reducing viral load (Zaidi et al., 2019; Kumar et al., 2021).

Advances in plant transformation technologies, such as Agrobacterium-mediated transformation and particle bombardment, have facilitated the development of transgenic crops expressing antiviral constructs. These technologies provide scalable solutions to introduce resistance traits into susceptible plant species, further contributing to long-term ToLCNDV management.

IV. Role Of Natural Resistance And Breeding

The exploration of natural resistance sources remains fundamental to combating ToLCNDV. Wild relatives of cucurbits and tomatoes have been extensively screened to identify genetic loci conferring resistance. For instance, resistance genes such as **Ty-1, Ty-2**, and **Ty-3** in tomatoes, which encode proteins that restrict viral replication and movement, have been effectively incorporated into commercial varieties through conventional and molecular breeding (Verlaan et al., 2013; Lapidot et al., 2015).

Similarly, the **Cy-1** locus in cucumbers has shown promising resistance against ToLCNDV infection (Yamamoto et al., 2022). Techniques like **quantitative trait locus (QTL) mapping** and **genome-wide association studies (GWAS)** have enabled the identification of key loci linked to resistance. These methods provide breeders with precise markers to develop resistant cultivars through marker-assisted selection (MAS) (Hanson et al., 2016).

The integration of molecular tools such as CRISPR/Cas with conventional breeding strategies has accelerated the development of resistant varieties. Genome editing technologies allow researchers to modify susceptibility genes (S-genes), rendering plants less prone to infection (Zaidi et al., 2019; Ji et al., 2020). For example, editing of the host transcription factors that interact with viral proteins has resulted in enhanced resistance to ToLCNDV in experimental models (Ali et al., 2016).

High-throughput phenotyping platforms and bioinformatics tools have further revolutionized breeding programs by enabling real-time monitoring of resistance traits and understanding host-pathogen interactions (Sinha et al., 2019). Integrating these techniques with traditional breeding practices offers a sustainable and long-term solution to ToLCNDV management.

V. Conclusion

The emergence and rapid spread of ToLCNDV have underscored the critical need for innovative and multidisciplinary approaches to manage this devastating pathogen. Molecular tools such as CRISPR/Cas systems, RNA interference, and biotechnological interventions have demonstrated significant potential in reducing viral loads and curbing disease progression. Advances in genome editing technologies and synthetic biology offer precise and scalable solutions for enhancing host resistance to ToLCNDV.

The development of resistant varieties through marker-assisted selection, genome-wide association studies, and genome editing tools provides a sustainable foundation for integrated disease management. Leveraging natural resistance genes from wild relatives of cucurbits and tomatoes has been pivotal in developing resilient cultivars. Furthermore, omics technologies, including genomics, transcriptomics, and proteomics, have provided deeper insights into host-virus interactions, facilitating the identification of novel resistance targets.

Future efforts should focus on combining molecular tools with eco-friendly approaches such as topical RNAi sprays, optimized agricultural practices, and biological control of whitefly vectors. Collaborative research involving breeders, biotechnologists, and policymakers will be essential to implement these technologies at the field level. By embracing a holistic and multidisciplinary approach, we can mitigate the economic impact of ToLCNDV and ensure global food security for cucurbit crops.

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