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MOLECULAR CHARACTERIZATION OF CHILLI LEAF CURL VIRUS AND ITS ASSOCIATED SATELLITES INFECTING MEDICINAL CHILLI

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ABSTRACT

Chilli leaf curl disease (ChiLCD) is a major constraint in medicinal chilli production in India. The present study aimed to identify and molecularly characterize the causal virus and its associated satellite molecules infecting medicinal chilli in Karnataka. Severe leaf curl symptoms with high disease incidence (up to 92.50%) were recorded in Kolar district. PCR using universal Deng primers confirmed the presence of begomovirus infection, while ChiLCV-specific coat protein primers amplified an approximately 1000 bp fragment from all symptomatic samples. Sequencing and phylogenetic analysis revealed up to 99% nucleotide identity with previously reported Indian isolates of Chilli leaf curl virus (ChiLCV), confirming its genetic affiliation within the ChiLCV clade. Screening for sub-genomic components detected a ~1.4 kb β-satellite showing strong sequence similarity to Tomato leaf curl Bangladesh betasatellite (ToLCBDB), whereas α-satellites were not detected. Whitefly-mediated transmission assays demonstrated 100 percent transmission efficiency, with typical leaf curl symptoms developing within 6–15 days after inoculation. The findings confirm that ChiLCV in association with ToLCBDB is responsible for severe ChiLCD in medicinal chilli in Karnataka. This study provides essential molecular insights into the virus–satellite complex and supports the development of accurate diagnostic tools and effective disease management strategies.

***Key words*:** Chilli leaf curl disease, Begomovirus**,** Satellite molecules

Introduction

Chilli (*Capsicum annuum* L.) is an economically and nutritionally important spice crop cultivated widely across tropical and subtropical regions (Dhaliwal *et al*., 2014; Thakur *et al*., 2018). India is one of the leading producers and exporters of chilli globally (FAO, 2021; Anonymous, 2022). Despite its economic importance, chilli production is severely constrained by viral diseases (Kenyon *et al*., 2014; Jagdale and Ghosh, 2019).

Among the reported viral pathogens, Chilli leaf curl virus (ChiLCV) has emerged as one of the most economically devastating begomoviruses infecting chilli (Kumar *et al*., 2011; Thomas *et al*., 2021). ChiLCD can cause yield losses up to 100% under severe infection (Arunkumar, 2007; Shingote *et al*., 2022). Infected plants exhibit upward leaf curling, puckering, vein thickening, internodal shortening and drastic fruit reduction (Senanayake *et al*., 2012).

ChiLCV belongs to the genus *Begomovirus* in the family *Geminiviridae*, characterized by circular single-stranded DNA genomes encapsidated in twinned icosahedral particles (Thomas *et al*., 2021). Begomo viruses are transmitted exclusively by the whitefly *Bemisia tabaci* in a persistent manner (Bedford *et al*., 1994; Muniyappa *et al*., 2000). Differences in transmission efficiency among whitefly cryptic species have been widely reported (Chowda Reddy *et al*., 2005; Mishra *et al*., 2020).

The ChiLCD complex is further complicated by its association with β- and α-satellites (~1.3 kb), which influence symptom severity and viral accumulation (Briddon *et al*., 2003; Nawaz-ul-Rehman and Fauquet,

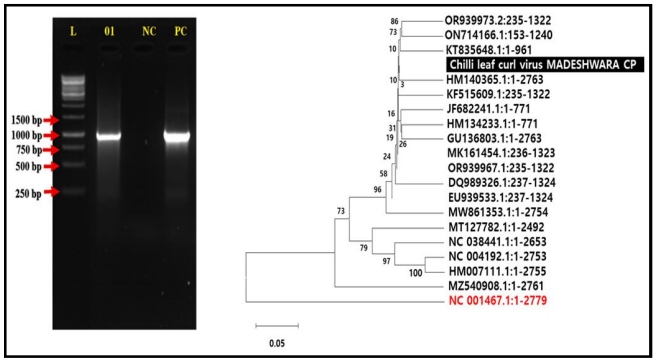

Fig. 1: PCR detection and phylogenetic analysis of ChiLCV-CP.

2009). The β-satellite encodes the βC1 protein, a key pathogenicity determinant responsible for symptom expression (Briddon *et al*., 2003), while α-satellites are known to attenuate disease severity (Briddon and Stanley, 2006).

Several ChiLCV variants such as Chilli leaf curl India virus, Chilli leaf curl Vellanad virus and Chilli leaf curl Kanpur virus have been reported across different agroecological regions of India (Hussain *et al*., 2004; George *et al*., 2014). Recent studies highlight extensive genetic variability and recombination events driving the evolution of ChiLCD complexes in the Indian subcontinent (Mishra *et al*., 2020; Pandey *et al*., 2022).

Considering the increasing incidence of ChiLCD in medicinal chilli cultivars, molecular characterization of the virus–satellite complex is essential for accurate diagnostics, epidemiological understanding and effective disease management strategies.

Materials and Methods

A severe leaf curl disease was observed on medicinal chilli plants during March 2023 in Kolar district, Karnataka, India. Field surveys were conducted following standard disease incidence assessment methods (Chaubey and Mishra, 2017; Raju, 2010).

Leaves exhibiting geminivirus-like symptoms were collected, and total genomic DNA was extracted using

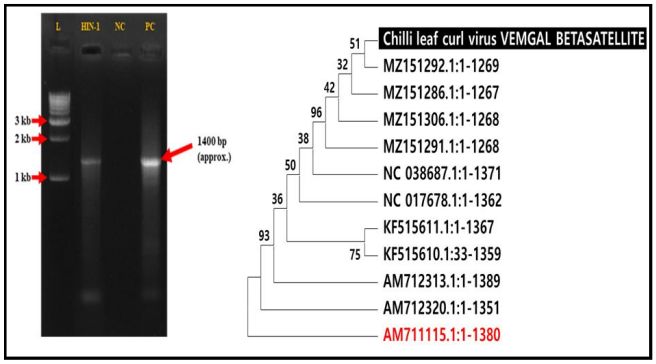

Fig. 2: PCR detection and phylogenetic analysis of ChiLCV-associated betasatellite.

the CTAB method (Lodhi *et al*., 1994; Murray and Thompson, 1980).

Initial detection of begomovirus infection was performed using universal Deng primers targeting the conserved coat protein region (Deng *et al*., 1994; Venkataravanappa *et al*., 2012). Further confirmation was achieved using ChiLCV coat protein-specific primers (Senanayake *et al*., 2012; Madhu Kiran *et al*., 2021).

β-satellite detection was carried out using universal β-satellite primers (Briddon *et al*., 2002; Kumar *et al*., 2010). Amplified products were sequenced, and nucleotide identity was determined using BLASTn. Sequence alignment was performed using Clustal W, and phylogenetic analysis was conducted using the neighbor-joining method with 1,000 bootstrap replicates in MEGA X software (Hall, 1998; Kumar *et al*., 2018).

Results and Discussions

Field surveys revealed severe leaf curl symptoms in chilli plants, consistent with earlier reports describing ChiLCD symptomatology (Senanayake *et al*., 2012; Shingote *et al*., 2022). The highest disease incidence (92.50%) was recorded in Vemgal, Kolar district, indicating significant disease pressure similar to earlier

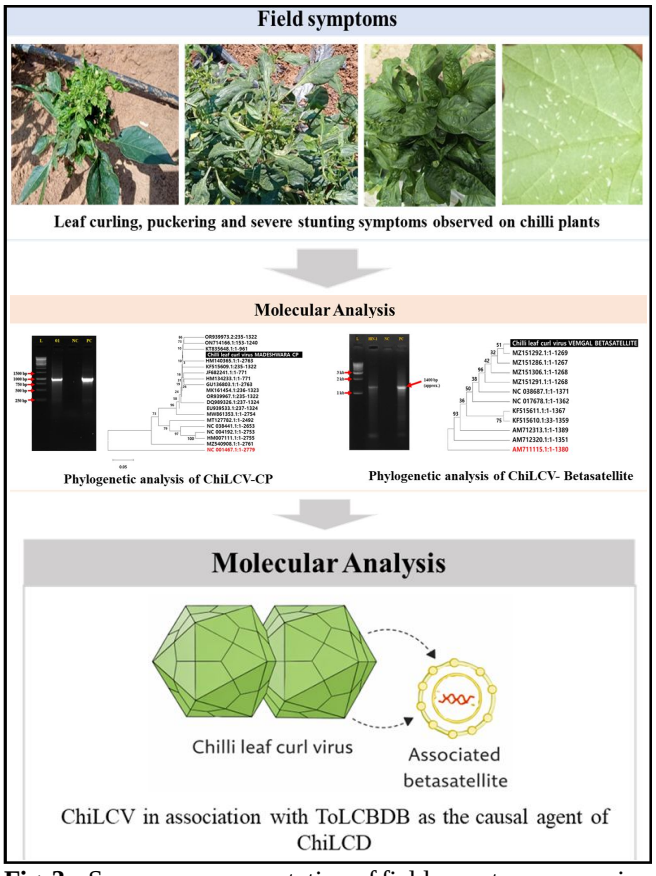

Fig. 3: Summary representation of field symptom expression and molecular characterization confirming ChiLCV–betasatellite association in medicinal chilli.

outbreaks reported in India (Kumar *et al*., 2014; Misal *et al*., 2022).

PCR amplification using Deng primers produced the expected ~550 bp fragment, confirming begomovirus infection (Deng *et al*., 1994). ChiLCV coat protein-specific primers amplified a ~1000 bp fragment exclusively in symptomatic samples, in agreement with previous studies (Senanayake *et al*., 2012; Mishra *et al*., 2020).

BLAST analysis revealed 99% nucleotide identity with Indian ChiLCV isolates, including the Raichur isolate, indicating close genetic affinity (Mishra *et al*., 2020; Pandey *et al*., 2022). Phylogenetic analysis clustered the present isolate within the Indian ChiLCV clade, supporting earlier findings of regional clustering (Khan *et al*., 2013) (Fig.1).

A ~1.4 kb β-satellite fragment was consistently amplified, while α-satellites were absent. Sequence analysis revealed 97% identity with Tomato leaf curl Bangladesh betasatellite (ToLCBDB), consistent with earlier associations of ToLCBDB with ChiLCV infections in India (Senanayake *et al*., 2012; Venkataravanappa *et al*., 2016; Bhatt *et al*., 2016). The absence of α-satellites supports earlier reports indicating that monopartite begomovirus–β-satellite complexes predominantly drive ChiLCD severity in India (Hussain *et al*., 2004; Kumar *et al*., 2015) (Fig.2).

Whitefly transmission assays demonstrated 100% transmission efficiency, with symptoms developing within 6–15 days post-inoculation. Similar high transmission efficiencies have been documented in previous studies (Muniyappa *et al*., 2000; Senanayake *et al*., 2012; Madhu Kiran *et al*., 2021), emphasizing the epidemiological significance of *Bemisia tabaci* in rapid disease spread.

Conclusion

The present study confirms that Chilli leaf curl virus (ChiLCV) in association with Tomato leaf curl Bangladesh betasatellite (ToLCBDB) is the causal agent of severe leaf curl disease in medicinal chilli in Karnataka. High nucleotide identity and phylogenetic clustering with Indian isolates indicate genetic conservation with possible regional adaptation (Mishra *et al*., 2020; Pandey *et al*., 2022). The consistent association of β-satellite and absence of α-satellites suggest that disease severity is driven primarily by the monopartite ChiLCV–β-satellite complex (Briddon *et al*., 2003; Kumar *et al*., 2015). High whitefly transmission efficiency further underscores the need for integrated vector management strategies. These findings provide critical molecular insights into the ChiLCV–satellite complex and form a foundation for improved diagnostics, resistance breeding and sustainable management of ChiLCD in medicinal chilli cultivation (Fig.3).

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