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RESEARCH ARTICLE

Molecular characterisation of ToLCNDV isolates causing yellow mosaic disease in gourds of Karnataka

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Abstract

Yellow mosaic disease (YMD) is a major constraint in cucurbit production in India. The present study was undertaken to detect and characterise begomoviruses associated with YMD in ridge gourd (*Luffa acutangula* (L.) Roxb.), bitter gourd (*Momordica charantia* L.), ivy gourd (*Coccinia grandis* (L.) Voigt) and bottle gourd (*Lagenaria siceraria* (Molina) Standl.) from Chikmagalur, Davanagere and Shivamogga districts of Karnataka in the year 2021. Polymerase chain reaction (PCR) amplification using degenerate primers DengA/DengB and PAL1v/PAR1c confirmed the presence of begomovirus, amplifying expected fragments of ~560 bp and ~1.2–1.3 kb, respectively. Sequencing and BLASTn analysis confirm the presence of Tomato leaf curl New Delhi virus (ToLCNDV). Phylogenetic analysis showed clustering of isolates based on host and region. The ridge gourd (MZ664277), bitter gourd (MZ664278) and ivy gourd (MZ664279) isolates grouped closely, while the bottle gourd (MZ664281) formed a distinct cluster. Identity analyses confirmed 96–100 % sequence similarity with known ToLCNDV strains. The study establishes ToLCNDV as the predominant etiological agent of YMD in these cucurbits in Karnataka.

Keywords: begomovirus; cucurbits; PCR diagnosis; phylogenetics; tomato leaf curl; yellow mosaic virus

Introduction

The Cucurbitaceae family, encompassing more than 900 species, includes important vegetable crops such as cucumber, ridge gourd, bitter gourd and bottle gourd (1). Ridge gourd (*Luffa acutangular* (L.) Roxb.), commonly known by several names, including Chinese okra and angled gourd, is a nutrient-rich vegetable widely cultivated in tropical climates (2). Bitter gourd (*Momordica charantia* L.), a staple in Indian diets, particularly in eastern Uttar Pradesh, is valued for both its culinary and medicinal properties (3). Ivy gourd (*Coccinia grandis* (L.) Voigt), recognised in traditional medicine, is a perennial vine found predominantly in tropical Asia and Africa (4). Bottle gourd (*Lagenaria siceraria* (Molina) Standl.), native to Africa, is extensively grown in India's hot and humid regions (5). Despite their economic importance, these gourds face significant constraints to productivity due to fungal and viral infections, including powdery mildew, downy mildew and particularly yellow mosaic disease (YMD) caused by begomoviruses (6). Tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus widely distributed across Asia, the Mediterranean and parts of Africa, is a major causal agent of YMD (7). The characteristic symptoms like mild to severe yellow mosaic, leaf curl, networking of yellow veins, followed by thickening of veins and veinlets, puckering, leaf distortion, stunting of the whole plant and reduced fruit yield in ridge gourd and bitter gourd (8). On ivy gourd, symptoms were mosaics, blistering, reduction in leaf size and stunted growth of plants (9). On the bottle gourd, symptoms include mosaic and yellowing (10).

The Begomovirus ToLCNDV is transmitted by whitefly *Bemisia tabaci* in ridge gourd, bitter gourd and pumpkin (11–13). The virus accumulates in the midgut and salivary glands of viruliferous whiteflies. The transmission of ToLCNDV is persistent in nature; once the whiteflies acquire the virus from the infected plants, they will transmit the virus to the healthy plants during feeding. The single whitefly can efficiently transmit the virus, but the speedy infection and increase in infection rate depend on the number of viruliferous whiteflies, susceptibility of the host, virus vector relationship and time (11). The virus has a complex genome composed of DNA-A and DNA-B segments that encode essential replication and movement proteins (14). Its adaptability through recombination facilitates infections in diverse plant hosts. Several ToLCNDV strains have been implicated in cucurbit viral epidemics across India (15, 16). Recent outbreaks in ridge gourd, bitter gourd, ivy gourd and bottle gourd highlight their expanding host range (17–20). Given their perennial nature and ability to harbour viruses, gourds serve as reservoirs that sustain the begomovirus inoculum. This study aims to identify the begomovirus variants affecting these four cucurbits in Karnataka and investigate their genetic diversity through molecular and phylogenetic analyses.

Materials and Methods

Field surveys were conducted during the summers of 2020 and 2021 across the Chikmagalur, Davanagere and Shivamogga districts in Karnataka to assess YMD prevalence in ridge gourd, bitter gourd, ivy gourd and bottle gourd. Symptomatic leaf samples were collected for further molecular investigation. Disease incidence was calculated as the proportion of infected plants to the total number of plants observed, expressed as a percentage.

Disease incidence (%) = (Number of diseased plants / Total plants observed) × 100 (Eqn. 1)

Genomic DNA extraction

Total genomic DNA was isolated from symptomatic and healthy leaves using a modified Cetyltrimethylammonium bromide (CTAB) method (21, 22). Fresh, infected leaf tissue (100 mg) was homogenised in pre-warmed CTAB buffer and processed through chloroform: isoamyl alcohol extraction, isopropanol precipitation, ethanol washing and resuspension in Tris–EDTA (TE) buffer. DNA quantity and quality were verified via Nanodrop and 1 % agarose gel electrophoresis.

Polymerase chain reaction (PCR) detection of Begomoviruses

Polymerase chain reaction (PCR) was performed using degenerate primers DengA/DengB targeting the begomovirus conserved region: Deng A 5'- TAA TAT TAC CKG WKG VCC SC- 3', Deng B 5'- TGG ACY TTR CAW GGB CCT TCA CA -3' (23) and PAL1v/PAR1c primers specific to the replication-associated protein and coat protein genes PAL1v 5'- GCATCTGCAGGCCCACATYGTCTTTYCCNGT-3' and PAR1c 5'-AATACTGCAGGGCTTYCTRTACATRGG-3' (24). Reactions were set up in a 50 µL volume with standard components and thermal cycling conditions optimised separately for each primer set. Amplicons were resolved by agarose gel electrophoresis.

Sequencing and phylogenetic analysis

PCR products were sequenced bidirectionally at Biokart India Pvt. Ltd., Bengaluru. Nucleotide sequences were aligned using BioEdit v7.2 to remove low-quality and chimeric sequences. The obtained sequences were identified for the closest homology using NCBI BLASTn. Phylogenetic trees were constructed using the neighbour-joining method with 1000 bootstrap replicates in MEGA 6.0. (25). Pairwise identity analysis was conducted using the SDT v1.2 software (26).

Results

Field surveys across Chikmagalur, Davanagere and Shivamogga during the summers of 2020 and 2021 revealed the widespread occurrence of YMD in ridge gourd, bitter gourd, ivy gourd and bottle gourd. Visual assessments indicated characteristic symptoms such as mosaic, mottling, blistering, leaf deformation (Both upward and downward curling), plant stunting and fruit malformation. Disease incidence ranged from 7.96 % to 58.18 % in ridge gourd and 15.25 % to 59.21 % in bitter gourd. In ivy gourd and bottle gourd, the disease incidence was slightly lower, ranging from 25.5 % to 32.5 % and 20 % to 42.5 %, respectively. Symptomatic samples from 22 locations were subjected to PCR analysis using begomovirus-specific degenerate primers DengA/DengB. All symptomatic samples produced the expected ~ 560 bp amplicon, confirming begomovirus infection. Further PCR using PAL1v/PAR1c primers amplified a ~ 1.2–1.3 kb region, targeting the replication-associated protein (Rep) and coat protein (CP) genes. These findings validated the presence of a whitefly-transmitted geminivirus (WTG), most likely ToLCNDV (Fig. 1, 2).

Sequenced PCR products from ridge gourd, bitter gourd, ivy gourd and bottle gourd (all from Davanagere) were analysed using BLASTn against the NCBI GenBank database. The results revealed the following identities based on conserved region sequences:

Ridge gourd showed 95.64 % similarity to Tomato leaf curl Joydebpur virus, Bitter gourd showed 96.79 % identity with Tomato leaf curl Bangladesh virus, while Ivy gourd and bottle gourd showed 100 % and 99.38 % identity respectively with Tomato leaf curl New Delhi virus. Rep and CP gene sequences from the same samples showed high sequence identity with ToLCNDV isolates as follows: Ridge gourd 99.72 %, Bitter gourd 98.94 %, Ivy gourd 99.50 % and Bottle gourd 100 %.

The Phylogenetic tree constructed for genes specific to rep and cp of virus infecting gourds like MZ664277 (ridge gourd), MZ664278 (bitter gourd), MZ664279 (ivy gourd) and MZ664281 (bottle gourd) isolates (amplified by PAL1v/PAR1c primer) revealed that the virus (ToLCNDV) infecting ridge gourd, bitter gourd and ivy gourd were most closely related and formed a single cluster, in contrast to bottle gourd. Whereas, bottle gourd forms a separate cluster (Fig. 3). From this, it was clear that YMD is caused by ToLCNDV and its strains in gourds, viz. ridge gourd, bitter gourd, ivy gourd and bottle gourd. The heat map generated using SDTv

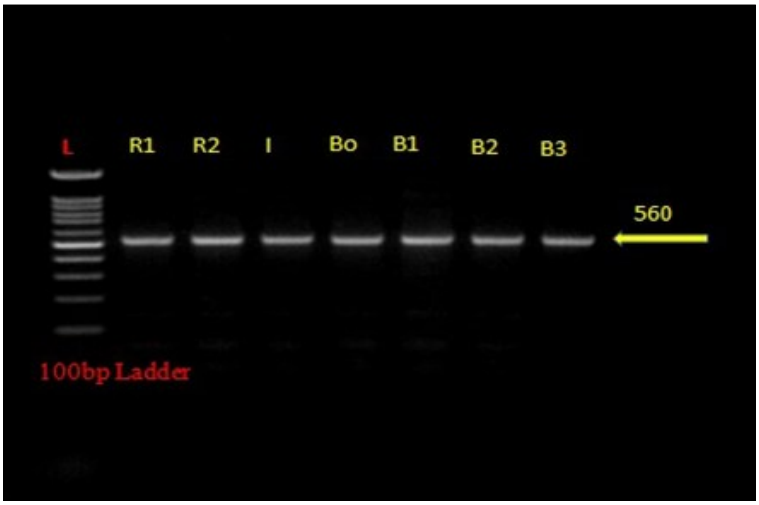

Fig. 1. Agarose gel electrophoresis of PCR products obtained from bitter gourd (B1, B2, B3), bottle gourd (Bo), ivy gourd (I) and ridge gourd (R1 and R2) YMD samples showing positive amplicon of ~ 560 bp in L= 100 bp ladder Genei.

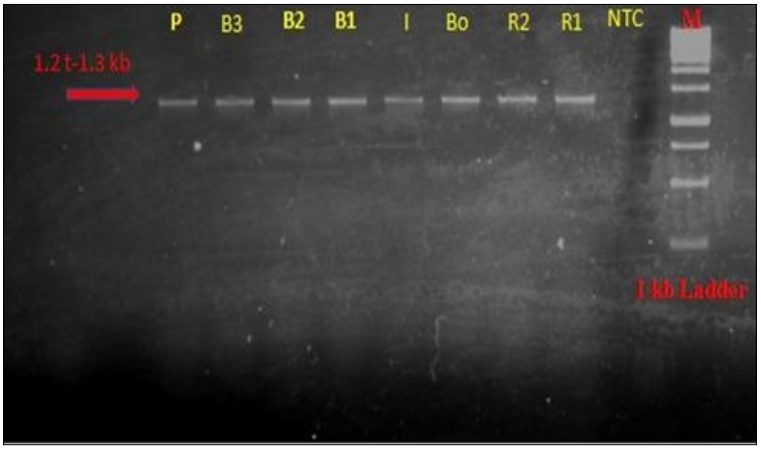

Fig. 2. Agarose gel electrophoresis of PCR products obtained from bitter gourd (B1, B2, B3), bottle gourd (Bo), ivy gourd (I) and ridge gourd (R1 and R2) YMD samples showing positive amplicon of ~1.2–1.3 kb, NTC- Non template control (serves as negative control), P- positive control Chilli leaf curl virus and M = 1kb ladder Thermofisher.

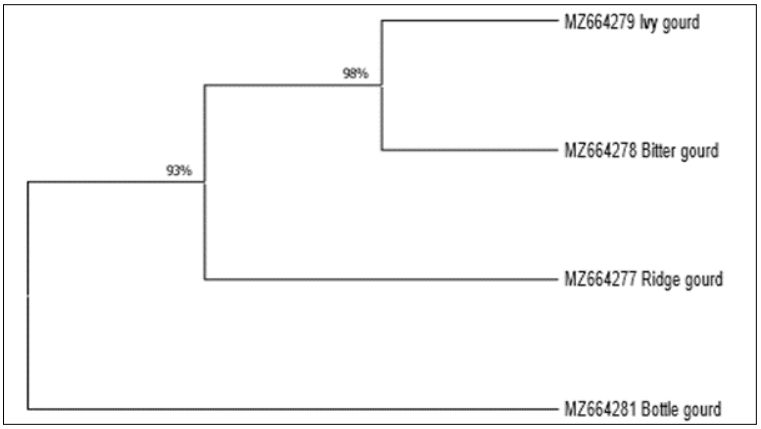

Fig. 3. Phylogenetic tree obtained from comparison of nucleotide sequences of Rep-associated protein and CP gene of ToLCNDV in ridge gourd, bitter gourd, ivy gourd and bottle gourd.

1.2 showed clearly that the virus which has been isolated from ridge gourd, bitter gourd, ivy gourd and bottle gourd showed the highest similarity range among the other 19 known ToLCNDVs recorded was 96–100 % (ridge gourd), 96–100 % (bitter gourd), 97–100 % (ivy gourd) and 99–100 % (bottle gourd), as shown in Fig. 4A–4D. As per the International Committee on Taxonomy of Viruses (ICTV) study group on Geminiviridae, a threshold value of 89 % sequence identity was used as the basis for demarcation of species. If pairwise sequence comparison analysis showed a percentage similarity between 89–93 % to all known members, then it is demarcated as a member of the new strain of the species. As the present virus has shown the similarity ranges from 96–100 % for all four gourds with known other ToLCNDV isolates, the present study reveals virus belongs to the ToLCND species as a new strain of the same species.

Discussion

Cucurbitaceous crops such as ridge gourd, bitter gourd, ivy gourd and bottle gourd play a significant role in Indian agriculture, but they face a growing threat from begomovirus infections, particularly ToLCNDV. These viruses, transmitted by the whitefly (*B. tabaci*), have become a major limiting factor in cucurbit cultivation because of their broad host range and high potential for recombination and emergence of new strains (7). The host range of the virus is very broad and known to infect many cucurbitaceous vegetables, such as bottle gourd, bitter gourd, cucumber, long melon, pumpkin, ridge gourd and watermelon in northern and north-western India (27). The losses incurred by this virus amount to 100 % in the cucumber crop. The present study confirms the association of ToLCNDV with YMD symptoms in gourds across different agro-climatic zones of Karnataka. PCR-based detection followed by sequencing revealed a high nucleotide similarity (96–100 %) with ToLCNDV reference strains, confirming the virus as a predominant pathogen across all the surveyed crops. These results highlight the urgency of continuous monitoring and genomic characterisation of begomoviruses in cucurbits. Given their perennial nature, they can act as reservoirs for ToLCNDV, thereby increasing the risk of interseasonal virus persistence and spread.

Conclusion

The current study confirms ToLCNDV as the primary pathogen responsible for YMD in major cucurbits—ridge gourd, bitter gourd, ivy gourd and bottle gourd across Karnataka. Molecular detection and sequence analysis confirmed the occurrence of ToLCNDV and related begomoviruses, with high sequence similarity (96–100 %) to known strains. Phylogenetic analysis revealed both host and region-specific clustering, indicating localised viral adaptation. Overall, the results provide crucial insights in understanding of the diversity and spread of viruses linked to YMD in cucurbits, which helps in expanding the surveillance programme to track the viral variants and understand their evolutionary dynamics. The findings enhance the understanding of begomovirus diversity and epidemiology in cucurbits and support the development of more effective targeted disease management strategies.

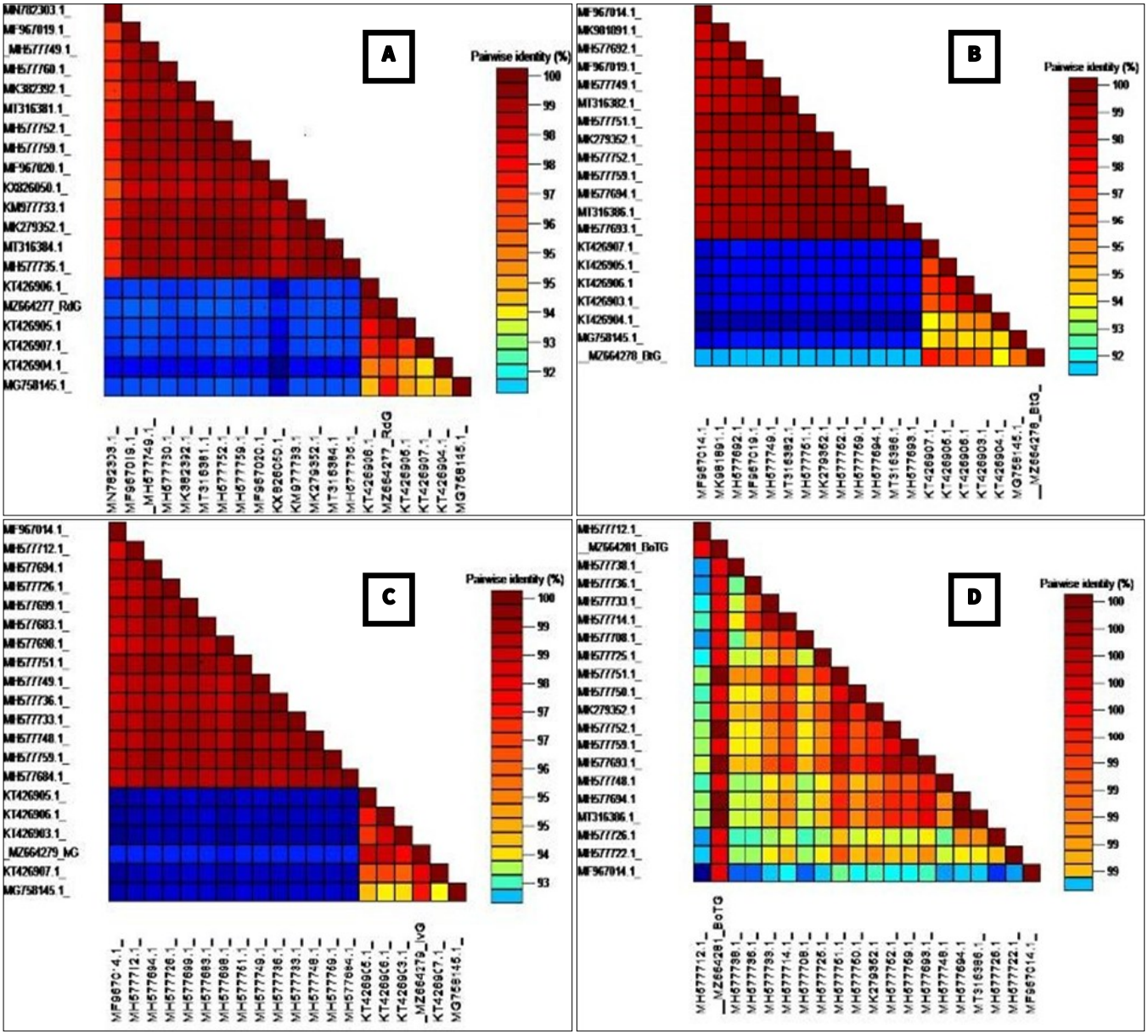

Fig. 4. Generated by SDTv 1.2 for ToLCNDV isolates: A. Ridge gourd (RdG); B. Bitter gourd (BtG); C. Ivy gourd (IvG); D. Bottle gourd (BoTG).

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Authors' contributions

SP designed the experiments. SB carried out the experiment. RGN provided technical guidance for the study. MH contributed to the manuscript preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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