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Characterization of begomoviruses associated with emerging mosaic disease of ivy gourd (*Coccinia grandis* L.) in Tamil Nadu, India

Vignesh S, Renukadevi P and Karthikeyan G*

Abstract

A systematic field survey was conducted in ivy gourd fields of Tamil Nadu during the year 2019-2021. The infected plants manifesting prominent chlorosis, mosaic, puckering, and yellow mosaic symptoms on leaves were tested through polymerase chain reaction (PCR) assay for the infection of begomoviruses. Amplicons of PCR from 5 samples were sequenced. 4 samples were infected with tomato leaf curl New Delhi virus (ToLCNDV) and one with Coccinia mosaic Tamil Nadu virus (CoMoTNV). The nucleotide sequences from isolates IGT-1 (OR041745) and IGP-1 (OR041746) have an identity of 96-98% with ToLCNDV isolate infecting chrysanthemum (MG758145) from Karnataka. Another isolate IGD-1 (OR041747) had 99.3% identity with tomato infecting ToLCNDV isolate (MW538662) from Raipur and isolate IGE-1 (OR041748) had 98.4% highest identity with the previously reported ToLCNDV isolates of India infecting bitter gourd. The IGS-1 (OR028863) isolate 98.9% identity at the nucleotide level with previously reported CoMoTNV (KY860899) infecting ivy gourd from Tamil Nadu. Phylogenetic analysis revealed that ToLCNDV isolates infecting ivy gourd were grouped under three different clusters reported previously reported from different parts of the globe. The present investigation reports the emergence of begomoviruses (ToLCNDV and CoMoTNV) on ivy gourd growing in different parts of Tamil Nadu.

Keywords: Ivy gourd, *Coccinia grandis*, PCR, ToLCNDV and CoMoV.

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Introduction

Cucurbits are one of the economically important vegetable crops cultivated for its edible fruits. These crops are susceptible to viral disease and fifty-nine viruses are recorded worldwide (Lecoq *et al.* , 2012). Ivy gourd (*Coccinia grandis* (L.) Voigt) is an indispensable perennial vegetable crop widely cultivated for culinary purposes in Southeast Asian countries belonging to the family *Cucurbitaceae*. Mature and immature fruits used as green vegetables which possess several medicinal properties to cure fever, bronchitis, leprosy and jaundice. Begomovirus infection on ivy gourd plays a significant role in reducing its fruit production. Begomoviruses are the only member of the family *Geminiviridae* include circular single-stranded DNA molecules of ~2.7 which are encapsulated in an icosahedral twinned particles of 20 × 30 nm size and transmitted by whitefly (*Bemisiatabaci*) (Bottcher *et al.* 2004; Krupovic *et al.* 2009; Zhang *et al.* 2001). There are about 24 begomovirus species are reported to be infecting cucurbits globally (Kumari *et al.* 2022). Among them, tomato leaf curl New Delhi virus (ToLCNDV) and Coccinia mosaic virus (CoMoV) were previously reported be infecting ivy gourd in India

(Venkataravanappa *et al.* 2018; Nagendran *et al.* 2016). Among these, ToLCNDV has emerged as a significant problem in several cucurbit crops (Zaidi *et al.* 2017) and other traits (Garg 2020, Rahman *et al.* 2020, Karmakar *et al.* 2021, Pandey *et al.* 2021). In this study, we have attempted to document the begomovirus associated with the samples collected from various places of Tamil Nadu from the symptomatic ivy gourd samples.

Materials and Methods

Survey and disease incidence

The symptomatic field samples were collected from ivy gourd fields of Coimbatore, Erode, Dindigul and Salem districts of Tamil Nadu. During our survey, each location's symptomatic leaf samples and asymptomatic leaf samples were collected and brought to the plant pathology laboratory under cooler box conditions to store -20°C for further molecular studies. The percent disease incidences were assessed simultaneously by visual observation of counting symptomatic plant in randomly selected with five square meter area at ten different spots of each location using the following formula,

$$\text{Percent mosaic incidence} = \frac{\text{No. of mosaic infected plants}}{\text{Total no. of plants in the plot}} \times 100$$

DNA extraction and molecular detection of begomovirus

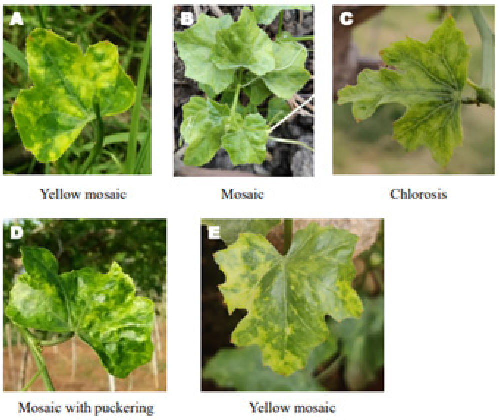
The total genomic DNA was extracted from virus like mosaic infected leaf samples of ivy gourd and as well as asymptomatic leaf sample of ivy gourd by using cetyl trimethylammonium bromide (CTAB) method as described by (Doyle and Doyle, 1990). About 100 to 200 mg of leaf tissue was finely grounded using liquid nitrogen (LN_2) in order to add one mL of pre-warmed (CTAB) extraction buffer (2% CTAB, 10mM EDTA, 2M NaCl, 100 mM Tris-HCl (pH 8) and 2% β-mercaptoethanol) to obtain total genomic DNA. The total DNA quality was analyzed through 0.8% agarose gel and the DNA was stored -80°C for further use. For detection of begomovirus, the stored DNA was subjected to PCR assay using begomovirus universal primer pair (PAL1c1960: 5'-GGNAARATHTGGATGGA -3' and PAR1v722: 5'-ACNGGNAARACNATGTGGGC -3') corresponding to AC1 and AC2 genes of begomovirus genome with an amplicon of ~1200 bp (Chatchawankanphanich and Maxwell, 2002). The PCR reaction was performed in a reaction volume of 25 µL consisting 12.5 µL of master mix (EmeraldAmp GT PCR Master Mix 2X (Takara bio, USA), 2.5 µL of 10 mMprimer (each), 2.5 µL of total genomic DNA and double distilled sterile was used to make the volume of PCR reactions. The amplification was performed in an Eppendorf-Mastercycler with PCR conditions as starting with an initial denaturation for 2 minutes at 94°C, followed by 35 cycles of denaturation for 1min at 94°C, annealing for 2 minutes at 55°C, and extension for 3 minutes at 72°C and the reaction was set upto final extension at 72°C for 10 minutes. Amplification of PCR products was separated on a 1.2% agarose gel in 1X TAE buffer at ~85V for 1 hour. The agarose gel was visualized and results were documented in Bio-Rod Plastic EZ Gel Documentation System.

Sequence Analysis

The amplified fragments were sequenced in both forward and reverse directions with M/S. Syngenome Private Limited, Bangalore, India. The nucleotide sequences were trimmed and BLAST searched. Homologous nucleotide sequence from NCBI GenBank database (http:// www. ncbi. nlm. nih. gov) reported previously from different parts of the world were retrieved and used in the analysis using the Molecular Evolutionary Genetic Analysis (MEGA) 11 (Kumar *et al.* , 2016). The phylogenetic relationship was derived for begomoviruses infecting ivy gourd with other previously reported isolates using the neighbor-joining method and CLUSTALW program (Kimura 2 parameter model) with bootstrap 1000 replications to generate the phylogenetic tree and also estimated evolutionary distances of all sequences. Moreover, Sequence Demarcation Tool 1.2 (SDT v1.2) (Muhire *et al.* 2014) was used to determine the percent identity matrix among the begomovirus sequences.

Results and Discussion

Viruses severely infected the ivy gourd plants showed characteristic symptoms such as mosaic, yellow mosaic, mosaic with puckering and chlorosis in all surveyed districts of Tamil Nadu (Figure 1) with the disease incidence ranging from 43 to 96%. A minimum incidence of 43% was documented in Ambilikai village of Dindigul district (IGD-1), whereas a maximum of 96% disease incidence was documented in Vettaikaranpudhur (IGP-1) of Coimbatore district (Table 1). A total of 26 samples were collected from the 5 different locations (11 from Coimbatore, 6 from Salem, 4 from Dindigul and 5 from Erode). ToLCNDV infection was



Yellow mosaic Mosaic Chlorosis

Mosaic with puckering Yellow mosaic

Figure 1: Symptomatology of begomovirus infection on ivy gourd. A. Yellow mosaic; B. Mosaic; C. Chlorosis; D. Mosaic with puckering; E. Yellow mosaic

Table 1: Survey on begomovirus infection in ivy gourd fields of different districts in Tamil Nadu

S. No.	*Isolates*	*Location*	*No. of samples*	*GPS location*	*Begomovirus disease incidence (%)*	*Gen Bank Accession*
1	IGT-1	Coimbatore (TNAU-Orchard)	6	11.0122° N, 76.9354° E	65	OR041745
2	IGS-1	Salem (Thalaivasal)	6	11.65916°N 78.72717° E	72	OR028863
3	IGD-1	Dindigul (Ambilikai)	4	10.56774°N 77.72321°E	43	OR041747
4	IGP-1	Coimbatore (Vettaikaranpudhur)	5	10.6292°N 76.8771° E	96	OR041746
5	IGE-1	Erode (Anthiyur)	5	11.6133°N 77.5692° E	45	OR041748

first observed in tomato (Padidam *et al.* , 1995) and later identified in different solanaceous and cucurbitaceous vegetables (Arguello-Astorga *et al.* , 2001 and Hanley-Bowdoin *et al.* , 1999). Venkataravanappa *et al.* , (2018) documented 60 to 70% disease incidence of ToLCNDV on ivy gourd collected from New Delhi and Uttar Pradesh, showing mosaic, blistering and stunted incidence. Begomoviruses are extending their host range to infect 98.6% in major cucurbits such as ash gourd, pumpkin, bitter gourd, bottle gourd, snake gourd, cucumber, ivy gourd and watermelon from Tamil Nadu (Nagendran *et al.* 2017). Similarly, Kumar *et al.* (2021) reported 24 to 60% disease severity on cucurbits infecting mosaic, puckering, yellowing and vein clearing in Bihar.

The total DNA obtained from all the symptomatic leaf samples were amplified an expected fragment size of ~1200 bp in the PCR assay with begomovirus universal primer pair (PAL1v722/PAL1c1960) targeting AC1 and AC2 region. In addition, asymptomatic plants of IGP-1 isolate were also yielded an amplicon of ~1200 bp fragment. Venkataravanappa *et al.* (2018) used begomovirus specific primer for amplifying 1.2 kb genome fragment for sixteen ToLCNDV isolate infecting ivy gourd samples. Nagendran *et al.* (2016) used coat protein specific (Deng 540/541) primer for identifying begomovirus in ivy gourd mosaic infected samples and subsequently cloned the full length genome through RCA and identified new Begomovirus species CoMoV in Tamil Nadu. The positive amplicons obtained from the ivy gourd samples in this study were sequenced from selective samples in both the end and aligned sequences were submitted to the NCBI GenBank database (Table 1).

In BLASTn analysis, IGT-1 and IGP-1 isolate from Coimbatore shared the highest identity of 96.2 to 98.2% with ToLCNDV isolate infecting chrysanthemum in Karnataka; IGD-1 isolate from Dindigul had 99.3% nucleotide identity with tomato ToLCNDV isolate from Raipur; and IGE-1 isolate showed 98.4% sequence identity towards the ToLCNDV isolates of Tamil Nadu infecting bitter gourd. Interestingly, isolate of IGS-1 from Salem, have highest nucleotide identity (98.9%) with the Coccinia mosaic Tamil Nadu virus infecting ivy gourd of Tamil Nadu. Nagendran *et al.* (2016) reported distinct bipartite *Coccinia* mosaic virus (CoMoV) infecting ivy gourd associated with mosaic disease in Tamil Nadu. Neoh *et al.* (2023) confirmed the mixed infection of Squash leaf curl China virus (SLCCNV) and Squash leaf curl Philippines virus (SLCuPV) in pumpkin and bottle gourd. In this study both ToLCNDV and CoMoTNV found to be associated with the mosaic diseases on ivy gourd plants in Tamil Nadu. The nucleotide identity matrix generated using the SDT tool was determined for the ivy gourd isolates of Tamil Nadu

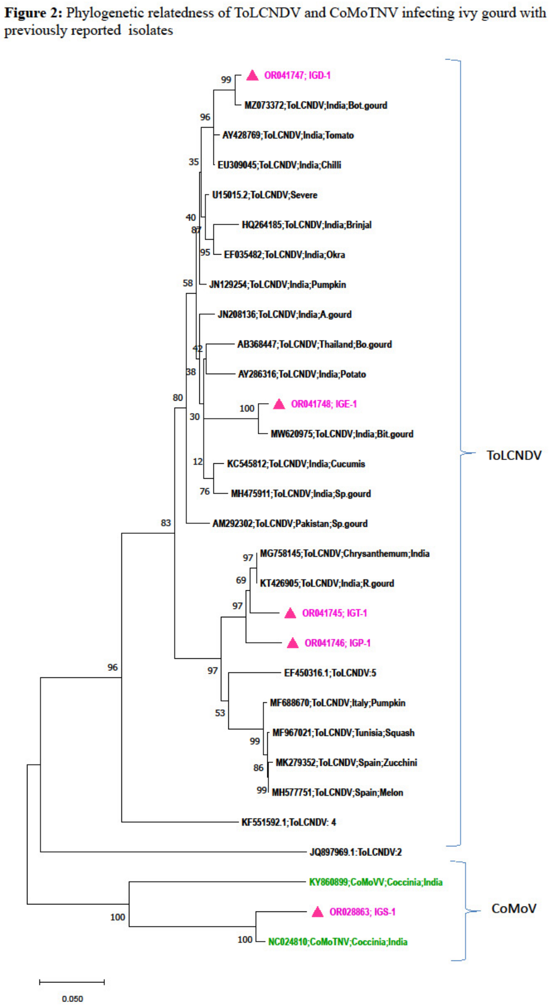


Figure 2: Phylogenetic relatedness of ToLCNDV and CoMoTNV infecting ivy gourd with previously reported isolates

Table 2: Per cent identity matrix based on nucleotide sequences of ToLCNDV and CoMoTNVisolates infecting ivy gourd isolate with other previously reported isolates

Accession number	*Virus*	*IG-ER*	*IG-DIN*	*IG-TNAU*	*IG-Pol*	*IG-Sal*
JQ897969	ToLCNDV-2	71.8	72.8	69.6	71.6	67.4
KF551592	ToLCNDV-4	82.2	84.5	78.2	77.7	69.3
MW620975	ToLCNDV;India;Bit.gourd	98.5	91.1	86.4	86.4	69.6
AB368447	ToLCNDV;Thailand;Bo.gourd	93.0	93.5	89.6	88.8	70.1
MZ073372	ToLCNDV;India;Bot.gourd	91.1	99.0	88.1	88.3	69.8
AY428769	ToLCNDV;India;Tomato	92.8	97.6	89.3	89.6	70.8
HQ264185	ToLCNDV;India;Brinjal	91.6	94.0	87.6	87.4	68.1
U15015	ToLCNDV-Severe	93.5	96.4	89.6	89.3	69.8
JN129254	ToLCNDV;India;Pumpkin	94.0	96.1	90.1	89.6	69.6
KC545812	ToLCNDV;India;Cucumis	93.5	95.2	88.3	88.6	70.8
MH475911	ToLCNDV;India;Sp.gourd	93.8	94.2	88.6	88.1	69.8
JN208136	ToLCNDV;India;A.gourd	93.5	95.0	89.1	88.6	69.8
MG758145	ToLCNDV;ChrysanthemumIndia	88.8	89.8	97.2	96.5	73.3
KT426905	ToLCNDV;India;R.gourd	88.8	89.8	97.2	96.5	73.3
MF688670	ToLCNDV;Italy;Pumpkin	87.6	90.1	93.3	92.5	71.3
MK279352	ToLCNDV;Spain;Zucchini	87.1	89.6	93.5	92.3	71.1
MH577751	ToLCNDV;Spain;Melon	87.4	89.8	93.8	92.5	71.3
EF450316	ToLCNDV;5	88.6	88.6	91.1	90.1	70.8
NC024810	CoMoTNV;Ivy gourd;India	72.8	72.3	74.8	73.8	95.5
KY860899	CoMoVV;Ivy gourd; India	71.6	70.4	73.8	72.8	77.0
OR041748	IGE-1	100	91.1	87.4	87.4	70.6
OR041747	IGD-1	91.1	100	88.1	88.3	70.3
OR041745	IGT-1	87.4	88.1	100	94.8	72.5
OR041746	IGP-1	87.4	88.3	94.8	100	71.6
OR028863	IGS-1	70.6	70.3	72.5	71.6	100

with other reported begomovirus sequences from different countries (Table 2). The phylogeny revealed the grouping of ToLCNDV and CoMoTNV in a separate group. ToLCNDV isolates infecting ivy gourd were grouped under three major clusters. The IGT-1 and IGP-1 isolates were grouped with *Chrysanthemum* (MG758145) and ridge gourd (KT426905), infecting ToLCNDV isolates along with the ToLCNDV-5 species. Isolate IGE-1 had close relationship with Indian ToLCNDV bitter gourd isolate (MW620975) whereas isolate IGD-1 had grouped with bottle gourd ToLCNDV of India (MZ073372) along the reference strain ToLCNDV-Severe (U150152). The isolate IGS-1 formed a separate group along with *Coccinia* mosaic Tamil Nadu virus (NC024810) and *Coccinia* mosaic Virudhunagar virus (KY860899) (Figure 2). Our results on occurrence of virus isolates related to ToLCNDV and CoMoV are similar to previous reports. Begomovirus species are known to cause diseases in many cucurbits. They are ToLCNDV in pumpkin (Phaneendra *et al.* 2012), bitter gourd (Tiwari *et al.* 2010), sponge gourd (Sohrab *et al.* 2003; Tiwari *et al.* 2012). Nagendran *et al.* (2016) documented CoMoV, a novel bipartite begomovirus associated with the mosaic disease of ivy gourd from the samples collected from Tamil Nadu. Previously, Nagendran *et al.* (2014) reported the SLCCNV and ToLCNDV cause yellow mosaic disease on pumpkin in Tamil Nadu. Similarly, full length genome of bipartite ToLCNDV associated with mosaic disease of ivy gourd was characterized by Venkataravanappa *et al.* (2018) from the samples collected from Uttar Pradesh. Squash leaf curl China virus (SLCCV) and Tomato leaf curl New Delhi virus (ToLCNDV) cause mosaic disease of cucurbits in Indonesia (Subiastuti *et al.* 2019).

In the present investigation, the infection of ToLCNDV and CoMoTNV on ivy gourd causing mosaic and yellowing disease in Tamil Nadu was documented. Since ivy gourd is a perennial and vegetatively propagated crop, once the virus enters the plant, it acts as a reservoir host and transmits the virus to other cucurbit crops. The occurrence of begomovirus species in ivy gourd will limit the vegetative propagated crop improvement programs. Many transgenic strategies are available for clonally propagated plants, but the techniques must be standardized for complete protection from begomovirus. Therefore, an effective management strategy is needed to prevent the begomovirus in ivy gourd by educating the farmers to plant virus-free propagative material. Our further investigation will focus on complete genome characterization, distribution and diversity of begomovirus associated ivy gourd in Tamil Nadu.

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सारांश

वर्ष 2019-2021 के दौरान तमिलनाडु में कुन्दरू के खेत में एक व्यवस्थित प्रक्षेत्र सर्वेक्षण किया गया। ऐसे संक्रमित पौधे जिनमें प्रभावी पीलापन मोजैक सिकुड़न तथा पीले मोजैक वाली पत्तियों कापरीक्षण पालीमरेज चेन रिएक्शन से बिगोमो वायरस हेतु किया गया। पी.सी.आर के एम्पलीकान्स से 5 प्रतिदर्शों का सिक्वेन्स किया गया। इनमें 4 प्रतिदर्शों को टोमैटो लीफ कर्ल, नई दिल्ली वायरस (टी.एल.ओ.सी.एन.डी.वी.) तथा 1 प्रतिदर्श काक्सिनिया मोजैक तमिलनाडु वायरस (सी.ओ.एम.ओ.टी.एन.वी.) संक्रमित पाया गया। आइसोलेट आई.जी.टी.-1 (ओ.आर.041745) व आई.जी.पी.-1 (ओ.आर.041746) के न्यूक्लिपोटाइड सिक्वेन्स में 96-98 प्रतिशत के साथ टी.ओ.एल.सी.एन.डी.वी. आइसोलेट क्राइसैन्थियम संक्रमण (एम.जी.758145) कर्नाटक में पाया गया। दूसरे आइसोलेट आई.जी.डी. (ओर.आर.041747) में 99.3 प्रतिशत समरूपता टमाटर को संक्रमित करने वाले टी.ओ.एल.सी.एन.डी.वी. आइसोलेट (एम.डब्ल्यू. 538662) रायपुर तथा आइसोलेट आई.जी.ई.-1 (ओ.आर. 41748) में 98.4 प्रतिशत समरूपता पहले से स्पष्ट की गयी टी.ओ.एल.सी.एन.डी.वी. आइसोलेट भारतवर्ष में करेला को संक्रमित करने वाला पाया गया। आई.जी.एस.-1 (ओ.आर.028863) आइसोलेट में 98.9 प्रतिशत समरूपता न्यूक्लियोटाइड स्तर पर पहले स्पष्ट हुआ सी.ओ.एम.ओ.टी.एन.वी. (के.वाई 860899) तमिलनाडु में कुन्दरू को संक्रमित करता है। वंशावली विश्लेषण से स्पष्ट हुआ कि टी.ओ.एल.सी.एम.डी.वी. आइसोलेट जो कुन्दरू को संक्रमित करता है, पहले से विश्व स्तर पर स्पष्ट हुआ परिणा के समूह में स्थापित होते हैं। वर्तमान परीक्षण में स्पष्ट हुआ कि विगोमोवायरस की व्युत्पत्ति (टी.ओ.एल.सी.एन.डी.वी. व सी.ओ.एम.ओ.टी.एन.वी.) तमिलनाडु के विभिन्न क्षेत्रों में कुन्दरू के पौधों पर संक्रमण करते हैं।