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RESEARCH ARTICLE

Molecular detection and characterisation of bipartite begomoviruses associated with cucurbitaceous vegetables in Sri Lanka

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Abstract: Begomovirus infection is one of the major problems associated with cucurbit cultivation in Sri Lanka. A study was conducted to detect bipartite begomoviruses associated with cucurbits in the country. Cucumber, gherkin, pumpkin, bitter gourd, ridge gourd and snake gourd samples showing viral like symptoms of leaf mosaic, upward leaf curling, stunting, rosetting, puckering and fruit malformation were found to be infected with begomovirus. However, only cucumber and gherkin samples were detected to be infected with bipartite begomoviruses with both A and B genomes. All the other samples were detected as infected with monopartite begomoviruses with A genome. The sequence comparison of the coat protein gene of DNA-A in bipartite begomovirus associated with cucumber and gherkin showed a 97 % nucleotide identity with the Tomato Leaf Curl New Delhi Virus (ToLCND) (KP868764). The phylogenetic analysis of the cucumber isolate was distinct from all the other viruses; however clustered with different ToLCND viruses. DNA-B showed 90 % identity with the Bhendi Yellow Vein Mosaic Virus (BYVMV) (JQ359517). This new isolate was named as Tomato Leaf Curl New Delhi Virus-Cucumber [Sri Lanka: 2014]. This is the first known bipartite begomovirus associated with cucurbits in Sri Lanka.

Keywords: Begomovirus, Bhendi Yellow Vein Mosaic Virus, cucurbits, Tomato Leaf Curl New Delhi Virus.

INTRODUCTION

Begomoviruses belonging to the family *Geminiviridae* are among the most devastating pathogens of a variety of cultivated crops including maize, cassava, bean, squash, cucurbits, tomato, sweet potato, cotton and grain legumes in tropical and subtropical regions of the world. All begomoviruses are transmitted by the whitefly *Bemisia tabaci* in a persistent, circulative manner to dicotyledonous plants (Rey *et al*., 2012). Begomoviruses are generally considered to be either monopartite (one ssDNA component called DNA-A) or bipartite (two circular ssDNA components called DNA-A and DNA-B). DNA-A encodes the proteins required for replication, control of gene expression, overcoming host defenses and encapsulation, and insect transmission inducing strong disease symptoms. DNA-B encodes two proteins with functions in intra- and inter-cellular movement in host plants enhancing symptom severity (Padidam *et al*., 1995; Engel *et al*., 1998). Most of the monopartite begomoviruses are associated with ssDNA satellite molecules known as alpha and beta satellites (Rey *et al*., 1995).

DNA-A and DNA- B components of the begomoviruses have very distinct molecular evolution. The analysis highlights that component exchange has played a greater role in diversification of the begomoviruses. Scientists have proposed a hypothesis that DNA-B originated as a satellite that was captured by the monopartite progenitor of all extant bipartite begomoviruses and subsequently evolved to become the integral genome component that is recognised today (Briddon *et al*., 2010).

Begomoviruses can be subdivided into New World and Old World members according to their geographical origin of isolation. New World includes the geographical regions of Latin America and Meso America; Old World includes Japan, Africa, India and Asia. Nearly all New World viruses have bipartite genomes and the majority of begomoviruses in the Old World apparently have

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monopartite genomes (Zubiaur *et al*., 1998). However, in the Indo-Pak sub-continent the begomoviruses are highly diverse. A small number of them are bipartite such as the Tomato Leaf Curl New Delhi Virus (ToLCNDV) (Padidam *et al*., 1995). ToLCNDV from the North India have bipartite genomes while those from the South have monopartite genomes (Borah & Dasguptha, 2012).

Begomovirus infection is a serious threat to crop cultivation in Sri Lanka and they have been reported from many crops (bean, cassava, tomato and chilli) as Bean Yellowing Virus (Wickramaarachchi *et al*., 2012), Sri Lanka Cassava Mosaic virus (SLCMV) (Saunders *et al*., 2002), Tomato Yellow Leaf Curl Gannoruwa Sri Lanka Virus (TYLCGSLV) (Samarakoon *et al*., 2012) and Chilli Leaf Curl Sri Lanka Virus (ChiLCSLV) (Senanayake *et al*., 2013). Most of them were reported as closely related with the Indian isolates, indicating trans-boundary movement of the virus due to the close geographical location. Identification of the diversity among begomoviruses in Sri Lanka is important for the management of the disease. The present study was conducted to detect bipartite begomoviruses associated with cucurbits.

METHODOLOGY

Sample collection

Leaf samples were collected from infected bitter gourd, ridge gourd, snake gourd, cucumber, gherkin and pumpkin plants, which showed viral like symptoms of leaf mosaic, upward leaf curling, stunting, rosetting, puckering and fruit malformation, from research fields of the Horticultural Crop Research and Development Institute in Gannoruwa, Makandura, Wanathawilluwa and from famer fields (Figure 2). Healthy plant samples were collected from greenhouse-grown plants, which were apparently free from viral symptoms. Total DNA was extracted from infected and healthy plant samples using the modified cetyl trimethyl ammonium bromide (CTAB) method (Lodhi *et al.,* 1994; Wickramaarachchi *et al.*, 2012).

PCR detection of begomovirus DNA-A genome using degenerate primers

A set of degenerate primers (Deng 540-5'TGGACYTTRCAWGGBCCTTCACA 3' and Deng 541-5'TAATATTACCKGWKGVCCSC 3') (Deng *et al*., 1994) was used to amplify virus DNA-A in the samples. Ten microlitres of reaction mixture comprised 5 µL of master mixture (PCR Master mixture-Qiagen), 0.8 µL of each primer (10 mM), 0.5 µL of diluted (1:25) test DNA suspension and sterile distilled water. The PCR programme used was: one cycle of initial denaturation at 94 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 1 min, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. PCR products were electrophorileat in 1.5 % agarose gel for 1 h at 80 V and visualised under UV light using gel documentation apparatus (Figure 1a).

PCR detection of DNA-B genome in begomovirus

A set of degenerate primers (PCRc1-5'CTAGCTGCAGCATATTTACRARWATGCCA 3' and PBL1v2040-5'GCCTCTGCAGCARTGRTCKATCTT CATACA 3') (Rojas *et al*., 1993) was used to amplify DNA-B in the samples. The same test procedures used for the amplification of DNA-A were used to amplify the DNA-B except the primers. The results have been given in Figure 2b.

Sequencing and phylogenetic analysis of bipartite begomoviruses in cucumber and gherkin

The PCR products for DNA-A and DNA-B of cucumber and gherkin samples were sent to GeneTech Pvt. Ltd., 54, Kitulwatte Road, Colombo 08, Sri Lanka for bidirectional sequencing. FASTA forms of the resulted sequences were edited and analysed using basic local alignment search tool (BLAST) (Altschul *et al.*, 1990) based on the close sequence identity and the length of the sequences. The phylogenetic analysis was performed to find the relationship of the begomovirus associated with cucumber with other published Gemini viruses using molecular evolutionary genetic analysis (MEGA) 4.0 software by neighbour-joining method (Tamura *et al.,* 2007) at 1000 bootstrap value.

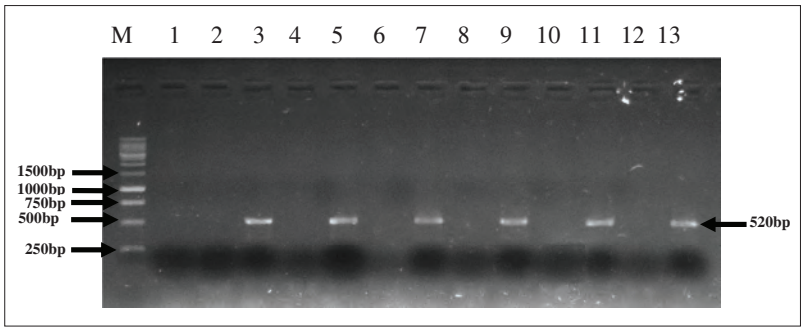
RESULTS

PCR detection of begomovirus and DNA-A genome

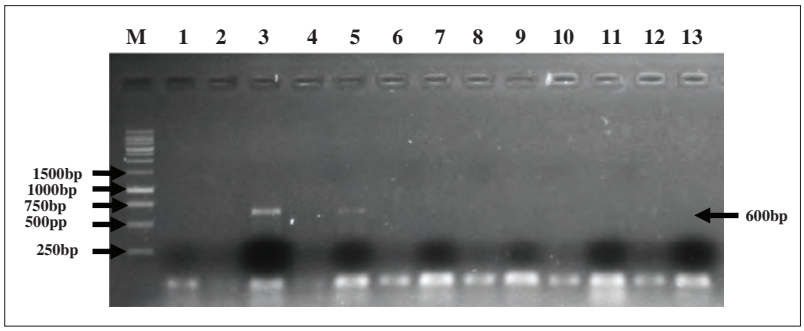
The PCR assay carried out using a set of degenerate primers successfully amplified the expected DNA-A fragment of 520 bp (Figure 1a) from infected bitter gourd, ridge gourd, snake gourd, cucumber, gherkin and pumpkin samples, confirming the association of begomoviruses in the diseased samples.

PCR detection of DNA-B genome in begomovirus

Only the infected cucumber and gherkin samples provided the expected DNA-B fragment of 600 bp (Figure 1b). It confirmed the presence of bipartite



(a)



(b)

Figure 1: Molecular detection of (a) DNA-A genome and (b) DNA-B genome in cucurbit samples using degenerate primers
Lane M – 10,000 Kb DNA ladder (promega-G571A); lane 1 - water control; lane 2 - healthy cucumber; lane 3 - infected cucumber; lane 4 - healthy gherkin; lane 5 - infected gherkin; lane 6 - healthy pumpkin; lane 7 - infected pumpkin; lane 8 - healthy bitter gourd; lane 9 - infected bitter gourd; lane 10 - healthy ridge gourd; lane 11 - infected ridge gourd; lane 12 - healthy snake gourd; lane 13 - infected snake gourd

Figure 2: Infected cucumber plants showing leaf yellowing, mosaic, mottling, distortion and upward leaf curling

begomoviruses in the infected cucumber and gherkin samples (Figure 2).

Sequencing and phylogenetic analysis of bipartite begomovirus associated with cucumber and gherkin

BLAST analysis of the DNA-A coat protein gene fragment of cucumber and gherkin virus isolates showed 97 % nucleotide identity with the ToLCNDV-BG1 (KP868764). A 96 % nucleotide identity was reported with ToLCNDV [Solanum:Pakistan] (DQ116885), ToLCNDV (KC914896), ToLCNDV-Bitter gourd (AM747291) and ToLCNDV - Raebareli (JX232220) viruses reported from India.

BLAST analysis of the DNA-B segment of the cucumber isolate showed 90 % nucleotide identity with the Bhendi Yellow Vein Mosaic Virus movement protein gene (JQ359517) reported from Karnataka, India (Venkataravanappa *et al*., 2012). This new virus isolate was named as Tomato Leaf Curl New Delhi Virus-Cucumber [Sri Lanka: 2014] (ToLCNDV-Cucumber [Sri Lanka: 2014]).

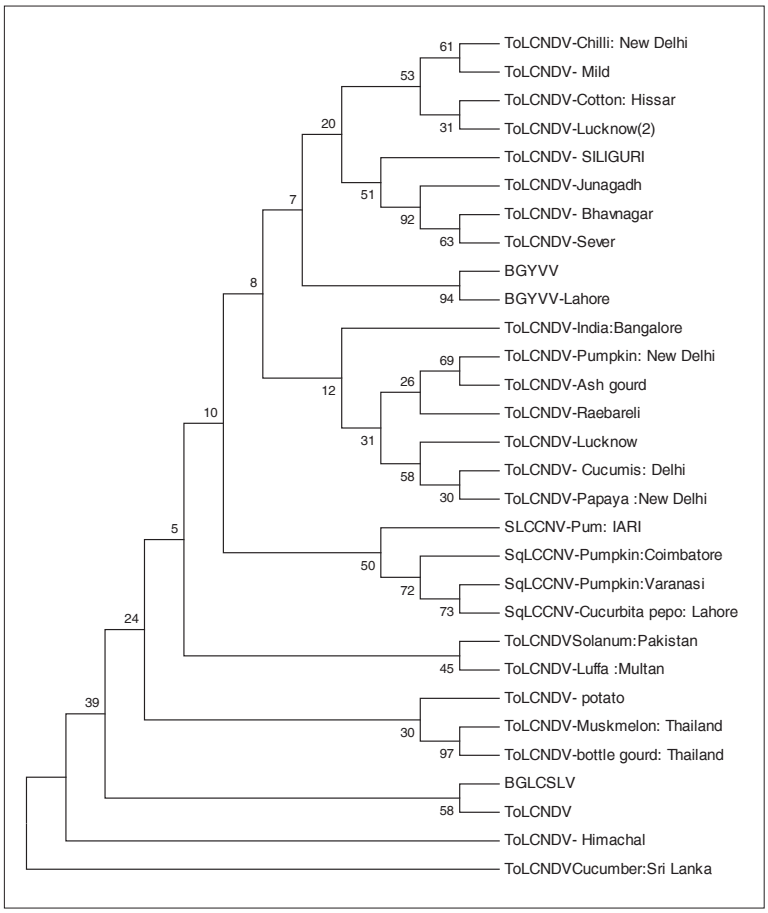


Figure 4: Phylogenetic tree constructed using MEGA 4.0 showing the relationship of cucumber virus isolate (BCSLV) with other begomoviruses

The phylogenetic analysis shows that the present isolate was distinct from all the other viruses. However, it clustered with ToLCNDV isolates from India, Thailand and Pakistan infecting different crops, and Squash Leaf Curl China Virus (SqLCCNV) from India and Pakistan infecting different crops indicating the comparative genetic similarity (Figure 4).

DISCUSSION

The PCR detection of begomoviruses using degenerate primers confirmed the association of begomoviruses and its DNA-A fragment with the infected bitter gourd, ridge gourd, snake gourd, pumpkin, cucumber and gherkin samples showing viral symptoms of leaf mosaic, upward leaf curling, stunting, rosetting, puckering and fruit malformation. The PCR detection of DNA-B genome using degenerate primers confirmed the association of bipartite begomoviruses with the infected cucumber and gherkin samples. This is the first evidence of the presence of bipartite begomoviruses in cucurbits in Sri Lanka. This result indicates the association of monopartite begomoviruses with infected bitter gourd, ridge gourd, snake gourd and pumpkin samples. The bittergourd sample was previously sequenced and found to be related to Tomato Leaf Curl New Delhi Virus-Bitter Gourd (AM747291) (Bandaranayake *et al*., 2014).

DNA-A genome of the bipartite ToLCNDV-Cucumber [Sri Lanka: 2014] was distinct from all the other viruses in phylogenetic analysis, but clustered with the ToLCNDV group infecting different crops. The BLAST analysis reported 97 % neucleotide identity with ToLCNDV, and therefore the ToLCNDV-Cucumber [Sri Lanka: 2014] can be a strain of ToLCNDV. If the sequence shares 94 % genome-wide pairwise identity to all the isolates described for that species, a strain name should then be proposed (Brown *et al*., 2015).

DNA-B of the ToLCNDV-Cucumber [Sri Lanka: 2014] showed 90 % nucleotide identity with the Bhendi Yellow Vein Mosaic Virus movement protein gene (BYVMV) (JQ359517) from Karnataka, India (Venkataravanappa *et al*., 2012). This BYVMV DNA-B showed the highest sequence identity with the ToLCNDV (AY158080). The phylogenetic analysis of the BYVMV isolate was distinct from all the other viruses; but clustered with the ToLCNDV group infecting different crops. The recombination analysis revealed that this isolate has sequences originated from ToLCNDV (Venkataravanappa *et al*., 2012).

Since BLAST analysis of the ToLCNDV-Cucumber [Sri Lanka: 2014] DNA-A has not shown any relationship with its DNA-B, it can be suggested that the monopartite begomovirus related to ToLCNDV has captured the DNA-B of BYVMV to infect cucumber. Component exchange and recombination has played a far greater role in the diversification of begomoviruses (Kumar *et al*., 2008; Briddon *et al*., 2010; Tahir *et al*., 2010; Venkataravanappa *et al.,* 2015). This study revealed the association of both bipartite and monopartite begomoviruses related to TLCNDV in cucurbit cultivations in Sri Lanka and the risk of trans-boundary movement of viruses from Indian subcontinent due to the close geographical location.

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