Virus Research 174 (2013) 126–136

Contents lists available at SciVerse ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

# Asymmetric synergism and heteroencapsidation between two bipartite begomoviruses, tomato leaf curl New Delhi virus and tomato leaf curl Palampur virus

S. Kanakala[a], P. Jyothsna[a], Richa Shukla[a], N. Tiwari[a], B.S. Veer[a], P. Swarnalatha[b], M. Krishnareddy[b], V.G. Malathi[a,*]

[a] Advanced Centre for Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India
[b] Division of Plant Pathology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru 560089, Karnataka, India

ARTICLE INFO

*Article history:*
Received 8 January 2013
Received in revised form 15 March 2013
Accepted 17 March 2013
Available online 8 April 2013

*Keywords:*
Begomovirus
Whitefly
Pseudorecombination
Synergism

## 1. Introduction

Whitefly-transmitted geminiviruses belonging to the genus *Begomovirus*, family *Geminiviridae*, cause severe diseases in vegetables worldwide. The genome of begomoviruses is composed of one (monopartite) or two (bipartite) single-stranded DNA molecules designated as DNA A and DNA B (Stanley, 1985, Stanley et al., 2005 and Fauquet et al., 2008), The majority of Old World viruses have only DNA A, which may be associated with satellite DNA components referred to as alphasatellites and betasatellites (Briddon et al., 2003, 2008). The DNA A component encodes genes for viral encapsidation and movement on the viral strand and genes responsible for replication (Rep), regulation of gene expression and suppression of post transcriptional gene silencing (PTGS) on the complementary sense strand. In the bipartite genomes, DNA B encodes genes for nuclear transport (nuclear shuttle protein [NSP], BV1) in the viral sense strand and genes for cell-to-cell movement (movement protein, [MP] BC1) in the complementary sense strand (Stanley et al., 2005). In DNA A and DNA B, there exists a noncoding region of ~150 nt length with high sequence similarity. This region, which is called the common region (CR), consists of the origin of replication, Rep-binding iterative sequences (iterons) and several *cis*-regulatory elements required for leftward and rightward gene expression. The iterons are specific sites recognised by the cognate Rep protein. Binding of the Rep protein to these is considered essential to initiate replication (Hanley-Bowdoin et al., 1999). The replication of DNA B is facilitated by the Rep protein encoded by DNA A, and it is mediated by the recognition of the same iteron sequences in DNA B as in DNA A (Hanley-Bowdoin et al., 1999; Argüello-Astorga and Ruiz-Medrano, 2001). The ability or the ease with which Rep encoded by DNA A binds to these iterons in DNA B determines whether viable exchange of DNA components (pseudorecombination) occurs between different species or strains of begomoviruses (Harrison and Robinson, 1999). Recombination and pseudorecombination during mixed infection of begomoviruses are the major factors leading to the emergence of new viruses. In this context, tomato is an ideal host as a large number of begomoviruses infect and produce severe diseases in this species.

Tomato leaf curl disease is a major challenge in the cultivation of tomato in India (Chakraborty, 2008). The disease is caused by 10 monopartite begomoviruses and two bipartite begomoviruses (Tiwari et al., 2012). The begomovirus, tomato leaf curl Gujarat virus (ToLCGuV) exists in both mono and bipartite form (Chakraborty et al., 2003; Jyothsna et al., 2012). Until 2008, the only bipartite begomovirus known to infect tomato was tomato leaf curl New Delhi virus (ToLCNDV). The virus was shown to have a wide host range and infect tomato (Padidam et al., 1995), eggplant (Pratap et al., 2011) cucurbits (Sohrab et al., 2003), cotton (Pandey

* Corresponding author. Fax: +91 011 25843123.
*E-mail address:* vgmalathi@rediffmail.com (V.G. Malathi).

0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.03.011

et al., http://www.ncbi.nlm.nih.gov/nuccore/EF063145) and weeds (Haider et al., 2005). In 2008, surprisingly, a whitefly-transmitted bipartite virus, tomato leaf curl Palampur virus (ToLCPalV) was reported from a sub-temperate region, Palampur, in the state of Himachal Pradesh, which was not previously known to harbour any begomoviruses or the whitefly vector (Kumar et al., 2008). Since the recording in 2008, ToLCPalV has been detected in different hosts: in cucurbits in India (Namrata et al., 2011, 2012); in tomato, cucumber and melon in Iran (Heydarnejad et al., 2009); and in melon in Pakistan (Malik et al., 2011). It appears that the virus may have a broader host range and be distributed in more areas.

In a survey of tomato leaf curl diseases in 2008–2010, we found mixed infection of ToLCPalV with ToLCNDV (our unpublished results). For example, in the state of Punjab in India, in samples collected from nine fields, ToLCPalV alone was present in four fields, whereas it was present with ToLCNDV in three fields and with tomato leaf curl Karnataka virus (ToLCKaV) in two fields. Similarly, in the Junagadh district in the state of Gujarat, in samples from five fields, two fields had only ToLCPalV, two had ToLCPalV and ToLCGuV and one had ToLCPalV and ToLCKaV.

The survey of natural infection in the tomato fields suggests that mixed infection between different species of begomoviruses is common. Usually, the symptom phenotype observed in the mixed infection in tomato was severe leaf distortion, bright yellow banding alternating with a green mosaic, vein thickening and extreme stunting. Mixed infection of two viruses and their variants in a crop genotype have been implicated in outbreaks of epidemics in crops such as cassava (Harrison et al., 1997). Mixed infection has implications for productivity and epidemiological consequences. Thus, we investigated the interaction between the two bipartite begomoviruses ToLCNDV and ToLCPalV to shed light on how they complement each other in viral pathogenesis. The present communication discusses the results of genetic reassortment between the two viruses. An isolate of ToLCPalV was cloned, and its infectivity was established in tomato and cucurbits. Viable pseudorecombinants were formed between DNA A and DNA B components of ToLCNDV and ToLCPalV. The results revealed that the interaction was more of the asymmetric synergism type, with ToLCNDV DNA A co-inoculated with ToLCPalV DNA B producing more severe symptoms but not vice versa. For the first time, whitefly transmission of pseudorecombinants was demonstrated clearly, indicating the possibility of heteroencapsidation.

## 2. Materials and methods

### 2.1. Virus source

Symptomatic leaves associated with leaf curling, severe yellow banding and vein thickening symptoms were collected from a farmer's fields in the Punjab and maintained at −80 °C before DNA extraction.

### 2.2. Isolation, virus amplification and cloning

Total DNA from symptomatic leaves of naturally infected tomato was extracted using the GEM–CTAB method (Rouhibakhsh et al., 2008). Two sets of primers, PAR1v772/PAL1c1960 (Paximadis et al., 1997), were first used to detect the presence/absence of the begomovirus. PCR was carried out in volumes of 25 μl with the following programme: a cycle of 2 min at 94 °C, then 30 cycles at 95 °C for 1 min, 55 °C for 2 min, 72 °C for 3 min and a final extension at 72 °C for 10 min. Samples positive for the begomovirus were selected for further characterisation. The complete length of the viral genome was amplified with the rolling circle amplification (RCA) method (Haible et al., 2006). The RCA products were digested with *Hin*dIII and cloned into the vector pUC18 linearised with the same enzyme. Two full-length clones were sequenced (ABI sequencer at Delhi University) by the primer walking strategy. The sequence data were assembled and analysed with the BLAST programme in the NCBI (www.ncbi.nlm.nih.gov) to find the maximum identity of the sequences.

### 2.3. Analysis of the complete sequences

The sequences of the full-length clones were aligned using Clustal W (Thompson et al., 1994). Nucleic acid and amino acid sequence identities were analysed using BioEdit v7.0.9.0 (www.mbio.ncsu.edu/BioEdit). Full-length sequence pair-wise alignment, together with its colour-coded matrix, was produced using the species demarcation tool SDTv1.0 program (http://web.cbio.uct.ac.za/SDT). The nomenclature and descriptions of the species follow those in Fauquet et al. (2008) and Kings et al. (2011).

### 2.4. Generation of infectious clones

Partial tandem repeat (PTR) constructs of the cloned DNA A and DNA B components were prepared to establish the infectivity. The procedure was as follows. A partial fragment [*Hin*dIII (103)/*Eco*RI (1764), 1095 bp] containing the origin of replication was released from ToLCPalV DNA A (PA) and ligated with pBIN19 restricted with *Hin*dIII/*Eco*RI to generate PA0.4mer. The full-length viral DNA fragment was inserted as a *Hin*dIII fragment in pBinPA0.4mer to produce PA1.4mer. Tandem orientation of the construct was verified by restriction with *Eco*RI and *Xba*I, which was expected to release a 2.7 kb fragment. For generating PTR of ToLCPalV DNA B (PB), the partial fragment [*Bgl*II (573)/*Hin*dIII (2511), 1136 bp] was cloned into pBIN19 and restricted with the *Bam*H1/*Hin*dIII enzymes to produce PB0.4mer. The full-length fragment was then inserted at the *Hin*dIII site to produce PB1.4mer. Insert tandem orientation was confirmed by restriction digestion with *Kpn*I and *Eco*RI, which was expected to release 1.3 kb and 1.5 kb fragments.

In this work, PTR constructs of ToLCNDV DNA A (HQ141673) and DNA B (HQ141674) described in Jyothsna et al. (2013) were used for the synergistic studies. To simplify the presentation of the results, the constructs are labelled as follows: ToLCNDV DNA A as NA, ToLCNDV DNA B as NB, ToLCPalV DNA A as PA and ToLCPalV DNA B as PB.

### 2.5. Agroinoculations

Partial tandem repeat constructs of all the viral components were transformed to the *Agrobacterium tumifaciens* strain EHA105 by direct transformation. *Agrobacterium* cultures were prepared for homologous, heterologous and mixed inoculations. Each virus construct was mixed in equal concentrations and inoculations of 2-week old tomato seedlings were performed by stem–prick method (Tiwari et al., 2010). The cucurbitaceous hosts were inoculated by seed sprout method (Kanakala et al., 2012) The plants were maintained under 16/8 h light/dark periods 28 ± 2 °C, 18,000 lx and 85% relative humidity at the National Phytotron Facility, IARI, New Delhi. Symptoms were observed and recorded periodically.

### 2.6. Analysis of viral replication

Symptomatic leaves were collected at 10-day intervals, from 10-day post-inoculation (dpi) and stored at 4 °C for further analysis. Total genomic DNA was extracted from newly emerging leaves using the GEM–CTAB method (Rouhibakhsh et al., 2008), and the replicative level was assessed by standard and quantitative real-time PCR.

Download English Version:

https://daneshyari.com/en/article/3428599

Download Persian Version:

https://daneshyari.com/article/3428599

Daneshyari.com