Association of tomato leaf curl Gujarat virus and tomato leaf curl Bangladesh betasatellite on papaya showing typical leaf curl symptoms in North India

Priyanka Varun¹ · Sangeeta Saxena¹

Received: 1 December 2017 / Accepted: 23 April 2018 / Published online: 8 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Papaya leaf curl is an economically important disease occurring in papaya growing tropical and subtropical areas. Papaya leaf curl virus, a begomovirus, is the main causative agent for the disease, but many other begomoviruses as well as betasatellites have also been reported on papaya leaf curl disease. Rapidly evolving host range of begomoviruses is a major issue for developing successful resistance strategies against begomoviral infection considering their expanding host range and mixed infection. In our study, we have identified the presence of begomovirus and associated satellite molecule on papaya showing severe leaf curl symptoms in Lucknow, India. Analysis of complete DNA-A component of this isolate (MG757245) revealed the highest similarity (91%) with tomato leaf curl Gujarat virus (ToLCuGuV), while sequence data of betasatellite (MG478451) showed maximum (89%) identity with tomato leaf curl Bangladesh betasatellite (ToLCuBB). This is the first report on identification of ToLCuGuV and ToLCuBB coinfecting papaya plants in Lucknow, Uttar Pradesh (India).

Keywords Betasatellite · Coat protein gene · Geminiviruses · Papaya leaf curl disease · ToLCuGuV · ToLCuBB

Introduction

Papaya is a significant horticultural crop of tropical and subtropical areas in the world having a great nutritional and economical importance. India is the major papaya producer contributing 44.6% of global papaya production (http://nhb.gov.in/area-pro/horst_galance_2016.pdf). Leaf curl disease is one of the major threats for papaya cultivation, resulting in huge yield loss and marketability of fruits and is known to be caused by whitefly transmitted geminiviruses (Saxena et al. 1998a). Plant viruses are a group of plant pathogens that have enormous negative impact on

horticultural crop production throughout the world. Begomovirus is the largest among all nine genera in the family geminiviridae comprising of whitefly transmitted geminate shaped virus particles having ~2.7 kb long circular single-stranded DNA genome (Zerbini et al. 2017). Begomoviruses may have monopartite (DNA-A) or bipartite (DNA-A and DNA-B) genome. DNA-A component encodes the genes required for replication, encapsidation, and control of gene expression and transmission, whereas DNA-B component encode the genes responsible for virus movement. The majority of begomoviruses are frequently found with association of a satellite DNA molecule (i.e., betasatellite) that induces symptom severity in plants infected with begomoviruses. Although DNA-A component of begomoviruses is capable to cause infection, but associated satellites enhance their virulence and increase symptom development of disease on plants. Betasatellites are DNA molecules that depend on ‘helper’ begomoviruses for their replication and encapsidation. Betasatellites encode a single protein (i.e., β C1) that works as pathogenicity determinant and silencing suppressors (Bridson et al. 2008). Begomoviruses are known to adapt new host very easily in different geographic conditions for their multiplication and survival. As papaya leaf curl virus (PaLCuV), a leaf

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-018-1254-7>) contains supplementary material, which is available to authorized users.

✉ Sangeeta Saxena
dr_sangeeta_saxena@yahoo.com

Priyanka Varun
varunpriyanka1@gmail.com

¹ Department of Biotechnology, School of Bioscience and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India

curl causing begomovirus has been reported in many crops in different geographic locations (Varun et al. 2017; Varun and Saxena 2017a) similarly, many begomoviruses may also choose papaya plants for continuing their existence in several unpredictable conditions. A diversity study on begomoviruses associated with papaya leaf curl disease in India highlighted the rapid evolution of begomoviral host range by giving an extensive list of begomovirus species and associated satellites reported in papaya (Singh-Pant et al. 2012). Presence of many begomoviruses have been reported on papaya like papaya leaf curl virus (PaLCuV) (Saxena et al. 1998b), tomato leaf curl New Delhi virus (ToLCNDV) (Raj et al. 2008), Papaya leaf crumple virus (Singh-Pant et al. 2012) and ageratum yellow vein virus (AYVV) (Shahid et al. 2013). Likewise, association of many betasatellites has also been reported in papaya leaf curl disease, e.g., papaya leaf curl betasatellite (PaLCuB), tomato leaf curl betasatellite (ToLCB), croton yellow vein mosaic betasatellite (CroYMB) (Singh-Pant et al. 2012) and tomato leaf curl java betasatellite (ToLCuJB) (Shahid et al. 2013). Above reports on different begomoviral infection on same crop represent their extensive host selection and co-existence with several betasatellites. Occurrence of mixed viral infection is becoming the major issue in developing management strategies specially based on RNAi where the technology is totally dependent on sequence homology. As many begomoviruses are found to be associated with papaya plant, papaya crop can be considered as an alternate host for many begomoviruses.

Tomato leaf curl Gujarat virus (ToLCuGuV) is a distinct species of begomovirus reported by Chakraborty et al. (2003) for the first time. Association of this virus with tomato leaf curl disease has been reported in Nepal, Pakistan and many Indian states like Gujarat, Maharashtra and Uttar Pradesh (Ranjan et al. 2013). Studies on host range of ToLCuGuV revealed the six known host, i.e., *Capsicum*, *Solanum lycopersicum*, *Nicotiana tabacum*, *Nicotiana benthamiana* (<http://www.genome.jp/virushostdb/219299>), *Xanthium*, a weed (Mubin et al. 2012) and cotton (Zaidi et al. 2015). In the same way, association of ToLCuBB has also been reported with chilli leaf curl virus (ChiLCuV) on chilli plants (Khan and Khan 2016) and with tomato leaf curl Bangladesh virus on *G. niveum* plants (Datta et al. 2015).

Results of this present study document the association of tomato leaf curl Gujarat virus (ToLCuGuV) and tomato leaf curl Bangladesh betasatellite (ToLCuBB) thus, reporting a new complex of begomovirus and betasatellite molecule infecting papaya plant in Lucknow, India. Being a perennial crop, infected papaya serving as an off-season reservoir for ToLCuGuV as well as ToLCuBB is a new challenge to viral disease management in papaya growing regions.

Materials and methods

Papaya plants showing typical leaf curl symptoms like upward and downward curling, leaf deformation, thickening of veins, crumpling and mottling of leaves were observed during survey carried out in Lucknow (Uttar Pradesh) India. Leaves of infected papaya plants (var. Pusa delicious) mainly grown in kitchen gardens were collected from six different locations of Gomti Nagar, RajniKhand, Aliganj, Indira Nagar, Cantonment area and BBA University campus in Lucknow. To identify the causal begomovirus total genomic DNA was isolated from leaf tissue using CTAB method (Murray and Thompson 1980) and were subjected to rolling circle amplification (RCA) using illustra™ TempliPhi DNA amplification kit (GE Healthcare, lifesciences) to amplify all circular DNA molecules. Furthermore, concatameric RCA products were diluted and used to conduct polymerase chain reaction (PCR) for amplification of ~560 bp fragment of core region of coat protein gene to confirm the begomoviral presence using begomovirus coat protein specific primers (Wyatt and Brown 1996). Subsequently, collected samples were also screened for the presence of associated satellite molecule using betasatellite specific primers (Bridson et al. 2002) and primers designed against DNA-B were used to screen the presence of DNA-B (Rojas et al. 1993). Amplification products of both coat protein gene and betasatellite were eluted from the agarose gel and purified using the invitrogen™ purelink™ quick gel extraction kit. Purified samples were sent to chromous biotech pvt. ltd. for sequencing from both orientations to identify the begomovirus and associated satellite molecule. RCA product of begomovirus was completely sequenced by primer walking to analyze the information of complete genome of isolate. Sequencing data was assembled and evaluated from BLAST online (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Furthermore, related begomoviruses and satellite sequences were retrieved from GenBank database and multiple sequence alignment of isolates was carried out using a built-in CLUSTALW implementation in Mega v6.0 software. Resulting alignments were further used for their evolutionary study by constructing the phylogenetic tree using neighbor-joining method by giving 1000 bootstrap replications [Mega version 6.0 (Tamura et al. 2013)].

Results and discussion

Several sites in Lucknow were visited to collect leaf samples of papaya plants having typical symptoms of leaf curl disease. Six samples were collected from different sites

Fig. 1 Plant leaves exhibiting typical leaf curl disease symptoms like upward curling, downward curling, vein thickening, mottling and crumpling

in Lucknow (Gomti Nagar, RajniKhand, Aliganj, BBAU University, Indira Nagar and Cantonment area) along with one healthy sample. Among all collected samples one sample collected from Gomti Nagar was found to be very interesting as it showed highly distinctive symptoms of leaf curling (upward, downward), crumpling and vein thickening (Fig. 1). For molecular identification total plant DNA was isolated from collected leaf tissues along with one healthy sample and extracted DNAs were used as template for RCA. High-molecular weight DNA produced from RCA was further used for PCR detection of existing begomovirus using coat protein specific degenerate primer pair (Wyatt and Brown 1996). Out of six only four samples (Gomti Nagar, Rajnikhand, Aliganj, BBA University) showed the amplification product of coat protein gene (~560 bp) that specifies the begomoviral infection on symptomatic plants, while samples collected from Aliganj and Indira Nagar were not found positive for begomoviral infection. Subsequently, the samples found positive were also tested for the presence of associated betasatellite and DNA-B to identify their presence in the samples. Interestingly, among these tested samples out of four isolates only two (BBAU and Gomti Nagar, Lucknow) showed amplification product of ~1.3 kb, thus indicating the presence of satellite molecule with existing begomovirus. No amplification products were obtained for DNA-B component in repeated attempts. To do the sequence analysis amplified products of coat protein gene, betasatellites and RCA product (Fig. 2) were eluted from the gel and sequenced from both orientations. Coat protein sequences of all the four samples were analyzed through blast online tool. Coat protein sequences of three samples showed the maximum similarity with papaya leaf curl virus and one representative sequence among them has been submitted in GenBank as papaya leaf curl virus strain PSB14 (accession MG021174). Coat protein sequence of

Fig. 2 Screening of collected leaf curl diseased samples through PCR amplification on 1.2% agarose gel. Amplicons of ~560 bp of coat protein (CP) gene of begomovirus using begomovirus CP gene specific primers (Wyatt and Brown 1996) (a), ~1.3 kb amplified product of betasatellites associated with existing begomoviruses (b), high-molecular weight rolling circle amplified DNA of begomovirus isolates associated with leaf curl disease of papaya (c). Lane 1–6 represent the samples, –ve (negative control—without template), +ve (positive control—pUC19 DNA taken as positive control for RCA screening), H (healthy plant), L (100 bp DNA ladder), L1 (Lambda HindIII/EcoRI digested DNA as marker)

sample collected from Gomti Nagar revealed the highest (94–96%) sequence homology with several tomato leaf curl Gujarat virus (ToLCuGuV) isolates available in database. To know the whole genome information of the isolate showing similarity with tomato leaf curl Gujarat virus, RCA product of the same isolate was completely sequenced by primer walking. The obtained sequence data of complete genome was subjected to BLAST online tool showing similarity in a range of (85–91%) with 17 different isolates of ToLCuGuV available in database and highest (91%) similarity with ToLCuGuV (KP164863.1, Maharashtra) which is above from species demarcation threshold to classify begomoviruses so that it cannot be considered as a new species. These results suggest it to be the strain of ToLCuGuV identified on papaya in Lucknow. To observe the phylogenetic relationship of begomovirus under study (accession MG757245) with some selected ToLCuGuV isolates, tomato leaf curl viruses along with other begomovirus species reported on papaya available in database (Table S1) were analyzed through Mega version 6.0 software. A neighbor-joining (NJ) phylogenetic tree constructed using the software depicts the clear clustering of isolate of this study with other ToLCuGuV isolates, while remaining begomovirus species used in this study showed separate groups. Distribution of different begomoviruses in phylogenetic tree reveals their evolutionary background and relatedness which again demonstrates the close relationship of isolate of the study with ToLCuGuV isolates (Fig. 3). Nucleotide sequence homology and phylogenetic analysis of isolate of this study clearly indicates the association of ToLCuGuV with the leaf curl disease

Fig. 3 Phylogenetic analysis based on complete DNA-A sequence of selected begomoviruses available in database using neighbor-joining method with MEGA software version 6.0. Numbers below branches indicate bootstrap value percentages from 1000 replicates. The ToLCuGuV-Lucknow isolate sequenced in present study (MG757245) is indicated with symbol in bold font

of papaya in Lucknow, India. Although, there are many reports of Tomato leaf curl New Delhi virus (ToLCNDV) on papaya, guar, bitter gourd, chilli and opium poppy identified in Lucknow. Tomato leaf curl Karnataka virus infection on soybean, tomato leaf curl Pakistan virus on mentha spp, tomato leaf curl Patna virus on *Mentha piperita* and tomato leaf curl virus on guar has also been documented in Lucknow. Present study is also an addition in pre-existing information about tomato leaf curl virus strains in Lucknow. These results also provide the explanation for expanding host range of ToLCuGuV to papaya. This is the first report of papaya as an alternative host for ToLCuGuV which is an extra threat for papaya cultivation.

In our next step amplified betasatellite fragment of ~1.3 kb was sequenced from two samples giving positive results, i.e., BBAU and Gomti Nagar, Lucknow isolates. Blast was performed and the betasatellite sequence from BBAU campus sample showed maximum similarity with papaya leaf curl betasatellite, while the betasatellite amplified from GomtiNagar, Lucknow sample showed maximum similarity with tomato leaf curl Bangladesh betasatellite. In silico analysis of betasatellite sequence using BLAST programme showed highest identity (89%) with tomato leaf curl Bangladesh betasatellite—Bijnour (ToLCuBB accession,

HM007107) which is greater than species demarcation threshold (i.e., 78% sequence identity) for betasatellites (Bridson et al. 2008) indicating that the associated betasatellite is related to ToLCuBB found in Bijnour, UP, India. It was interesting to note that virus isolated from Gomti Nagar sample showing 91% similarity with ToLCuGuV in its DNA-A component is associated with tomato leaf curl Bangladesh betasatellite (ToLCuBB) bearing 89% similarity in its betasatellite component. Phylogenetic analysis of ToLCuBB from papaya of present study with earlier reported betasatellite sequences available in database (Table S2) was carried out with the help of mega version 6.0 software. Tree constructed through the software represents the close association among similar betasatellite species that sub-clustered on the basis of their geographical origin. Several isolates of papaya leaf curl betasatellites were grouped together in the tree and found to be clustered with the isolates from the same origin. Similarly, ToLCuBB identified in present study shows clear clustering with other ToLCuBB and close relationship with the isolate of Bijnour (Fig. 4). Close relationship of betasatellite of this study (accession MG478451) with other ToLCuBB isolates represent the presence of tomato leaf curl Bangladesh betasatellite with leaf curl disease of papaya. The presence of ToLCuBB together with

Fig. 4 An evolutionary relationship of different betasatellite sequences reported from different geographical locations inferred using neighbor-joining method with Mega v6.0 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Betasatellite sequence isolated in present study (MG478451) is indicated with symbol

ToLCuGuV-Lucknow supports their co-existence on papaya and this study represents papaya as a natural host for these viruses.

Conclusion

As a general practice begomoviruses are named after their host and symptoms followed by place of occurrence. More than 320 species are reported in the genus begomovirus and frequently found to be associated with DNA satellites (Zerbini et al. 2017). Coat protein of begomoviruses is the structural protein that forms the geminate envelope of begomoviruses as well as plays a role in viral movement within plant cells and virus- vector interaction during transmission (Varun and Saxena 2017b). Betasatellites are ssDNA molecules which can combine with many begomoviruses and are known for rapidly evolving their host range that helps in promoting symptom induction of disease. Whole genome sequence as well as coat protein amino acid sequence analysis of isolate of our study follows the guidelines of virus taxonomy to consider it as a Lucknow isolate of tomato leaf curl Gujarat virus. Phylogenetic analysis of begomoviruses

has proven out to be useful for understanding of their relationships and evolutionary background. Emergence of new epidemics can also be determined by phylogenetic studies. Begomoviruses as well as betasatellites are expanding their host range in new geographic origins for their survival and their highly adaptive nature in different geographic locations as well as plant hosts can be seen.

To our knowledge, this is the first report on presence of tomato leaf curl Gujarat virus (ToLCuGuV-MG757245) as well as the first identification of tomato leaf curl Bangladesh betasatellite ToLCuBB (MG478451) on papaya that signifies a new begomovirus–betasatellite complex infecting papaya in Lucknow, India. This property of expanding range of hosts is a major challenge for controlling and minimizing the losses due to mixed begomoviral infection. There is an urgent need to monitor the impact of begomoviral infection on commercially grown crops like papaya and development of sustainable disease management approach to minimize the crop losses due to begomoviruses.

Acknowledgements Authors acknowledge Babasaheb Bhimrao Ambedkar University—A Central University, Lucknow, India for the support and necessary infrastructure facilities provided for the

above research. Priyanka Varun gratefully acknowledges Rajiv Gandhi National Fellowship (F1-17.1/2014-15/RGNF-2014-15-SC-UTT-61433) from University Grants Commission (UGC), Govt. of India.

Author contributions PV carried out the work under the guidance of SS. PV and SS analyzed the data and wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

- Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002) Universal primers for the PCR-mediated amplification of DNA β . *Mol Biotechnol* 20(3):315–318
- Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X, Fauquet CM (2008) Recommendations for the classification and nomenclature of the DNA- β satellites of begomoviruses. *Arch Virol* 153:763–781
- Chakraborty S, Pandey PK, Banerjee MK, Kalloo G, Fauquet CM (2003) Tomato leaf curl Gujarat virus, a new begomovirus species causing a severe leaf curl disease of tomato in Varanasi India. *Phytopathology* 93(12):1485–1495
- Datta S, Budhaujiya R, Das B, Chatterjee S, Bora A, Vairale MG, Gogoi HK, Veer V (2015) First report of tomato leaf curl Bangladesh virus (ToLCBV) infecting *Gomphostemma niveum* plants in Assam, India. *New Dis Rep* 31:19. <https://doi.org/10.5197/j.2044-0588.2015.031.019>
- Khan ZA, Khan JA (2016) Characterization of a new begomovirus and betasatellite associated with chilli leaf curl disease in India. *Arch Virol* 162:561. <https://doi.org/10.1007/s00705-016-3096-0>
- Mubin M, Akhtar S, Amin I, Briddon RW, Mansoor S (2012) *Xanthium strumarium*: a weed host of components of begomovirus-betasatellite complexes affecting crops. *Virus Genes* 44:112–119
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8:4321–4325
- Raj SK, Snehi SK, Khan MS, Singh R, Khan AA (2008) Molecular evidence for association of tomato leaf curl New Delhi virus with leaf curl disease of papaya (*Carica papaya* L.) in India. *Australas Plant Dis Notes* 3:152–155
- Ranjan P, Kumar RV, Chakraborty S (2013) Differential pathogenicity among tomato leaf curl Gujarat virus isolates from India. *Virus Genes* (2013) 47:524–531. <https://doi.org/10.1007/s11262-013-0977-0>
- Rojas MR, Gilbertson RL, Russell DR, Maxwell DP (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted geminiviruses. *Plant Dis* 77:340–347
- Saxena S, Hallan V, Singh BP, Sane PV (1998a) Leaf curl disease of *Carica papaya* from India may be caused by a bipartite geminivirus. *Plant Dis* 82(1):126
- Saxena S, Hallan V, Singh BP, Sane PV (1998b) Evidence from nucleic acid hybridization tests for a geminivirus infection causing leaf curl disease of papaya in India. *Indian J Exp Biol* 36(2):229–232
- Shahid MS, Yoshida S, Khatri-Chhetri GB, Briddon RW, Natsuaki KT (2013) Complete nucleotide sequence of a monopartite Begomovirus and associated satellites infecting *Carica papaya* in Nepal. *Virus Genes* 46:581–584
- Singh-Pant P, Pant P, Mukharjee SK, Mazumdar-Leighton S (2012) Spatial and temporal diversity of begomoviral complexes in papayas with leaf curl disease. *Arch Virol* (2012) 157:1217–1232. <https://doi.org/10.1007/s00705-012-1287-x>
- Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Varun P, Saxena S (2017a) Leaf curl disease of *Carica papaya*. Begomoviruses: occurrence and management in Asia and Africa. Springer, New York. https://doi.org/10.1007/978-981-10-5984-1_547
- Varun P, Saxena S (2017b) Transmission of Begomoviruses. Begomoviruses: occurrence and management in Asia and Africa. Springer, New York. https://doi.org/10.1007/978-981-10-5984-1_544
- Varun P, Ranade SA, Saxena S (2017) A molecular insight into papaya leaf curl—a severe viral disease. *Protoplasma*. <https://doi.org/10.1007/s00709-017-1126-8>
- Wyatt SD, Brown JK (1996) Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology* 86:1288–1293
- Zaidi SSA, Iqbal Z, Amin I, Mansoor S (2015) First report of tomato leaf curl Gujarat virus, a bipartite Begomovirus on cotton showing leaf curl symptoms in Pakistan. *Plant Dis* 99(11):1655
- Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E et al (2017) ICTV virus taxonomy profile: Geminiviridae. *J Gen Virol* 98:131–133