Journal of Plant Pathology (2019) 101:307–314
https://doi.org/10.1007/s42161-018-0182-z

ORIGINAL ARTICLE

Characterization of leaf curl virus in chili and overwintering role of nightshade in linkage between chili and tomato

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Published online: 29 October 2018

Abstract

Diseases caused by begomoviruses are an emerging threat to many crops in tropical and sub-tropical regions of the world. Leaf curl of chili is one of the most destructive disease induced by begomoviruses causing substantial losses. Leaf curling, puckering and stunted growth of the plants are typical symptoms of leaf curl disease in chili and also in many other plants like tomato and nightshade (*Solanum nigrum*). Rolling circle amplification (RCA) was used to characterize the genome of the virus causing leaf curl disease in chili at Sabour, in northern state of Bihar, India. RCA product digested with *Bam*HI and *Hin*dII released ca. 2.7 kb DNA fragments. The causal virus of chili leaf curl disease at Sabour was found to have a monopartite genome consisting of 2742 nucleotides (nt) with genome organization similar to begomoviruses, having two ORFs in virion-sense and six ORFs in complementary sense, separated by an intergenic region. The complete genomic sequence (GenBank accession No. KY010624) showed highest nucleotide identity of 98% with tomato leaf curl Joydebpur virus (tomato isolate). Hence the virus isolate under study has been named as tomato leaf curl Joydebpur virus-Sabour. An associated betasatellite DNA was 1370 nt long with a single ORF and had 99% identity with tomato leaf curl Joydebpur betasatellite. Abutting primers successfully amplified the full genome of tomato leaf curl Joydebpur virus-Sabour confirming its presence in tomato, nightshade and whitefly. Based on the findings, it is hypothesized that nightshade acts as a reservoir of tomato leaf curl Joydebpur virus-Sabour and is involved in spreading the virus from chili to tomato through whitefly (*Bemisia tabaci*).

Keywords Begomovirus · Betasatellite · Chili · Leaf curl and whitefly

Introduction

The genus *Begomovirus* of the family *Geminiviridae* is the largest genus of whitefly (*Bemisia tabaci* Gennadius) transmitted plant viruses with more than 300 species (Zerbini et al. 2017). Some of the begomoviruses are responsible for many devastating diseases in different economically important crops throughout the world mostly in the tropical and subtropical regions infecting dicot plants including tomato, chili, pepper, cassava, beans, cotton and cucurbits (Singh et al. 2012; Kumari et al. 2010; Inoue-Nagata et al. 2016). The begomoviruses are assumed to have co-evolved with their hosts for a long period of time; however, these viruses appear to have become more interactive with economically important crops (Borah and Dasgupta 2012; George et al. 2014; Khan and Khan 2017). On the basis of genomic organization, three types of begomoviruses are recognized viz, Type I: with bipartite genome, Type II: with monopartite genome, and Type III: with monopartite genome and associated satellite DNA component (Malathi and John 2008; Fauquet et al. 2008; Pandey et al. 2010). Monopartite DNA is known to code for the replication associated protein (Rep) that is essential for viral replication, replication enhancer protein (REn), transactivator protein (TrAP) which controls the late gene expression and linked to

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RNAi suppression and the coat protein (CP) for encapsidation and whitefly transmission (Sunter et al. 1990). However, in bipartite viruses DNA-B encodes the nuclear shuttle protein (NSP) and the movement protein (MP), which play a vital role in intra- and inter- cellular movement of the virus in the host plant (Lazarowitz and Shephard 1992). Both DNA A and DNA B are essential for initiating infection by a bipartite begomovirus (Hamilton et al. 1983). The genome of monopartite begomoviruses consists of only one component functionally equivalent to DNA A and DNA B and homologous to DNA A of bipartite begomoviruses and alone causes disease in host plant (Bisaro 1994). The third type of begomoviruses known to occur in Old World have a monopartite genome but require association of a betasatellite DNA for induction of typical disease symptoms (Briddon et al. 2001). Betasatellites are approximately half the size of the helper virus genome, unrelated in sequence to their helper viruses and dependent on them for replication, movement in plants and insect transmission. Betasatellites are known to have a highly conserved organization consisting of an adenine-rich region, a region that is conserved among all betasatellites [known as the satellite conserved region (SCR)] and a single open reading frame (ORF) in the complementary strand that codes for the βC1 protein (Briddon et al. 2003, 2008). A potential hairpin structure with the loop sequence TAA/GTATTAC, similar to that of the origin of replication of geminiviruses is present in SCR (Briddon et al. 2003). Betasatellites have been shown to augment the accumulation of their helper begomoviruses and accentuate the symptoms induced in host plants (Patil and Fauquet 2010). This has been attributed to the silencing suppressor activity of the βC1protein (Cui et al. 2004).

Begomoviruses like chili leaf curl virus, tomato leaf curl New Delhi virus, chili leaf curl Palampur virus, papaya leaf curl virus, pepper leaf curl Bangladesh virus, chili leaf curl Salem virus, and chili leaf curl Bijnour virus and an associated betasatellites such as chili leaf curl betasatellite, tomato leaf curl Bangladesh betasatellite, croton yellow vein mosaic betasatellite, tomato leaf curl Joydebpur betasatellite and tomato leaf curl Ranchi betasatellite have been reported to be involved in leaf curl disease of chili crop (Kumar et al. 2011, 2012, 2015, 2016; Senanayake et al. 2012).

Weeds are extensively distributed all over the world and have great ecological adaptability and are known to act as reservoir or alternative hosts for many economically important begomoviruses (Roye et al. 1997; Sanz et al. 2000). Many weeds belonging to the genera *Solanum*, *Datura*, *Sinapis* and *Sonchus* in Cyprus (Papayiannis 2011) and *Corchorus* in Saudi Arabia (Sohrab 2016) have been shown to harbour tomato yellow leaf curl virus. *Ageratum conyzoides*, a common weed in India, harbors mungbean yellow mosaic India virus that is known to cause yellow mosaic disease in many leguminous crops (Naimuddin et al. 2014, 2016).

Chili (*Capsicum annum*) is grown in many parts of India as both a vegetable and spice crop. A leaf curl disease of chili characterized by curling and chlorosis in leaves and overall stunted plant growth is observed in the experimental field of Bihar Agricultural University, Sabour for the last five years. The present paper describes the findings of investigations undertaken to characterize the causal virus, its vector and weed hosts.

Materials and methods

DNA isolation and PCR assay Leaf samples from chili and tomato plants showing leaf curling symptoms and from weeds, *Ageratum conyzoides* (goat weed), *Euphorbia hirta* (asthma weed), *Solanum nigrum* (nightshade), *Acalypha indica* (Indian nettle) and *Nicotiana obtusifolia* (desert tobacco) growing in and around chili fields and showing curling and mosaic symptom were collected. One sample each was also collected from healthy plants of chili, tomato, and all five weeds. Whitefly adults observed feeding on chili, tomato and weed plants named above were also collected from the Vegetable Research Farm of Bihar Agricultural University, Sabour and nearby farmers' fields. Total DNA was extracted from symptomatic leaves of chili, tomato and weeds, and whiteflies using GeneJet DNA Isolation Kit (Thermo Scientific, USA). The polymerase chain reaction (PCR) was performed using primer pairs- Deng540/Deng541 (Deng et al. 1994), PVL1v2040/PCRc1 (Rojas et al. 1993), beta01/beta02 (Briddon et al. 2002) and an abutting primer pair (ToLJV1 F: 5' GGTCGCTTCGACATAATTTC 3′/ ToLJV2 R: 5' GGTCCTAAAGACCCTTAAGA 3′) designed from conserved sequences of monopartite genome of ToLCJV-SBO (GenBank accession No. KY010624), the virus identified as the causal agent of leaf curl disease in chili in the present study. The PCR was performed in a Master Cycler (Nexus, Eppendorf, Germany) programmed with one step of preheating at 95 °C for 3 min, 30 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 49 °C for Deng540/Deng541, at 60 °C for PVL1v2040/PCRc1, at 50 °C for beta01/beta02 and at 56 °C for ToLJV1 F/ToLJV2R, and a 1 min extension at 72 °C, followed by a one step final extension at 72 °C for 10 min. The PCR tests were performed using Dream *Taq* Green Master Mix (2X) (Fermentas, USA), in total reaction mixture of 50 μl which consisted of 4 μl (50 ng/μl) DNA template, 2 μl of each primer (20 pmol), 25 μl 2X master mix and 17 μl dH2O. Amplified products were analysed in 1% agarose gel with 1X TAE buffer containing 0.1% ethidium bromide and visualized in gel documentation system (UVITECH, UK).

Amplification and cloning of full length genome of the virus and associated betasatellite Total DNA from four randomly

selected samples of chili that gave positive amplification with Deng540/Deng541 primers was processed for full length genome amplification through RCA method using REPLI-g Mini Kit (QIAGEN GmbH, USA) following the manufacturer's protocol. RCA product was digested with five restriction enzymes viz, *Bgl*I, *Dra*II, *Hin*dII, *Eco*RV and *Bam*HI to obtain the linear ~2.7 kb DNA fragments. The digested RCA product was visualized in 1% agarose gel and the ~2.7 kb linearized DNA from one of the samples was randomly selected and purified using Gel extraction kit (Thermo Scientific), cloned into pJET/1.2 blunt vector using CloneJET PCR Cloning Kit (Fermentas) and custom sequenced (1st BASE, Malaysia, Xcelris Genomics, India). Similarly, one of the betasatellite DNAs amplified by PCR using primer pair beta01/beta02 was also cloned and sequenced.

Sequencing and analysis Sequences obtained were assembled through Bioedit and subjected to BLAST and ORF finder available at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The assembled sequences of the virus isolate from chili (hereinafter referred as ToLCJV-SBO) and betasatellite molecule (hereinafter referred as ToLCJB-SBO) were submitted to NCBI database. Sequences that had maximum identity with the genome of ToLCJV-SBO and ToLCJB-SBO in BLAST search with 100% query coverage were selected for comparison and phylogenetic relationship. Pairwise percent nucleotide identity of ToLCJV-SBO and an associated betasatellite was obtained using Clustal W software available at http://www.genome.jp/tools/clustalw/ following standard parameters.

Whitefly mediated sequential transmission Whitefly interceded transmission of leaf curl causing virus from chili to nightshade, and subsequently from nightshade to tomato and chili plants was attempted (Table 3). Healthy colonies of whiteflies were raised on caged eggplant seedlings by modified method of Muniyappa et al. (2000). Non-viruliferous status of the colony was ascertained by subjecting randomly collected whiteflies to PCR tests. Healthy seedling of chili, tomato and nightshade at 3–4 leaf stage grown in protrays (BioBlooms) individually under insect proof cages were used in transmission tests. Non-viruliferous whiteflies were released on caged infected chili plant for 12 h of acquisition. After acquisition feeding, whiteflies were collected, released onto healthy caged chili, nightshade and tomato seedlings (10 whiteflies/plant) and allowed 48 h inoculation feeding. Similarly, transmission through whitefly was also attempted from infected nightshade (through whitefly transmission) to tomato and chili. Inoculation feeding was terminated by spraying of systemic insecticide (Imidacloprid 17.8 SL, Bayer Crop Science). Whitefly inoculated plants were closely monitored for 30 days for development of any symptom.

Results

Sample collection and PCR analysis In PCR tests, 12 of the 14 chili samples tested gave positive result with Deng's primers as indicated by the presence of an amplicon of ca. 530 bp. Nine out of these 12 gave positive result with primer pairs ToLJV1 F/ToLJV2 R and beta01/beta02 and yielded DNA bands of ca. 2.7 kb and 1.4 bp, respectively. The PCR products obtained with Deng's primers were sequenced directly; BLAST analysis revealed that the sequences were closely similar to ToLCJV (data not shown). Presence of geminivirus infection in samples of all the plant species tested with Deng's primer was confirmed though the percentage of samples found positive differed. Of the 12 samples of chili, 11 of tomato and 8 of nightshade that were positive with Deng's primers, respectively 9, 5 and 5 were positive with primer pair ToLJV1 F/ToLJV2 R. PCR results with beta01/beta02 indicated betasatellite DNA to be present in 9 chili and 4 each of tomato and nightshade samples (Table 1). Results of PCR assays with DNA-B specific primer (PVL1v2040/PCRc1) were negative in all the samples.

Rolling circle amplification and genome organization Of the five restriction enzymes used to release the linearized genome of the virus from the RCA products, only *Hin*dII and *Bam*HI yielded ~2.7 kb linearized DNA without any undigested high molecular weight DNA in the agarose gel, indicating that only one DNA molecule was present in all the samples. *Bgl*I, *Dra*II and *Eco*RV did not linearize the viral genome (Fig. 1). Sequence of the ca. 2.7 kb DNA fragment produced by *Bam*HI revealed that the virus isolate under this study is 2742 nucleotides (nt) long. The sequence data was submitted

Table 1 PCR assay of collected samples from different hosts

Host plant	No. of sample collected	PCR assay (positive amplification)		
		Deng 540F/540R	ToLJV1 F/ ToLJV2 R	beta01/ beta02
Goatweed (*Ageratum conyzoides*)	12	07	00	00
Nightshade (*Solanum nigrum*)	13	08	05	04
Asthma plant (*Euphorbia hirta*)	12	02	00	00
Indian Acalypha (*Acalypha indica*)	15	07	00	00
Desert tobacco (*Nicotiana obtusifolia*)	14	02	00	00
Tomato (*Solanum lycopersicum*)	16	11	05	04
Chili (*Capsicum annuum*)	14	12	09	09

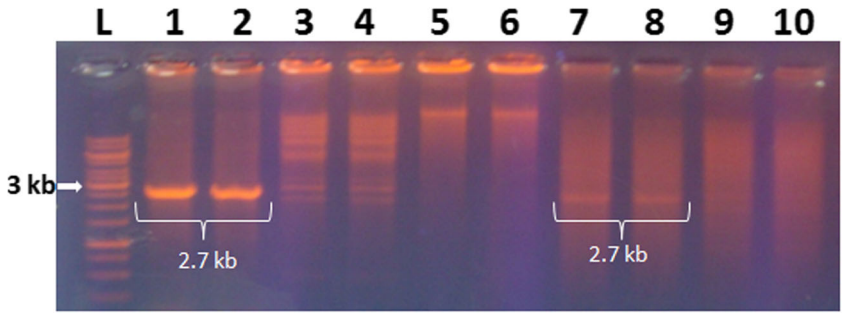

Fig. 1 RCA products digested with restriction enzymes (L: DNA ladder; lanes 1–2, *Bam*HI; lanes 3–4, *Dra*II; lanes 5–6, *Eco*RV; lanes 7–8, *Hin*dII; lanes 9–10, *Bgl*I)

to NCBI data base under the accession No. KY010624. Results of BLAST and ORF finder analyses showed that the chili virus isolate (KY010624) had a genome organization typical of geminiviruses and consists of eight ORFs, two (AV1 and AV2) in virion sense and six (AC1, AC2, AC3, AC4, AC5 and AC6) in the complementary sense separated by an intergenic region (IR) and a putative stem loop structure having a conserved nonanucleotide sequence.

Comparison of genomic sequence with other leaf curl viruses

The present virus isolate (KY010624) had nt identity ranging between 94 and 98% with isolates of tomato leaf curl Joydebpur virus, with the highest identity being with isolate HJP09 (JQ654463; 98% identity). Therefore the present virus isolate has been named as tomato leaf curl Joydebpur virus-SBO. ToLCJV-SBO has the genomic organization identical to ToLCJV- HJP09 (JQ654463) as both these isolates have the

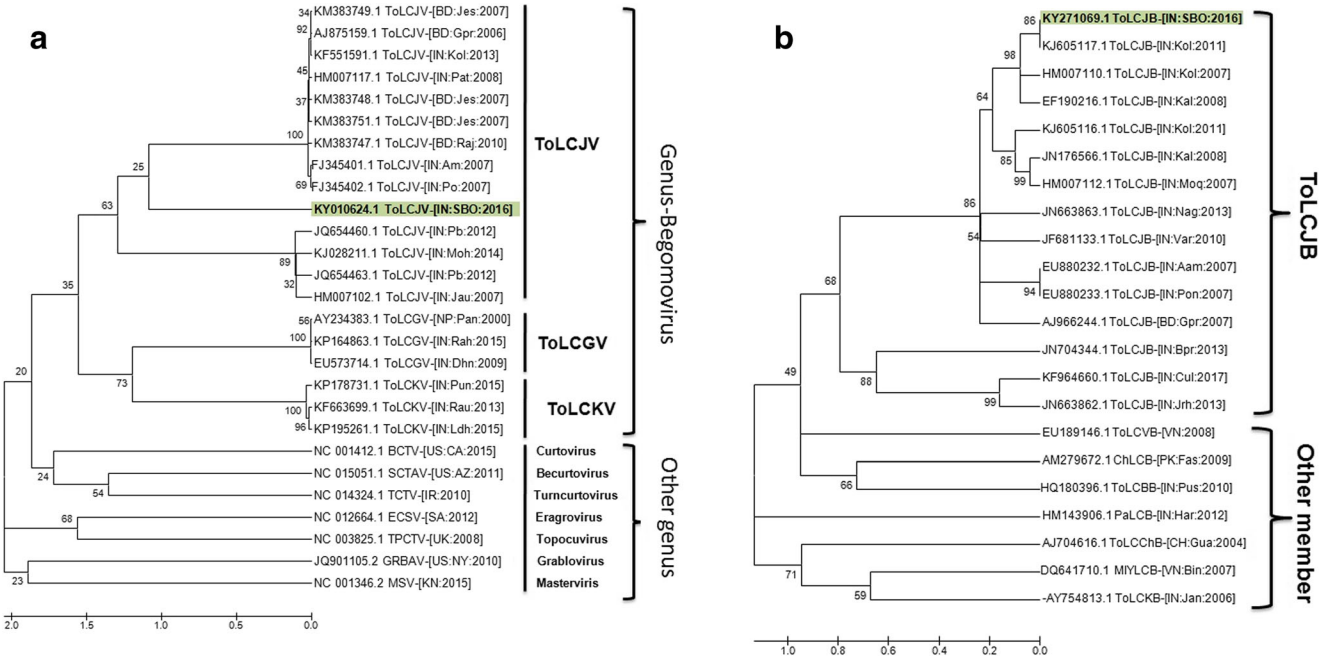

Fig. 2 **a** Phylogenetic dendogram depicting the relationship of tomato leaf curl Joydebpur virus (ToLCJV-SBO, KY010624, this study) with other isolates of tomato leaf curl virus and representatives of different genera of the family *Geminiviridae* using the Neighbor-Joining method in MEGA 5. The bootstrap accorded with the tree inferred from 1000 replicates. Virus identity are indicated as ToLCJV-tomato leaf curl Joydebpur virus [IN-India: Pat-Patna, Po-Ponduru, Pb-Punjab, Moh-Mohali, Jau-Jaunpur, SBO-Sabour, Kol-Kolkata, Am-Amadalavalasa], [BD-Bangladesh: Jes-Jessore, Raj-Rajshahi, Gpr-Ghajipur]; ToLCGV-tomato leaf curl Gujrat virus [Rah-Rahuri, Dhn-Dhanbad], [NP-Nepal: Pan-Panchkhal]; ToLCKV-tomato leaf curl Karnatka virus [Pun-Pune, Rau-Rauke, Ldh-Ludhiana]; BCTV-[US:CA] beet curly top virus, United States: California; SCTAV-[US:AZ] spinach curly top Arizona virus, United States: Arizona; TCTV-[IR] turnip curly top virus, Iran; ECSV-[SA] Eragrostis curvula streak virus, South Africa; TPCTV-[UK] tomato pseudo-curly top virus, United Kingdom; GRBAV-[US:NY] grapevine red-blotch associated virus, United States: New York; MSV-[KN] maize streak virus, Kenya. **b** Dendogram showing the relationship of an associated betasatellite with ToLCJV-SBO (ToLCJB, KY271069, this study) and other betasatellites of begomoviruses including ToLCJB-tomato leaf curl Joydebpur betasatellites [IN-India: Kol-Kolkata, Kal-Kalyani, Mog-Mograhat, Nag-Nagpur, Var-Varanasi, Asm-Amadalavalasa, Pon-Ponduru, Bpr-Baruipur, Cul-Calcutta, Jrh-Jorhat]; ToLCVB-[VN] tomato leaf curl Vietnam betasatellite, Vietnam; ChLCB-[PK:Fas] chili leaf curl betasatellite, Pakistan:Faislabad; ToLCBB-[IN:Pus] tomato leaf curl Bangladesh betasatellite, India:Pusa; PaLCB-[IN:Har] papaya leaf curl betasatellite, India: Haryana; ToLCChB-[CH:Gua] tomato leaf curl China betasatellite, China:Guangxi; MIYLCB-[VN:Bin] mimosa yellow leaf curl betasatellite, Vietnam: Binhduong; ToLCKB-[IN:Jan] tomato leaf curl Karnataka betasatellite, India:Janti

Table 2 Comparison of complete genome of ToLCJV-SBO, and its ORFs with closely related viruses

Virus	Accession	DNA-A	AV1	AV2	AC1	AC2	AC3	AC4	AC5	AC6
ToLCJV-IN:SBO	**KY010624**	**100/2762**	**[a]100/771** **[b]100/256**	**100/357** **100/118**	**100/1086** **100/361**	**100/405** **100/134**	**100/405** **100/134**	**100/294** **100/97**	**100/291** **100/96**	**100/843** **100/280**
ToLCJV-IN: PB	JQ654463	98/2768	96/771 92/256	91/303 84/100	99/1086 100/361	99/405 97/134	99/405 99/134	99/294 98/97	90/291 83/96	96/843 91/280
ToLCJV-BA: JP	KM383747	94/2760	92/771 91/256	89/357 85/118	96/1086 98/361	96/405 93/134	96/405 95/134	99/294 97/97	87/291 81/96	–
ToLCJV-IN: WB	EU431116	94/2761	91/771 91/256	88/366 81/121	96/1086 95/361	97/405 94/134	97/405 95/134	98/294 95/97	–	–
ToLCJV-IN: AP	FJ345402	94/2761	91/771 91/256	88/366 80/121	96/1086 95/361	97/405 94/134	97/405 95/134	98/294 94/97	–	–
ToLCJV-IN: PT	HM007117	94/2761	93/771 91/256	89/357 85/118	95/1086 96/361	97/405 91/134	97/405 95/134	98/294 98/97	–	–
ToLCJV-IN: KL	KF551591	94/2761	93/771 91/256	90/357 85/118	94/1086 96/361	97/405 93/134	97/405 97/134	98/294 96/97	–	–

Bold entries has been made in order to reflect the present virus isolate and their ORFs

[a] Percent nucleotide identity/ total number of nucleotide

[b] Percent amino acid identity/ total number of amino acid

ORF AC6 in addition to the AV1, AV2, AC1, AC2, AC3, AC4 and AC5. Further, all the ORFs of ToLCJV-SBO had maximum nt identity with the corresponding ORFs of ToLCJV-HJP09 (JQ654463). AV1 gene of ToLCJV-SBO was 771 nt long similarly to all the isolates of ToLCJV used for comparison study. AC5 gene was 291 nt long and had 87–90% nt identity with the corresponding genes of isolates HJP09 (JQ654463) and BD (KM383747). Interestingly, AC5 was not present in other isolates of ToLCJV. An unique feature, AC6 gene was also present in ToLCJV-SBO which is otherwise reported to be present only in ToLCJV-HJP09 (JQ654463) isolated from Punjab, India which is named as ToLCJV-IN:PB and had 96% nt identity among them (Table 2). Presence of a ca.1.4 kb band indicated the association of betasatellite with infected chili plant. The full length genome consisted of 1370 nt and the sequence was submitted at NCBI database (KY271069). It had all the structural features which are present in betasatellites, such as a single ORF (bC1), nonanucleotide sequence TAATATTAC, satellite conserved region (SCR), and adenine rich (A-rich) region (Mansoor et al. 2003). The complete nt sequence of betasatellite (KY271069) shared highest identity (96%) with tomato leaf curl Joydebpur betasatellite, ToLCJB (KJ605116). Its only ORF i.e. bC1 was 381 nt long and encoded a 126 amino acid product. The amino acid sequence of bC1 also had highest identity (99%) with ToLCJB KJ605116 (tomato isolate). The betasatellite found associated with ToLCJV-SBO has therefore been named as ToLCJB-SBO. The phylogenetic analysis grouped ToLCJV isolates reported from India, Bangladesh with other members of the family *Geminiviridae*; the monopartite DNA of ToLCJV-SBO and some other begomoviruses clustered with isolates of ToLCJV (Fig. 2a). The betasatellite sequence of the Sabour

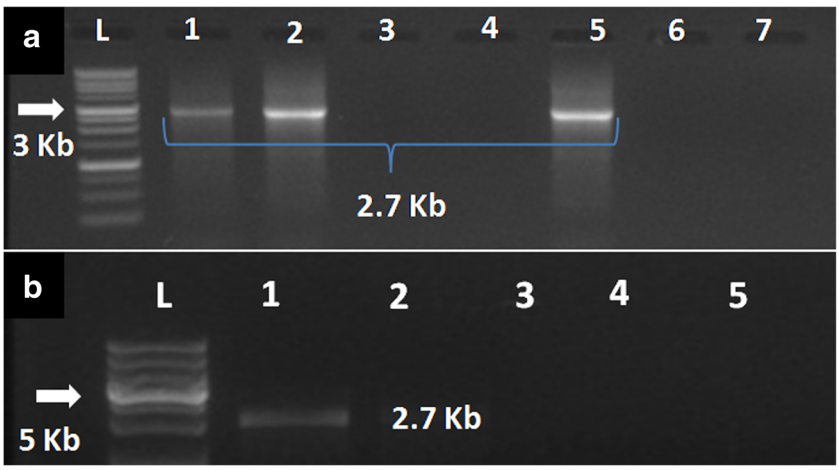

Fig. 3 Agarose gel electrophoresis of PCR products amplified from the genome of tomato leaf curl Joydebpur virus using abutting primers (ToLJV1 F/ ToLJV2 R). **(a)** L, DNA ladder; lane 1, chili- control; lane 2: tomato; lane 3, *Ageratum conyzoides*; lane 4: *Euphorbia hirta;* lane 5, *Solanum nigrum*; lane 6, *Acalypha indica*; lane 7, *Nicotiana obtusifolia*. **(b)** PCR amplified products of whiteflies collected from different weeds. L: ladder; lane 1, *Solanum nigrum*; lane 2, *Ageratum conyzoides*; lane 3, *Euphorbia hirta*; lane 4, *Acalypha indica*; lane 5, *Nicotiana obtusifolia*

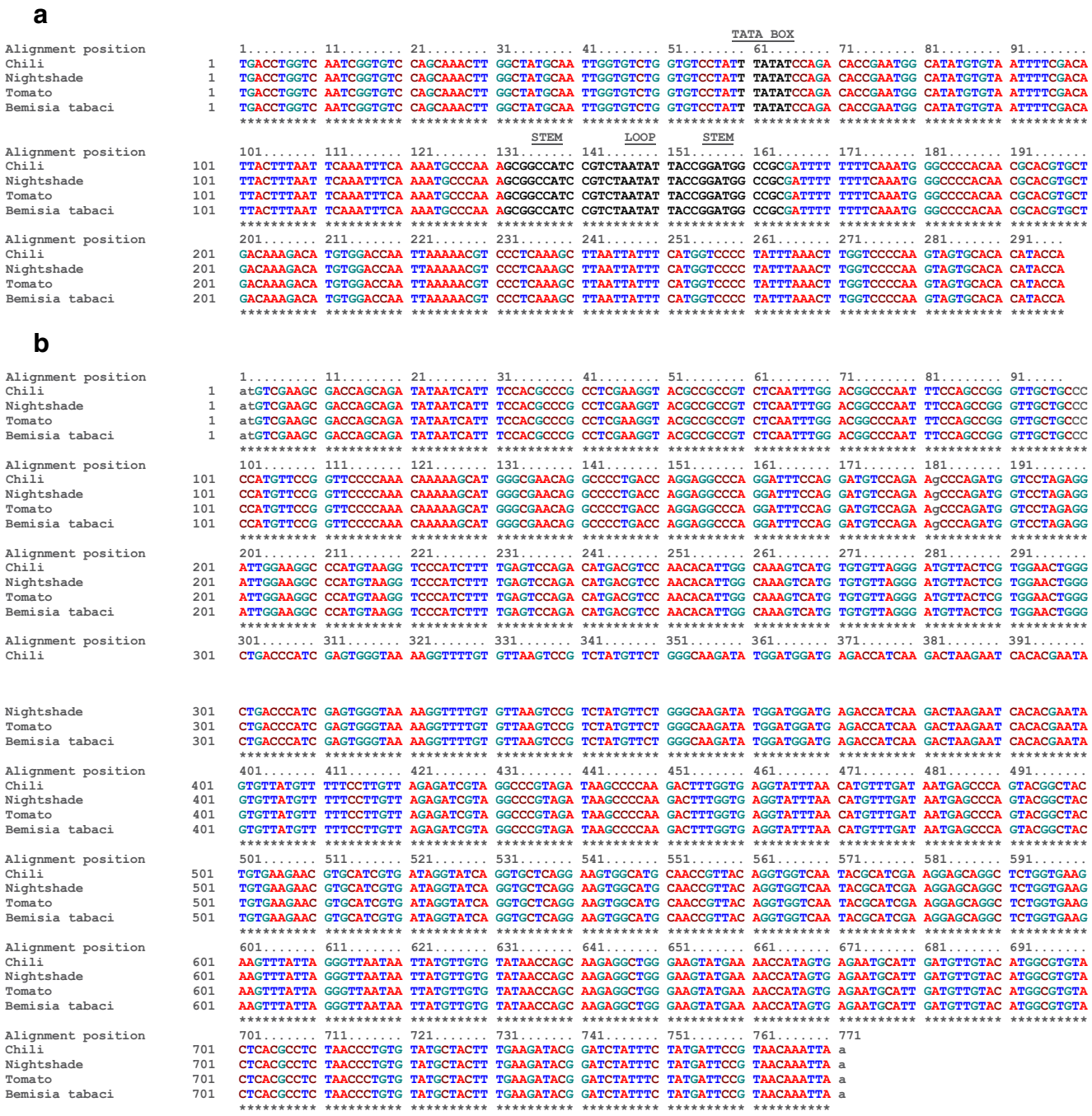

Fig. 4 Multiple sequence alignment of IR region (**a**) and AV1 gene (**b**) from PCR amplified fragments from ToLCJV-infected chili, nightshade, tomato and viruliferous whiteflies

isolate is closely related to ToLCJB sequences reported from India which formed a major clade (Fig. 2b).

PCR analysis of weeds, tomato and whiteflies The PCR reactions were successful in amplification of a 2.7 kb DNA fragment by using abutting primers. Positive amplification was found in symptomatic leaves of tomato and nightshade, whereas no amplification was obtained from *Ageratum conyzoides*, *Euphorbia hirta*, *Acalypha indica* and *Nicotiana obtusifolia* (Fig. 3a). Moreover, no amplification was detected in whiteflies collected from weeds other than nightshade (Fig. 3b).

Sequence analysis of IR region and AV1 gene AV1 gene and IR region were selected from full sequences of 2.7 kb genome and aligned from ToLCJV infected chili. Analyses of AV1 gene and IR region were undertaken from ToLCJV-infected chili, nightshade, tomato and *Bemisia tabaci*. The conserved

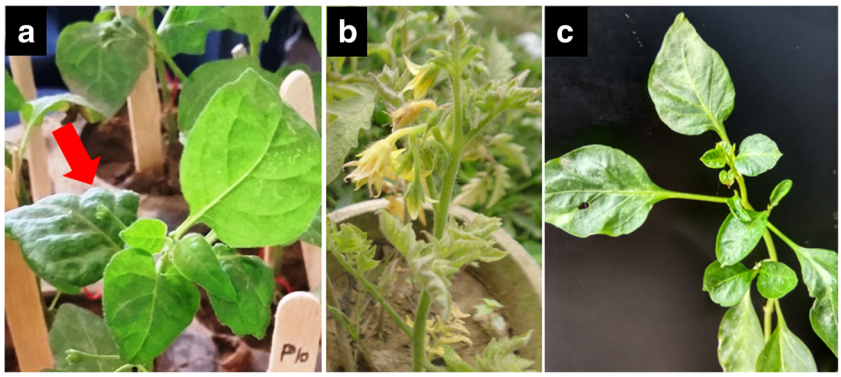

Fig. 5 Symptomatic appearance of experimentally whitefly-transmitted tomato leaf curl Joydebpur virus. **a**: nightshade; **b**: tomato and **c**: chili

nonanucleotide in the hairpin-loop, TAATAT TAC that is characteristic of the begomovirus of family *Geminiviridae* and TATA box, were identified in the IR sequences of all chili, nightshade, tomato and whitefly samples (Fig. 4a). The IR region was 297 nt long and identical in all infected samples. Similarly, AV1 gene sequence (771 bp) of chili isolate was compared with the nightshade, tomato and *Bemisia tabaci* samples which were also found identical and absolutely conserved (Fig. 4b).

Whitefly mediated transmission Under natural conditions, nightshade and tomato were found to be infected with ToLCJV. (Fig. 5). Results of transmission test through whitefly indicated that ToLCJV was transmitted from chili to nightshade and from nightshade to tomato. Transmission from tomato to chili was also successful (Table 3) producing typical leaf curl symptoms in more than 50 % of the plants tested.

Discussion

In Indian subcontinent leaf curl disease of chili is widely distributed and causes severe losses (Senanayake et al. 2007; Zehra et al. 2017; Khan et al. 2006). A number of whitefly transmitted geminiviruses are reported to cause leaf curl diseases in chili and other solanaceous vegetables (Kumar et al. 2008; Singh et al. 2011; Laufs et al. 1995; Lazarowitz and Shephard 1992). Association of betasatellites in all mono, mono-bi and bipartite begomoviruses causing leaf curl in tomato has been well documented (Chakraborty et al. 2003). A report suggests that tomato-infecting begomoviruses maintain diverse betasatellites through a host-driven process (Ranjan et al. 2014). The possible role of cross infection of different begomoviruses and their betasatellites is an interesting area of investigation. In the present study, an isolate of monopartite ToLCJV and an associated betasatellite DNA were found to be associated with leaf curl disease of chili at Sabour, in the north-east plain zone of Indian state of Bihar. In India, ToLCJV is known to cause leaf curl disease in chili and tomato (Shih et al. 2007; Tiwari et al. 2013). However, the present study not only confirmed the cause of leaf curl disease of chili and tomato in the region but also found the weed nightshade to be a natural host of ToLCJV-SBO. Positive results of transmission of ToLCJV-SBO through whitefly from nightshade to chili, nightshade to tomato and tomato to chili make it easy to explain the possible recurrence and disease cycle of the virus (ToLCJV-SBO) in the region. Removal of nightshade weed can therefore be a part of integrated management of leaf curl disease of chili and tomato in this area.

Table 3 Transmission series of tomato leaf curl Joydebpur virus by whiteflies in different hosts

Host plant	No. of infected/No. of inoculated plants			Mean transmission	Symptom appearance Day after inoculation
	Exp Set-1	Exp Set-2	Exp Set-3		
Chili → Nightshade	8/11	7/9	6/12	66.8[a]	14-19
Nightshade → Tomato	9/13	6/8	4/11	60.1[a]	38-47
Tomato → Chili	7/12	6/11	5/9	56.1[a]	21-27

[a] Means followed by the same letter are not significantly different

Acknowledgements The present work is supported by Science and Engineering Research Board, Department of Science and Technology, Government of India, Young Scientist Scheme-YSS/2015/000923.

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