First report of a begomovirus and associated betasatellite in *Rosa indica* and in India

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Abstract The complete genome sequences of a begomovirus and associated betasatellite was obtained by the rolling circle amplification from rose (*Rosa indica*) exhibiting dwarfing, leaf distortion and leaf curling symptoms from Rajasthan, India. The sequence of the begomovirus was determined to be 2,741 bp and showed the typical genome arrangement of an monopartite Old World begomovirus. The sequence showed the highest nucleotide sequence identity (98 %) to an isolate of *Rose leaf curl virus* (RoLCuV), confirming it as an isolate of RoLCuV. The sequence of the rose begomovirus associated betasatellite (1,349 bp) displayed greater than 97 % nucleotide sequence identity to *Digera* leaf curl betasatellite. Our analysis also demonstrated that recombination events shaped the evolutionary history of RoLCuV. To our knowledge, this is the first report of RoLCuV associated with betasatellite infecting rose in India.

Keywords Geminivirus · Begomovirus · Betasatellite · *Rosa indica*

Anurag Kumar Sahu and Avinash Marwal are equal contribution

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Geminiviruses are an emerging group of plant viruses infecting most of the economically important crops and ornamental plants throughout the world (Nawaz-ul-Rehman et al. 2009). Based on the genome organization, sequence phylogeny, host range and the vector, the geminiviruses are classified into seven genera: *Becurtovirus*, *Eragrovirus*, *Turncurtovirus*, *Topocuvirus*, *Curtovirus*, *Mastrevirus* and *Begomovirus* (Brown et al. 2012). The majority of members of this family belong to the genus *Begomovirus* and viruses of this genus are transmitted by the whitefly (*Bemisia tabaci*) (Briddon et al. 2001). The viruses of this genus are further divided into two sub-groups, bipartite begomoviruses (with genomes consisting of two components known as DNA-A and DNA-B) and monopartite begomoviruses (with genomes consisting of a single component homologous to the DNA-A component of the bipartite viruses). The discovery of betasatellite (ssDNA satellites previously known as DNA β) associated with monopartite members of the genus have increased in the last decades worldwide (Briddon et al. 2008). Betasatellites are half the size of their helper viruses (approximately 1,350 nucleotides in length) with a highly conserved structure gene encoded in the complementary-sense known as β C1. The satellite region also contains a hairpin-loop structure TAA/GTATTAC similar to the origin of replication of geminiviruses, and an Adenine rich region (A-rich) (Briddon et al. 2003).

Geminiviruses are an emerging group of plant-infecting viruses in the tropical and subtropical region of the world. Their emergence is thought to be caused by many factors including, but not limited to, the high insect vector populations, the existence of alternative hosts and response to changes in their environment (such as alterations in cropping systems and/or population dynamics of insect vectors). Previously, diverse begomoviruses isolated from different geographical locations were reported to have the capacity to interact with betasatellites (Briddon et al. 2003; Saunders et al.

Fig. 1 Symptoms of RoLCuV on rose (*R. indica*)

2008). Recently, Marwal et al. (2012) have reported a high rate of recombination and pseudo-recombination events that contribute in the evolution of new viral species in begomoviruses from ornamental plants in India.

Rose (*R. indica*) is a woody perennial plant that is cultivated mostly in gardens throughout India. In a survey for begomoviruses, leaves of twenty rose plants exhibiting dwarfing, leaf distortion and leaf curling typical of begomovirus symptoms with 50 to 80 % disease incidence were collected from 10 gardens around the Shekhawati region of Rajasthan, India in 2012 (Fig. 1). Total DNA was extracted from the collected symptomatic and two apparently healthy leaves by using a kit (viral DNA from serum/plants,

Chromous Biotech Pvt. Ltd., India) as per the manufacturer's instructions. Extracted DNA was used as a template for the rolling circle amplification (RCA) (Haible et al. 2006), yielding high molecular weight concatemers (data not shown). Digestion of RCA with *Pst*I, *Bam* HI, *Xho*I and *Eco*RI resulted in ~2.7 and ~1.3 kb fragments, respectively from infected plants but not from healthy plants. The restriction fragments of RCA products were cloned into pBluescript SK + vector (Stratagene, La Jolla, USA). All the clones obtained in each case showed the same restriction pattern and therefore only one clone for each RCA restriction product (pRLV10 for begomovirus and pRLVβ for betasatellite) was selected and sequenced completely (Xcelris Genomics, Ahmedabad, India). The attempts to detect alphasatellite and/or DNA-B either by PCR (by using universal primers for both alphasatellite and DNA-B components) or RCA were unsuccessful (Rojas et al. 1993; Briddon et al. 2002).

The complete nucleotide sequence of the begomovirus (pRLV10) isolated from rose was determined to be 2,741 bp (GenBank acc. KF584008). Analysis of the sequence of pRLV10 showed the virus to be a typical Old World begomovirus with two ORFs (V1-V2) in the virion-sense and four ORFs (C1-C4) in the complementary-sense and a predicted stem loop structure which is the origin of replication of the virion-sense strand (Rojas et al. 2005). The sequence comparison showed pRLV10 to be highly identical to *Rose leaf curl virus* (RoLCuV; GQ478342) reported from Pakistan in rose (Khatri et al. 2014) (97 % nucleotide sequence

Fig. 2 Phylogenetic dendrograms based on alignments of selected begomovirus (a) and betasatellite (b) sequences. Begomovirus sequences used for comparison are mentioned in the respective trees. Vertical branches are arbitrary, horizontal branches are proportional to calculated

mutation distances. Values at nodes indicate percentage bootstrap values (1,000 replicates). The isolates that were collected as part of this study are highlighted

Table 1 Whitefly transmission and host range study of RoLCuV

Test plant	Common name	Symptoms	PCR result*
<i>Raphanus sativus</i>	Radish	Leaf curling, yellowing	+
<i>Solanum lycopersicum</i>	Tomato	Leaf curling, yellow mosaic	+
<i>Carica papaya</i>	Papaya	Leaf curling, leaf stunting	+
<i>Capsicum annuum</i>	Chili	Leaf curling	+
<i>Abelmoschus esculentus</i>	Bhendi	Leaf curling	+
<i>Rosa indica</i>	Rose	Leaf curling	+
<i>Gossypium arboreum</i>	Cotton	Yellow mosaic stunting	+
	Health Control	—	—

*PCR was done by using universal coat protein (AC 1048 and AV 494; Marwal et al. 2012) and betasatellites primer (beta01 and beta02; Briddon et al. 2002)

identity). Further analysis confirmed that ORFs of the pRLCV10 displayed greater than 98 % nucleotide sequence identity with RoLCuV (GQ478342) confirming its identity as an isolate of RoLCuV. In the phylogenetic tree based on the genome monopartite, begomovirus pRLV10 grouped with RoLCuV (Fig. 2a). Based on the presently applicable species demarcation threshold of 89 % for begomoviruses (Fauquet et al. 2008), we conclude that the virus isolated from *R. indica* is an isolate of RoLCuV for which we propose the isolate descriptor RoLCuV-Sikar.

The complete nucleotide sequence of the associated betasatellite (pRLV β) was also determined to be 1,349 bp (GenBank acc. KF584009). The sequence of pRLV β shows the typical arrangement of earlier betasatellites, with a single ORF in the complementary sense (β C1; coordinates 513–187), a region rich in adenine residues (coordinates 719–1013), and a sequence motif highly conserved between all betasatellites, known as the satellite conserved region (SCR; coordinates 1265–14). The complete genome of pRLV β shown here has the highest levels of sequence identity (97.4 %) to a *Digera* leaf curl betasatellite (DaLCB; GQ478344). The predicted sequence of the β C1 of pRLV β displays the highest levels of amino acid sequence identity (97 %) to the β C1 of DaLCB. In phylogenetic analysis it grouped with DaLCB to be most closely related to an isolate identified in Faisalabad, Punjab, Pakistan, which is situated close to the border with India (Fig. 2b). These findings indicate that the betasatellite characterised here is an isolate of DaLCB for which the isolate descriptor DaLCB-Sikar is proposed. Previously, isolates of DaLCB have been identified in a *Digera arvensis* plant with yellow vein symptoms infected with *Cotton leaf curl Rajasthan virus* [FM202328] and in rose plant with *Rose leaf curl virus* [GQ478342].

To determine the transmission and host range of the begomovirus infection, the viruliferous whiteflies were inoculated on healthy rose and other plants as described in Table 1. Non-viruliferous whiteflies were allowed an acquisition access period (AAP) of 24 h on a symptomatic rose plant. The whiteflies were then transferred to the uninfected test plant and remained there for the 24 h inoculation access. The

whiteflies were then eliminated by treating plants with imidacloprid and symptoms on inoculated plants were monitored after 28 days (dpi). The experiments were conducted in the insect-proof cage maintained at 27–30 °C. The presence of virus in symptomatic plants was further confirmed through PCR by using universal coat protein (AC 1048 and AV 494; Wyatt and Brown 1996) and betasatellites primer (beta01 and beta02; Briddon et al. 2002) (Data not shown). Our results showed that the virus was transmitted by whiteflies in a persistent manner and caused systemic leaf curling and stunting on host plants (Table 1).

Recombination analysis for both pRLV10 and pRLV β was also done by RDP (Martin and Rybicki 2000). The analysis for pRLV10 shows that there are three recombination fragments (coordinates 216–1448, 1777–1796 and 2188–2589). The recombination in the first fragments was detected in VI, C2 and C3 regions with major parent *Eclipta yellow vein virus* (GQ478343) and minor parent *Pedilanthus leaf curl virus* (AM712436). The second recombination fragment (C1 region) showed the major parent *Radish leaf curl virus* (EF175733) and minor parent *Tomato leaf curl Pakistan virus* (FM164938) while the third recombination fragment (C4 region) showed major parent *Ageratum yellow vein virus* (JN809821) and minor parent *Euphorbia leaf curl virus* (KC852148) (Table 2). Similarly RDP analysis of betasatellite showed the five recombination fragments (Table 2).

Leaf curl disease of rose is a relatively recent phenomenon. This is the first time RoLCuV and associated betasatellite have

Table 2 Recombination analysis of DNA-A and betasatellite of RoLCuV by using Recombination detection program (RDP)

Recombination fragment and Coordinates	Major parents	Minor parents	p-value
DNA-A (KF584008)			
216–1448	GQ478343	AM712436	8.283×10^{-18}
2188–2589	JN809821	KC852148	4.408×10^{-03}
Betasatellites(KF584009)			
990–1243	EF175734	DQ020491	9.670×10^{-02}

been identified in India. RoLCuV has so far only been identified from *R. chinensis* (Chinese rose) in Faisalabad, Pakistan (Khatri et al. 2014). Further studies to determine the diversity of this virus and associated betasatellite from this country are needed.

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