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Associations of papaya leaf curl virus and betasatellite with yellow vein disease of pot marigold (*Calendula officinalis* L.)

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ABSTRACT

The calendula plants showing the yellow vein disease symptoms were collected from Madikeri dist. of Karnataka state, India. Total nucleic acid isolated from the infected Calendula plant leaf samples were subjected to PCR using begomovirus specific primer. The PCR diagnostic and whole genome sequencing indicated that the symptomatic calendula plants are associated with Papaya leaf curl virus (PaLCuV). The viral complete genome causing yellow vein disease had showed maximum nucleotide (nt) identity of 91.5–94.9% with begomovirus (PaLCuV) strains previously identified begomovirus from India infecting *Croton bonplandianum*, *Acalypha* sp. and chilli. Regarding to begomoviruses criteria for strain demarcation (91% nucleotide similarity), the virus infecting calendula in this report is considered as a strain of PaLCuV.

The betasatellite showed maximum nucleotide identity of 89.8% with croton yellow vein mosaic betasatellite (CroYVMB) infecting okra and *Croton*. According to our knowledge, this is the first report of PaLCuV infecting the Calendula, in India. The result also indicated that calendula plants infected with PaLCuV may act as an alternate host for other economically important plant pathogens.

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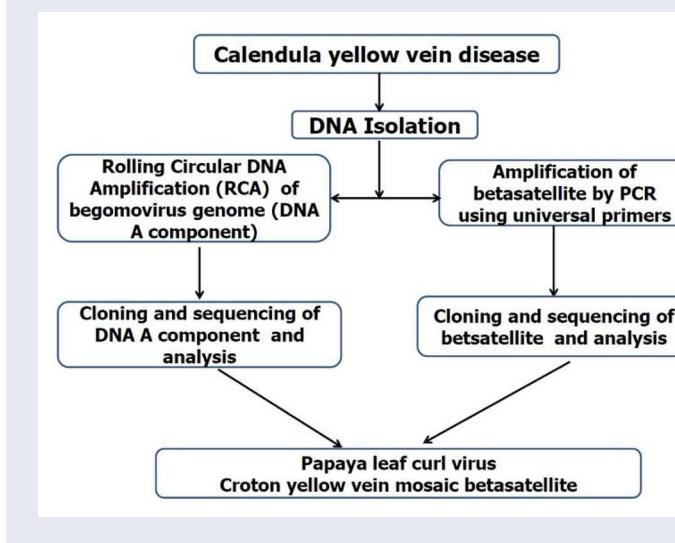
Calendula; betasatellite; begomovirus; PCR; phylogenetic analysis

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GRAPHICAL ABSTRACT



The ornamental flower plant, Pot marigold (*Calendula officinalis* L.) is an important ornamental flower crop belonging to the family Asteraceae. This plant grows during the winter season in different parts of Karnataka. It can also be used in the pharmaceutical industry. This plant is highly susceptible to different plant pathogens, many of which can affect the quality and quantity of the flowers. Different viruses such as Cucumber mosaic virus, Turnip mosaic virus and Tobacco mosaic virus were recorded worldwide causing huge losses on calendula plants that can be passed on the level of the floriculture industry (Lisa and Della-Valle 1979; Naqvi and Samad 1985; Hristova et al. 1994; Gupta and Verma 1983). Recently it also shown that Tomato leaf curl New Delhi virus is infecting *Calendula officinalis* in India (Khan et al. 2005). In the present investigation we characterize another begomovirus causing yellow vein disease of *Calendula officinalis* in India.

The ornamental Pot marigold plants showing the typical of virus-like symptoms of yellow vein were collected at Madikeri dist. of Karnataka state, India (Figure 1). In order to investigate the potential presence of begomovirus on calendula, total nucleic acid was isolated from five symptomatic and from one asymptomatic calendula plants using the CTAB method (Doyle and Doyle 1990), and subjected to polymerase chain reaction (PCR) using begomovirus specific primer pair (Venkataravanappa et al. 2012).

An expected PCR amplicon ~1.2 kb close to PaLCuV was detected in all five infected pants but not from the healthy plant sample. The partial amplified 1.2 kb PCR fragment from the five samples were cloned and



Figure 1. (a) Healthy Calendula plant, (b) begomovirus infected calendula plant showing yellow vein and leaf curl symptoms under natural conditions.

sequenced. The sequence analysis (1.2 kb) indicates five calendula plant samples are associated with monopartite begomovirus (more than 97% nt identity among themselves), close to PaLCuV. After confirmation of begomovirus infection in calendula plant samples, one sample was selected for full-length genome amplification using RCA method as recommended by the manufacturer's protocols. To identify enzymes a product of 2.8 kb size monomeric units, the RCA products amplified from the calendula were subjected to restriction endonucleases *EcoR* I, *Xba* I, *Bam* HI and *Hind* III digestion. Among these, the *Bam* HI enzyme gave 2.8 kb monomeric fragment which was cloned into pUC19 plasmid (Venkataravanappa et al. 2016). The transformed colonies were screened by PCR and restriction digestion using *Bam*HI and *Sca*I enzymes in order to confirm the DNA insert. The confirmed clones were sequenced.

BLASTn Program (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to search sequence similarity of DNA A and betasatellite to the virus. Results showed highest value of NSI (nucleotide sequence identity) with PaLCuV infecting different crops. These sequences were retrieved and used in analysis (Tables S1 and S2, Supplementary material). ORF Finder (www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to determine the genome organization. The sequences showing maximum NSI to the PaLCuV were aligned by Species Demarcation Tool (SDT) (Muhire et al. 2014) and pairwise per-cent nucleotide identity of PaLCuV and the sequences were generated. A maximum likelihood method was used to draw the phylogeny using MEGA X software (Kumar et al. 2018) with 1000 bootstrapped replications.

The DNA A component of PaLCuV infecting ornamental calendula plant was 2773 bp in length and it was submitted to GenBank database (Accession no. MK087120). The SDT analysis of complete genome of PaLCuV isolated from calendula showed the maximum NSI of



Table 1. Pairwise percent of nucleotide identities between the genomic components and amino acid sequence identities of encoded genes from the begomovirus infecting Calendula (Acc. no. MK087120) with the components and genes of selected other begomoviruses available in the databases.

Begomoviruses*	Accession	Crop	Genome	IR	AV2	Gene (percentage amino acid sequence identity)				
						CP(AV1)	Rep (C1)	TRAP (C2)	REn (C3)	C4
PaLCuV	JN831446	<i>Croton bonplandianus</i>	91.5	97.2	100	98.4	83.6	94.7	94.7	54.4
PaLCuV	AJ507777	<i>Croton bonplandianum</i>	93.6	97.9	98.3	98.4	87.8	95.5	95.5	87.0
PaLCuV	FN645902	<i>Acalypha</i> sp.	94.9	95.8	100	98.4	90.5	96.2	96.2	90.6
PaLCuV	JN663850	chilli	93.4	95.8	100	98.0	87.8	95.2	93.2	89.4
ToLCPuV	AY754814	Tomato	79.0	85.1	68.6	76.5	81.7	81.3	79.8	28.8
ToLCRaV	DQ339117	Tomato	77.3	85.1	87.2	83.5	76.1	61.1	21.1	21.1
ToLCBaV-C	AF165098	Tomato	78.7	77.7	68.6	74.0	81.1	80.5	82.0	35.0
ToLCKeV	EU910141	Tomato	81.9	79.3	85.5	80.4	78.3	88.0	88.0	37.5
ToLCPaV	EU862323	Tomato	76.6	76.5	74.7	81.3	76.4	62.9	64.1	40.0
ToLCNDV	U15015	Tomato	74.4	84.1	65.2	81.6	74.7	59.7	62.5	24.7
ToLCPaV	AM884015	Tomato	73.3	84.4	68.5	77.7	72.7	56.1	63.9	28.2
ToLCBV	AF188481	Tomato	82.2	63.3	91.5	82.8	82.8	85.8	82.8	34.0
PaLCuV	HM143914	<i>Nicotiana glutinosa</i>	84.2	79.1	88.1	82.8	83.9	82.8	85.0	90.5
AEV-IN	JX436472	Tomato	83.7	78.8	91.5	83.2	83.3	85.0	87.3	52.9
TbCSV	JN387045	Tomato	81.5	74.8	87.2	81.2	81.1	78.3	83.5	36.0
ChilCV	JO654460	Chilli	80.4	73.1	75.4	82.4	82.5	79.8	76.1	35.0
RaLCuV	GU732204	Radish	84.1	77.3	87.2	82.8	70.0	88.0	87.3	43.2
CLCuKoV	HM461862	Cotton	78.6	74.5	88.1	83.2	76.8	72.3	79.1	36.0
CLCuBaV	AY705380	Cotton	75.6	80.7	66.9	78.9	78.7	60.0	68.6	36.0
CLCuAIV-AI	AJ002452	Okra	75.4	79.4	66.1	79.6	80.1	58.6	71.6	77.6
CLCuMuV-IN	GQ220850	Cotton	76.7	56.2	57.6	81.2	79.0	60.0	71.6	44.0
OELCuV	GU111999	Okra	79.9	76.1	66.9	89.0	79.8	56.0	68.6	38.0
BYMVV	AF241479	Okra	74.1	69.6	65.2	80.0	74.9	60.8	72.3	37.0
FbLCV	JO866297	French bean	75.9	72.6	63.8	77.0	77.6	74.6	70.1	34.0
MYMIV	AF481865	Mungbean	66.6	57.3	39.4	73.5	70.1	48.5	41.7	26.2
PeLCV	AM948961	<i>Glycine max</i>	83.6	82.9	88.1	83.2	82.2	88.8	88.0	38.7

*The species are indicated as Papaya leaf curl virus (PaLCuV), Tomato leaf curl Pune virus (ToLCPuV), Tomato leaf curl Rajasthan virus (ToLCRaV), Tomato leaf curl Bangalore virus (ToLCBaV), Tomato leaf curl Kerala virus (ToLCKeV), Tomato leaf curl Patna virus (ToLCPaV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Palampur virus (ToLCPaV), Tomato leaf curl Bangladeshi virus (ToLCBV), Papaya leaf curl virus (PaLCuV), Ageratum enation virus (AEV), Tobacco curly shoot virus (TbCSV), Chilli leaf curl virus (ChilCV), Radish leaf curl virus (RaLCuV), Cotton leaf curl Kokhran virus (CLCuKoV), Cotton leaf curl Bangalore virus (CLCuBaV), Cotton leaf curl Alabad virus (CLCuAIV), Cotton leaf curl Multan virus (CLCuMuV), Okra enation leaf curl virus (OELCuV), Bhendi yellow vein mosaic virus (BYMVV), French bean leaf curl virus (FbLCV), Mungbean yellow mosaic India virus (MYMIV), Pedilanthus leaf curl virus (PeLCV). For each column the highest value is underlined.

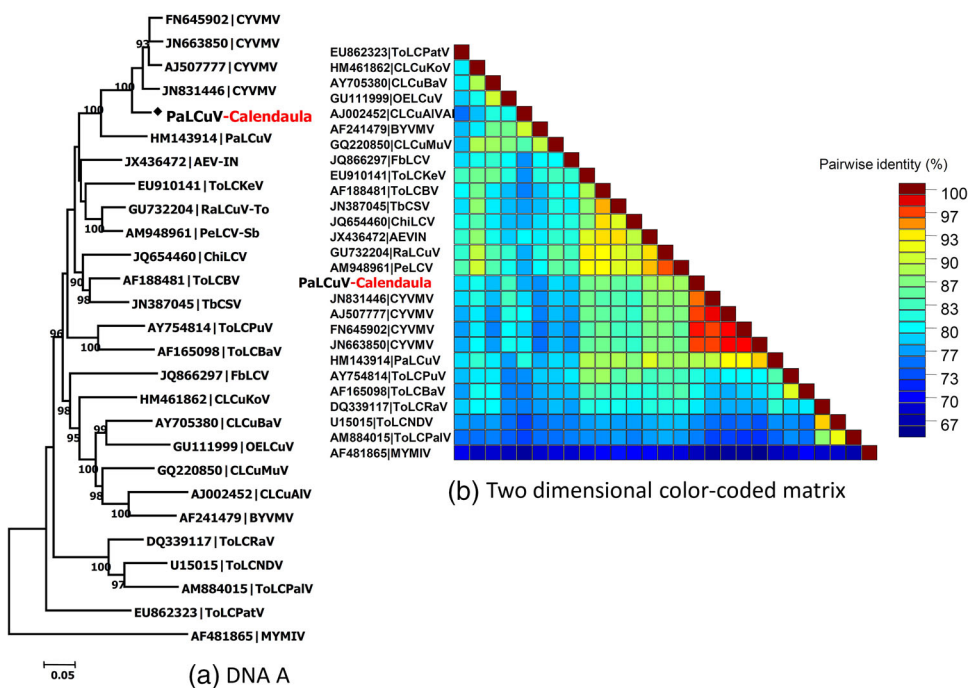


Figure 2. Phylogenetic trees constructed from aligned complete nucleotide sequences of (a) PaLCuV (Acc. no. MK087120) infecting *Calendula* with other begomoviruses using maximum likelihood method. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches. (b) The two dimensional color-coded matrix of pairwise identity scores of the begomovirus under study were obtained using Species Demarcation Tool (<http://web.cbio.uct.ac.za/SDT>).

91.5–94.9% with PaLCuV infecting *Croton bonplandianum*, *Acalypha* sp. and chilli previously described begomovirus from India (Table 1). This was supported by a graphical color-coded matrix of pairwise identity scores (Figure 2(b)) generated by Species Demarcation Tool (SDT) (Muhire et al. 2014). Based on the currently applicable demarcation criteria for begomoviruses species (91% nucleotide sequence identity, Brown et al. 2015) have outlined begomoviruses strains with nucleotide identities less than 95% across the complete genome and as variants with more than 95%. Since the virus infecting calendula showed 91.5–94.9% sequence identity with PaLCuV, it is likely to be a strain of this virus. Additional descriptor (India: Karnataka: Calendula: 2018) is proposed. A phylogenetic analysis of the DNA A of PaLCuV infecting calendula, confirms close similarity of PaLCuV infecting *Croton bonplandianum*, *Acalypha* sp. and chilli from India (Figure 2(a)).

Most of the old world begomoviruses are commonly found associated with ssDNA molecules known as satellite, for which PCR was performed with universal primers specific (Briddon et al. 2002). Amplicon of 1.3 kb

Table 2. Percentages of nucleotide or amino acid sequence identities between betasatellite of *Calendula* and betasatellites (Acc. No. MK087121) of other begomoviruses.

Betasatellites	Accession numbers	Country	Crop	Complete sequence of DNA β (percentage NSI)	ORF β C1
PaLCuB	KY825247	Pakistan	Chilli	88.5	77.4
PaLCuB	KY825246	Pakistan	Chilli	88.0	77.4
PaLCuB	KY825245	Pakistan	Chilli	87.8	76.6
PaLCuB	JN663874	India	Chilli	86.9	78.8
PaLCuB	KT253636	India	Cluster bean	86.0	77.5
PaLCuB	MH359169	India	wild sunflower	83.9	72.5
AEVB	AJ557441	India	Ageratum	71.2	55.9
CroYVMB	GU111995	India	Okra	89.8	55.0
CroYVMB	JN831447	India	<i>Croton bonplandianus</i>	<u>89.5</u>	<u>84.7</u>
CroYVMB	JQ354987	India	<i>Croton bonplandianus</i>	<u>88.7</u>	<u>83.0</u>
CroYVMB	KF964661	India	<i>Croton bonplandianus</i>	86.7	77.9
RaLCuB	JN663873	India	Chilli	74.1	76.2
ToLCB	KU500806	India	Tomato	72.2	60.1
ToLCB	KJ605115	India	Tomato	74.4	55.0
CLCuMuB	FJ159274	India	<i>Hibiscus cannabinus</i>	65.3	58.3
TobSCVB	AJ421485	China	Tobacco	70.4	27.9
ToLCJoB	JN663863	India	Chilli	66.2	54.2
ToLCBDB	AY438558	India	Tomato	67.5	46.8
ChILCB	AM279668	India	Chilli	65.7	57.5
ToLCPnB	HQ180393	India	Tobacco	70.7	58.3

#The species are indicated as Synedrella yellow vein betasatellite (SYVB), Ageratum yellow leaf curl betasatellite (AYLCB), Croton yellow vein mosaic betasatellite (CroYVMB), Radish leaf curl betasatellite (RaLCuB), Tomato leaf curl betasatellite (ToLCuB), Cotton leaf curl Multan betasatellite (CLCuMB), Tobacco curly shoot betasatellite (TbCSB), Tomato leaf curl Joydebpur betasatellite (ToLCJoB), Tomato leaf curl Bangladesh betasatellite (ToLCBDB), Chili leaf curl betasatellite (ChLCB), Tomato leaf curl Patna betasatellite (ToLCPnB). For each column the highest value is underlined.

obtained from infected calendula plants was cloned and sequenced. The betasatellite had typical features of other betasatellites (Briddon et al. 2002; Venkataravanappa et al. 2011). The betasatellite (1362 nt, Acc. No. MK087121) characterized in this study showed more than 89.8% nucleotide identity with croton yellow vein mosaic betasatellite (CroYVMB) infecting okra and *Croton bonplandianus* (Table 2). This was supported by a graphical color-coded matrix of pairwise identity scores (Figure 3(b)) generated by Species Demarcation Tool (SDT). The threshold value for classification betasatellites is set at 78% NSI (Briddon et al. 2008), the betasatellite isolated from calendula plant is an isolate of CroYVMB. The phylogenetic analyses also showed that the betasatellite associated with yellow vein disease in *Calendula* is closely cluster with Indian isolates of CroYVMB and PaLCuB infecting chilli, okra and *Croton bonplandianus* in Indian subcontinents (Figure 3(a)).

Begomovirus are important group of plant viruses (Mansoor et al. 2003) which infects number of crop pants and causes significance yield loss to agriculture and horticulture crops in worldwide (Lima et al. 2013). Ornamental plants are spread throughout the world and have wide environmental adaptability, which are reservoirs for new viruses

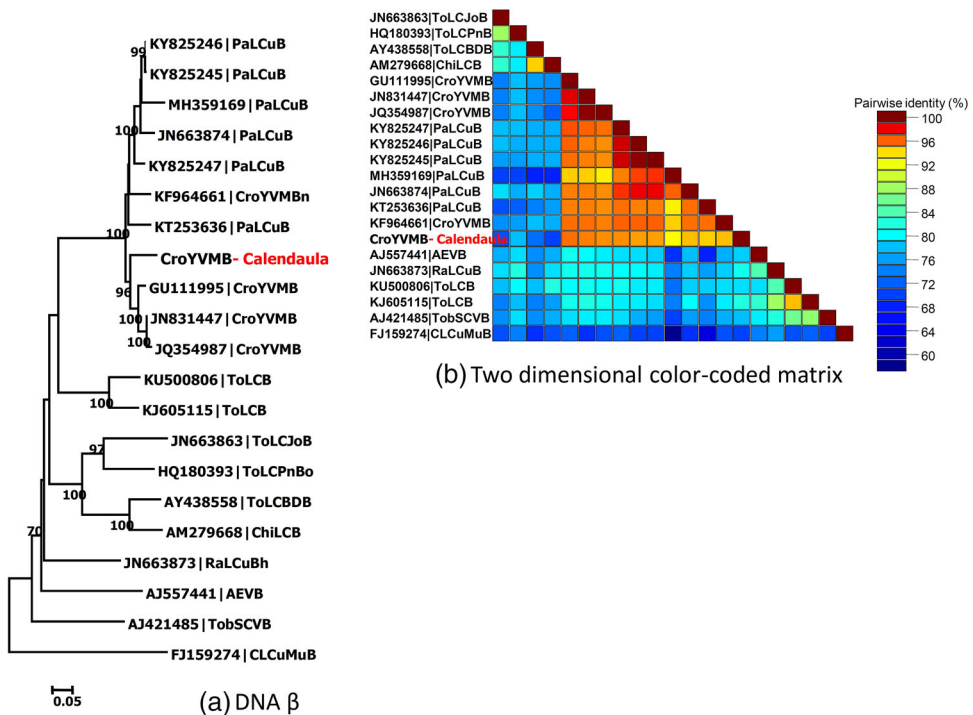


Figure 3. Phylogenetic trees constructed from aligned complete nucleotide sequences of CroYVMB betasatellite (Acc. No. MK087121) infecting *Calendula* (a) with other betasatellites retrieved from the database using maximum likelihood method. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches. (b) The two dimensional color-coded matrix of pairwise identity scores of the betasatellite under study were obtained using Species Demarcation Tool (<http://web.cbio.uct.ac.za/SDT>).

and unidentified viruses (Urbino et al. 2013). The literature showed that ornamental plants are alternative hosts for different virus survival and spread in the absence of the main hosts (Raj et al. 2007; Ilyas et al. 2013). In the present study the *Calendula* plant showing typical yellow vein disease symptoms are associated with a strain Papaya leaf curl virus, which is previously identified in India are infecting *Croton bonplandianum*, *Acalypha* sp. and chilli.

Most of the betasatellites were identified in different crops so far are associated with Old World monopartite DNA viruses (Briddon and Stanley 2006). In the present study the betasatellite associated with the yellow vein disease of *calendula* plant is belongs to croton yellow vein mosaic betasatellite (CroYVMB), which is previously identified in okra, papaya and radish, croton, crotalaria and jatropha in India subcontinents (Pramesh et al. 2013). The literature also showed that many betasatellites are trans-replicating the in the distinct begomovirus species (Jyothsna et al. 2013; Saunders et al. 2002, 2008; Mansoor et al. 2003). In this study

clearly indicates papaya leaf curl virus is expanding its host range from papaya to other ornamental hosts, which may cause a serious threat for cultivation of ornamental and horticulture crop plants. In-depth study is required to identify further spread of papaya leaf curl virus in the country. This will form the basis for further investigations.

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Disclosure statement

All the authors declare that they have no competing interests.

Compliance

This article does not contain any studies with human or animal subjects performed by any of the authors.

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References

- Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X, Fauquet CM. 2008. Recommendations for the classification and nomenclature of the DNA-satellites of begomoviruses. *Arch Virol.* 153(4):763–781.
- Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. 2002. Universal primers for the PCR-mediated amplification of DNA beta: a molecule associated with some monopartite begomoviruses. *MB.* 20(3):315–318.
- Briddon RW, Stanley J. 2006. Subviral agents associated with plant single-stranded DNA viruses. *Virology.* 344(1):198–210.
- Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Jose CF, Silva Fiallo-Olive E, Briddon RW, Hernandez-Zepeda C, Idris A, et al. 2015. Revision of begomovirus taxonomy based on pairwise sequence comparisons. *Arch Virol.* 160(6):1593–1619.
- Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12:13–15.
- Gupta M, Verma VS. 1983. Calendula rosette disease. *Gartenbauwissenschaft.* 48:106–107.
- Hristova D, Barkerdzhieva N, Svrakov K. 1994. Tomato mosaic virus isolated from *Calendula officinalis*. *Conf Plant Virol.* 32:153.
- Ilyas M, Nawaz K, Shafiq M, Haider MS, Shahid AA. 2013. Complete nucleotide sequences of two begomoviruses infecting Madagascar periwinkle (*Catharanthus roseus*) from Pakistan. *Arch Virol.* 158(2):505–510.
- Jyothsna P, Haq QMI, Priyanka S, Sumiya KV, Praveen S, Rawat R, Briddon RW, Malathi VG. 2013. Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus

- components and antagonistic interaction between DNA B and betasatellites. *Appl Microbiol Biotechnol.* 97(12):5457–5471.
- Khan A. A, Naqvi QA, Khan MS, Singh R, Raj SK. 2005. First report of a begomovirus infecting *Calendula* in India. *Plant Pathol.* 54(4):569.
- Kumar S, Stecher G, Tamura K. 2018. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 33(7):1870–1874.
- Lima AT, Sobrinho RR, Gonzalez-Aguilera J, Rocha CS, Silva SJ, Xavier CA, Silva FN, Duffy S, Zerbini FM. 2013. Synonymous site variation due to recombination explains higher genetic variability in begomovirus populations infecting non-cultivated hosts. *J Gen Virol.* 94(Pt_2):418–431.
- Lisa V, Della-Valle G. 1979. Isolation of two viruses from *Calendula officinalis*. *Inform Fitopatol.* 29:11–11.
- Mansoor S, Briddon RW, Bull SE, Bedford ID, Bashir A, Hussain M, Saeed M, Zafar MY, Malik KA, Fauquet CM, et al. 2003. Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA β . *Arch Virol.* 148(10):1969–1986.
- Mansoor S, Briddon RW, Zafar Y, Stanley J. 2003. Geminivirus disease complexes: an emerging threat. *Trends Plant Sci.* 8(3):128–134.
- Muhire BM, Varsani A, Martin DP. 2014. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE.* 9(9):e108277.
- Naqvi QA, Samad A. 1985. Purification and properties of *Calendula* yellow net virus. *Indian J Virol.* 1:143–146.
- Pramesh D, Mandal B, Phaneendra C, Muniyappa V. 2013. Host range and genetic diversity of croton yellow vein mosaic virus, a weed-infecting monopartite begomovirus causing leaf curl disease in tomato. *Arch Virol.* 158:531–542.
- Raj SK, Khan MS, Snehi SK, Kumar S, Khan AA. 2007. Natural occurrence of a begomovirus on *Dimorphotheca sinuate* in India. *Austral Plant Disease Notes.* 2(1):25–26.
- Saunders K, Briddon RW, Stanley J. 2008. Replication promiscuity of DNA- β satellites associated with monopartite begomoviruses; deletion mutagenesis of the *Ageratum* yellow vein virus DNA- β satellite localizes sequences involved in replication. *J Gen Virol.* 89(12):3165–3172.
- Saunders K, Salim N, Mali VR, Malathi VG, Briddon RW, Markham PG, Stanley J. 2002. Characterisation of Sri Lankan cassava mosaic virus and Indian cassava mosaic virus: evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virology.* 293(1):63–74.
- Urbino C, Gutiérrez S, Antolik A, Bouazza N, Doumayrou J, Granier M, Martin DP, Peterschmitt M. 2013. Within-host dynamics of the emergence of tomato yellow leaf curl virus recombinants. *PLoS ONE.* 8(3):e58375.
- Venkataravanappa V, Reddy CNL, Jalali S, Krishna Reddy M. 2012. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes.* 44(3):522–535.
- Venkataravanappa V, Reddy CNL, Swarnalatha P, Jalali S, Briddon RW, Reddy MK. 2011. Diversity and phylogeography of begomovirus-associated beta satellites of okra in India. *Virol J.* 8(1):555.
- Venkataravanappa V, Swarnalatha P, Reddy CNL, Chauhan N, Reddy MK. 2016. Association of recombinant Chilli leaf curl virus with enation leaf curl disease of tomato: a new host for chilli begomovirus in India. *Phytoparasitica.* 44(2):213–223.