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First report of sida yellow vein Madurai virus infecting Lisianthus (*Eustoma russellianum*)

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Abstract
Lisianthus (*Eustoma russellianum*) is one of the emerging cut flower crops in international flower market quickly ranked in top ten cut flowers worldwide. Despite its rising popularity, studies relating to the identification and characterization of viral diseases affecting it are lacking from India. Thus, the present study was focused on identification and characterization of virus in lisianthus plants samples collected from Vensai Floritech, Narasihmanahalli village, Tubagere hobli, Doddaballapur taluk, Bengaluru rural district, Karnataka state, India exhibiting symptoms similar to begomoviruses infections. Association of the begomovirus with sample was confirmed by PCR using begomovirus specific primers which resulted in the expected amplicon (~ 1.2 kb). Further, whole-genome amplification was done by rolling circle amplification (RCA) for one representative sample (LIS-1). The amplified RCA product was cloned, sequenced and analyzed. The phylogenetic and nucleotide (nt) sequence analysis revealed that the begomovirus associated with lisianthus plants showed the maximum nt identity of 91.0% with sida yellow vein Madurai virus (SIYVMV-TN:OM141480) infecting a weed, *Sida cordata*, reported from Tamil Nadu, India, which is geographically close to Karnataka. Based on species demarcation criteria for begomoviruses, the collected isolate is identified as a strain of sida yellow vein Madurai virus associated with leaf curl of lisianthus from India and proposed the name "Sida yellow vein Madurai virus -[India:Karnataka:Doddaballapura:Lisianthus:2023]" and designated as SIYVMV-[IN:Kar:Dod:Lis:23]. Further, recombination analysis revealed a single intra-specific recombination event in the genomic region. Hence, this study provides a one more evidence of expanding host range for begomoviruses in India.

Keywords Characterization · Detection · *Eustoma russellianum* · Lisianthus · India · Phylogeny · Recombination · Sequence demarcation tool

Eustoma russellianum commonly known as lisianthus, is an herbaceous flowering plant that belongs to the Gentianaceae family (Wazir et al. 2014). The popularity of lisianthus is increasing in India, and the area under cultivation is gradually expanding (Bhatia and Sindhu 2019; Lakshmaiah et al. 2019; Rehana and Bala 2022). Lisianthus cultivation faces challenges from various fungal (Bhatia et al. 2020; Wu et al. 2023; Hanagasaki et al. 2023) and viral diseases (Chen et al. 2000; Jan et al. 2003; Beikzadeh et al. 2011; Cohen et al. 2001; Taniguchi et al. 2023) worldwide leading to significant economic losses for growers. There are no reports of viral diseases affecting lisianthus in India. In this study we report the identification and characterization of a begomovirus affecting lisianthus in India.

Seven leaf samples from lisianthus plants exhibiting symptoms typical to begomovirus infection such as vein

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thickening, leaf curling, and reduction in leaf size, yellow mosaic and stunted growth were collected (designated as LIS-1 to 7 isolates) along with an asymptomatic leaf sample from Vensai Floritech (13°29'00.4"N 77°28'18.5"E) (Bengaluru, India) (Fig. 1). To confirm the causal agent, total genomic DNA was isolated from the lisianthus samples (Lodhi et al. 1994) and subjected to PCR amplification using degenerate primers specific to DNA-A (PAR1c496/PAL1v1978) and DNA-B (PBL1v2040/PCRc1) as described by Rojas et al. (1993). The amplification yielded PCR amplicon of ~1.2 kb, specific to begomoviruses with respect to DNA-A. However, there was no amplification with the DNA-B specific primers, indicating the virus associated with symptomatic lisianthus plants is a monopartite begomovirus. All the samples were also subjected to PCR for detecting the presence of satellite molecules i.e., betasatellite (Briddon et al. 2002) and alphasatellite (Bull et al. 2003) using specific primers but unsuccessful for amplification of both satellite molecules.

Further, one representative sample (LIS-1 isolate) was used for complete genome amplification by rolling circle DNA amplification (RCA) (Inoue-Nagata et al. 2004). The amplified RCA product (2 µL) was digested with HindIII restriction enzyme to obtain a monomeric unit of ~2.7 kb. The digested product was cloned into the linearized pUC19 plasmid with the respective sites. The recombinant clones were confirmed by HindIII restriction digestion and sequenced by the primer walking method at Medauxin Pvt. Ltd., Bengaluru, Karnataka, India. The obtained complete genome sequence was assembled using different bioinformatic tools (BioEdit, ClustalX2, and SeaView), and sequence similarities were checked at the NCBI database using BLASTn (http://www.ncbi.nlm.nih.gov).

The complete DNA-A genome sequence of LIS-1 isolate was deposited in NCBI GenBank under the accession number OR371601 and its genome consists of 2754 nt with seven open reading frames (ORFs), which were identified by using the ORF finder tool (http://www.Ncbi.nlm.nih.Gov/gorf/gorf.html). Analysis showed that viral genome (DNA-A) codes for seven potential ORFs, two [V2 and V1 (CP)] on virion sense strand and five [C1 (Rep), C2 (TrAP), C3 (REn), C4 and C5] on complementary sense strand. These two strands are separated by intergenic region (IR), which harbored a predicted stem-loop structure containing nonanucleotide sequence TAATATTAC which constitutes the origin of viral DNA replication (Supplementary Tables 1 and Supplementary Fig. 1). Pairwise nt and amino acid (aa) identities for the LIS-1 isolate from lisianthus were compared with selected begomoviruses sequences retrieved from GenBank using the Sequence Demarcation Tool (SDT) (SDT version 1.2) (http://web.cbio.uct.ac.za/brejnev/) (Muhire et al. 2014). Sequence analysis revealed that the LIS-1 isolate exhibited nt sequence identity of 87.8–91.0 per cent for the complete genome with sida yellow vein Madurai virus (SIYVMV). The highest nt identity (91.0%) indicates that the LIS-1 isolate represents a strain of SIYVMV infecting lisianthus (Brown et al. 2015; Table 1 and Suppl. Table 2). This is further supported by SDT analysis (Fig. 2b).

Based on the ICTV guidelines for begomovirus nomenclature (Fauquet and Stanley 2005), we propose the name "Sida yellow vein Madurai virus-[India:Karnataka:Doddaballapura:Lisianthus:2023]", abbreviated as SIYVMV-[IN:Kar:Dod:Lis:23], for the isolate.

Amino acid identities of the proteins encoded by the isolate with those encoded by other begomoviruses are listed in Table 1.

A phylogenetic tree generated using the Neighbor-Joining method by MEGA11 (Tamura et al. 2021) revealed that SIYVMV-[IN:Kar:Dod:Lis:23] (OR371601) clustered with sida yellow vein Madurai virus (SIYVMV) infecting sida plants reported from Tamil Nadu, India (Fig. 2a). Recombination analysis of SIYVMV-[IN:Kar:Dod:Lis:23] using RDP 5 (Martin et al. 2021) revealed a single significant

Fig. 1 Lisianthus infected plants showing begomoviral symptoms; **(A)** Reduction in leaf size, **(B)** Leaf curling as compare to **(C)** healthy under field conditions

Table 1 Pairwise nucleotide and amino acid identities between the begomovirus isolated from lishanthus (SIYVMV-[IN:Kar:Dod:Lis:23]) and selected begomoviruses

Begomoviruses*	Percent nt identity range (%)		Percent aa identity range (%)						
	DNA-A	IR	AC1**	AC2	AC3	AC4	AC5	AV1	AV2
AYVV	70.6 [1]	56.5 [1]	70.8 [1]	55.5 [1]	64.9 [1]	30.6 [1]	13.2 [1]	72.7 [1]	56.5 [1]
BYVIV	76.5 [1]	53.9 [1]	83.4 [1]	54.5 [1]	67.9 [1]	**86.6**[1]	14.5 [1]	78.1 [1]	53.9 [1]
CYVMV	78.2 [1]	54.8 [1]	84.0 [1]	64.2 [1]	64.9 [1]	82.5[1]	-	82.8 [1]	54.8 [1]
LELCV	74.5 [3]	55.6 [3]	75.2–75.5 [3]	61.5–62.2 [3]	68.6–69.4 [3]	44.8–45.8 [3]	-	82.8 [3]	55.6 [3]
MeYVMV	77.8 [1]	54.8 [1]	85.7 [1]	53.1 [1]	66.4 [1]	82.5 [1]	14.5 [1]	82.0 [1]	54.7 [1]
MYVYNV	77.9–78.1 [4]	57.1–58.0 [4]	83.7–84.0 [4]	54.5 [3]	60.4–61.9 [4]	79.4–80.4 [4]	13.6 [2]	82.0-83.2 [4]	54.8–58.0 [4]
OELCuV	77.8–78.2 [5]	54.8–55.6 [5]	**84.6–85.9**[5]	53.1–55.2 [5]	66.4 [5]	81.4–82.5 [5]	14.5 [3]	89.1–82.0 [5]	54.8–55.6 [5]
PaLCuBV	76.9 [1]	54. 8 [1]	82.6 [1]	61.9 [1]	63.4 [1]	80.0 [1]	**15.5**[1]	82.8 [1]	54.8 [1]
PaLCuGdV	73.2–77.7 [3]	53.0 [3]	72.7–73.5 [3]	59.2–60.0 [3]	70.9 [3]	45.9 [3]	-	72.7–72.7 [3]	53.0 [3]
SiLCV	85.2–87.9[7]	93.0-97.4 [7]	80.1–81.8 [7]	86.7–87.4 [7]	94.8–97.7 [7]	44.8–49.5 [7]	-	91.4–94.1 [7]	93.0-97.4 [7]
SIYVMV	**87.8–91.0**[4]	**34.8–100.0**[4]	33.8–83.7 [4]	**72.3–95.1**[4]	**42.5–98.5**[4]	45.3–48.4 [4]	-	**60.9–94.9**[4]	**34.8–100.0**[4]
SYLCuV	77.5–77.7 [4]	56.5 [4]	84.5–85.6[4]	59.7–61.2 [4]	59.7–61.2 [4]	79.4–80.4 [4]	13.6[3]	82.8–84.0 [4]	56.5 [4]
ToLCGV	78.1–78.4 [2]	57.4 [2]	84.0-84.9 [2]	61.2–61.9 [2]	61.9 [2]	82.4 [2]	-	82.8–82.2 [2]	57.4 [2]
TYLCV	72.4–72.9 [2]	55.6–56.5 [2]	75.9–76.9 [2]	55.5 [2]	67.1–67.9 [2]	67.0-71.1 [2]	-	66.8 [2]	55.6–56.5 [2]

Note: Numbers indicated in parenthesis are total sequences retrieved from databases for comparisons. *Ageratum yellow vein virus (AYVV); Bhendi yellow vein India virus (BYVIV); Croton yellow vein leaf curl virus (CYVMV); Lisianthus enation leaf curl virus (LELCV); Malvastrum yellow vein Yunnan virus (MeYVMV); Mesta yellow vein mosaic virus (MYVYNV); Okra enation leaf curl virus (OELCuV); Papaya leaf curl Bagalkote virus isolate (PaLCuBV); Papaya leaf curl Guandong virus (PaLCuGdV); Sida leaf curl virus (SiLCV); Sida yellow vein Madurai virus (SIYVMV); Synedrella leaf curl virus (SYLCuV); Tomato leaf curl Gujarat virus (ToLCGV); Tomato yellow leaf curl virus (TYLCV). **Genes are indicated as AV1:Coat protein (CP), AV2: Pre coat protein, AC1:Replication-associated protein (Rep), AC2:Transcriptional activator protein (TrAP) and AC3:Replication enhancer protein (REn). The products encoded by ORFs AC4 and AC5 have yet to be named

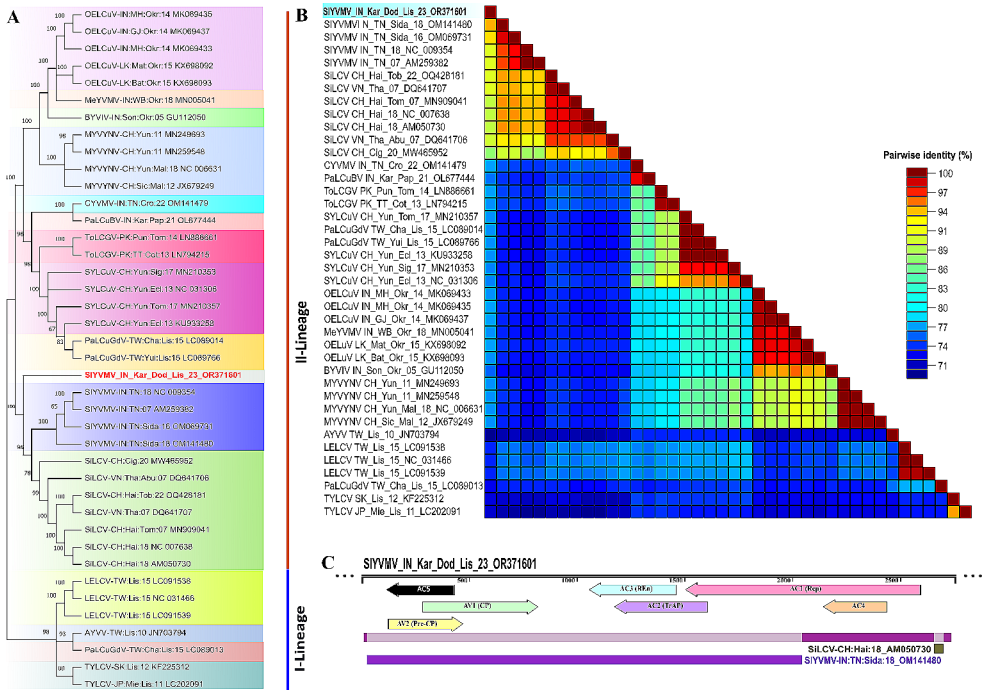

Fig. 2 **(A)** Phylogenic tree based on the complete nucleotide sequence of SIYVMV isolate from lisianthus (IN:Kar:Dod:Lis:23;OR371601) and other selected begomoviruses. **(B)** Nucleotide sequence identity matrix obtained from the same dataset using Sequence Demarcation Tool. and **(C)** recombination breakpoint analysis of SIYVMV (OR371601) isolate with other selected begomoviruses isolates reported across the world

intra-specific recombination event occurring at the 14th to 2087th nt position in the DNA-A genome of SIYVMV-[IN:Kar:Dod:Lis:23]. This recombinant fragment descends from okra enation leaf curl virus (OELCuV: MK069435) and sida yellow vein Madurai virus (SIYVMV: OM141480) as the major and minor parents, respectively (Table 2; Fig. 2c). This report provides evidence of the association of recombinant begomovirus with lisianthus for the first time in India. Furthermore, symptomatology, characterization, phylogeny and recombination analysis indicated that leaf curl disease of lisianthus is caused by a strain of SIYVMV.

Table 2 Recombination analysis of the begomovirus isolate from lisianthus (SIYVMV-[IN:Kar:Dod:Lis:23]).

Begomovirus	Break point (nt)		Recombination parents		*p*-value					
	Event	Begin	End	Major parent	Minor parent	RDP	Geneconv	Bootscan	MaxChi	Chimera	SiScan	3Seq
SIYVMV-[IN:Kar:Dod:Lis:23] (OR371601)	1	14	2087	OELCuV-[IN:MH:Okr:14] MK069435	SIYVMV-[IN:TN:Sida:18] OM141480	4.40E-19	1.47E-21	NS*	1.37E-20	1.63E-03	3.19E-57	1.88E-46

*Non significant value

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Declarations

Accession number OR371601.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests.

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