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GENOME REPORTS

Full-length genome sequence of *Cyrtanthus elatus virus-A* isolated from *Narcissus tazetta* in India

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Abstract

Narcissus tazetta L. is a bulbous ornamental plant popular for its notable fragrant flowers which make it the plant of high importance. In spite of its economic value, narcissus is found to be susceptible for a number of diseases borne by fungi, bacteria, nematodes, and viruses. A potyvirus, *Cyrtanthus elatus virus-A* isolate NBRI16 (CEVA-NBRI16), associated with leaf chlorotic stripe disease of *N. tazetta* cv. Paperwhite was reported for first time in India from our laboratory based on the partial coat protein gene sequence. In present study, the full-length genomic sequence of CEVA-NBRI16 is determined which consists of 9942 nucleotides, excluding the polyA tail, and encodes a single large polyprotein of 3102 amino acids with the genomic features typical of a potyvirus. It shares highest 93% nucleotide sequence identity and closest phylogenetic relationship with sequences of CEVA-Marijiniup7-1 and CEVA-Marijiniup7-2, both reported from Australia on *Cyrtanthus elatus* host. The full-length genomic sequence of CEVA from narcissus plant is being reported for the first time from India.

Keywords *Cyrtanthus elatus virus A* · Potyvirus · Sequence identity · *Narcissus tazetta* · Full-length genome

Introduction

Narcissus tazetta L. (family Amaryllidaceae) is known for attractive flowers with sweet fragrance, worldwide. It can easily be noticed anywhere by its pleasant sweet fragrance and notable cool white appearance. Due to this magnificent property, it is widely accepted as cut-flower crop and adapted by the floriculture and perfumery industry for economy. The narcissus plant is susceptible to several RNA viruses of genera *Carlavirus*, *Maculavirus*, *Nepovirus*, *Potexvirus,* etc. (Brunt 1995; Wylie and Jones 2012). Amongst all, potyviruses are the most prevalent in *N. tazetta* (Brunt 1995; Aminuddin and Raj 1999; Yadav and Khan 2007) and infected plants in general exhibit the symptoms of leaf chlorotic stripes, plant stunting, and flower colour breaking. The vegetative mean of propagating bulbs is a particular problem for virus disease dissemination in all bulbous crops including narcissus. These viral diseases are also reported to reduce the yield of such plants (Brunt 1977). An uncharacterized potyvirus was detected from *N. tazetta* cv. Paperwhite plants associated with yellow stripes symptoms (Aminuddin and Raj 1999) which was identified as Lycoris potyvirus, based on the partial 3′ sequence (Yadav and Khan 2007). Moreover, occurrence of a previously unidentified *Cyrtanthus elatus virus A* (CEVA) on *N. tazetta* cv. Paperwhite samples exhibiting chlorotic stripes were also reported on the basis of partial 3′ sequence (Kumar et al. 2015). In present study, the full-length genomic sequence of this CEVA isolate is determined. Information on complete genome sequence of CEVA from India will shed light on genetic makeup of indigenous isolate and will be a source to study the sequence diversity among potyviruses in narcissus plant.

Narcissus tazetta cv. Paperwhite plants exhibiting chlorotic stripes were observed at the experimental plot of CSIR-NBRI, Lucknow with 78% (105/137) disease incidence. Upon blooming, though the flowers in infected plants appear symptomless, the flower stalk showed light green to pale stripes (Fig. 1b, c) as compared to healthy plants

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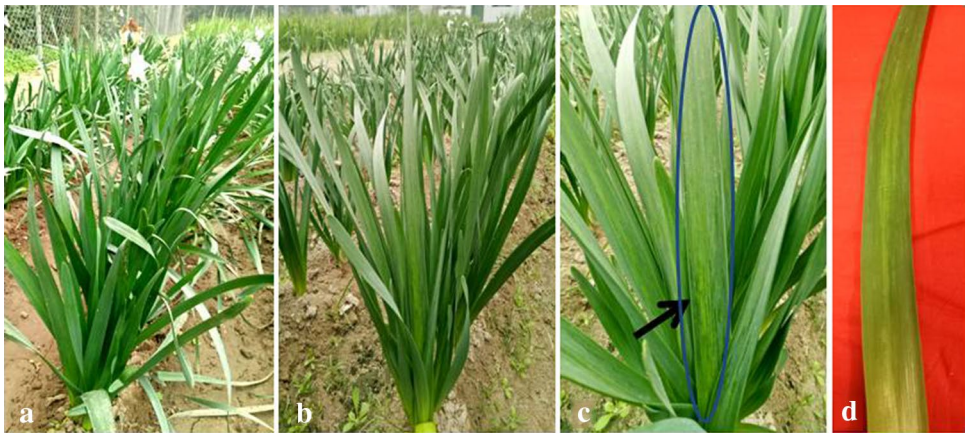

Fig. 1 **a** Healthy and **b** infected narcissus plant in field condition **c** close view of infected narcissus plant and **d** infected leaf showing the symptoms

Table 1 Virus isolates used in present study

[a]Virus	Isolate	Host	Location	Genome length (nt)	GenBank accession	Source
CEV-A	NBRI16	*Narcissus tazetta*	India	9942	KX575832	This study
CEV-A	Marijiniup7-1[b]	*Cyrtanthus elatus*	Australia	9908	NC_017977	Wylie and Jones (2012)
CEV-A	Marijiniup7-2[b]	*Cyrtanthus elatus*	Australia	9908	JQ723475	Wylie and Jones (2012)
NYSV	Zhangzhou-1	*Narcissus tazetta*	China	9650	AM158908	Chen et al. (2006)
NYSV	Zhangzhou-2	*Narcissus tazetta*	China	9650	NC_011541	Chen et al. (2002)
NYSV	ZZ2	*Narcissus tazetta*	China	9654	JQ911732	Unpublished
NYSV	Marijiniup3	*Narcissus* spp.	Australia	9647	JQ395042	Wylie and Jones (2012)
NLSYV	Zhangzhou	*Narcissus tazetta*	China	9651	JQ326210	Lin et al. (2012)
NLSYV	Marijiniup8	*Narcissus* spp.	Australia	9687	KC691259	Wylie et al. (2014)
NLSYV	Marijiniup9	*Narcissus* spp.	Australia	9577	JX156421	Wylie et al. (2014)
[b]ScaMV	− 1	*Allium chinense*	China	9324	NC_003399	Chen et al. (2002)
[b]ScaMV	− 2	*Allium chinense*	China	9324	AJ316084	Chen et al. (2002)
TuMV	TIGD	*Tigridia* spp.	Germany	9798	AB701735	Nguyen et al. (2013)
TuMV	NZ403B	*Lepidium oleraceum*	New Zealand	9796	AB989658	Yasaka et al. (2015)
TuMV	UK1	–	UK	9835	NC_002509	Jenner et al. (2000)
JYMV	M	*Dioscorea japonica*	Japan	9760	NC_000947	Chen et al. (2006)
PWV	MU2	*Passiflora caerulea*	Australia	9682	NC_014790	Wylie and Jones (2012)

[a]Virus Acronyms: *CEV-A Cyrtanthus elatus virus A*, *JYMV* Japanese yam mosaic virus, *NLSYV* narcissus late season yellows virus, *NYSV* narcissus yellow stripe virus, *ScaMV* scallion mosaic virus, *TuMV* turnip mosaic virus, *PWV* passion fruit woodiness virus

[b]Virus isolates abbreviated for the present study to avoid confusion

having green leaves (Fig. 1a). A closeup of a single leaf clearly shown the leaf stripes symptoms (Fig. 1d). Association of such symptoms in *N. tazetta* was also observed earlier with potyvirus in India (Aminuddin and Raj 1999; Yadav and Khan 2007) and other countries (Wylie et al. 2014; Chen et al. 2006). For characterization of virus, infected leaf samples of narcissus were collected and stored in − 80 °C till further used. The total RNA from 100 mg infected leaf sample was isolated using TRI reagent (Sigma-Aldrich Co., MO, USA) for Reverse transcription-PCR and 5′RACE (Thermo Fisher Scientific Inc., MA, USA). For virus detection, primers MJ-I and MJ-II targeting the conserved region from MVWCIEN to QMKAAA motifs in coat protein (CP) region, were used (Marie-Jeanne et al. 2000; Grisoni et al. 2006). All the 105 infected samples produced expected size ~ 300 bp DNA band in RT-PCR. Sequencing of 18 randomly selected RT-PCR products confirmed the occurrence of potyvirus in *N. tazetta*.

The occurrence of CEVA in 18 *N. tazetta* samples exhibiting chlorotic leaf stripes were confirmed by nucleic acid spot hybridization (NASH) assay in a replica nitrocellulose membrane. The 2 µg RNA from a healthy *N. tazetta* plant (as negative control) along with 200 ng cloned DNA of CEVA (as positive controls) was also blotted. The membranes were

Table 2 Percent identity of CEVA-NBRI16 isolate at the level of nucleotide and its deduced amino acid sequence of full-length genome and polyprotein, respectively, with other full length potyviruses (for which complete genome sequences are available)

GenBank accession	Virus	Isolate	Location	Percent identity										
				Complete genome (polypeptide)	Open reading frames									
					P1	HC-Pro	P3	PIPO	6K1	CI	6K2	VPg	NIa	NIb	CP
NC_017977	CEVA	Marijiniup7-1	Australia	93 (94)	97 (95)	97 (96)	97 (94)	–	92 (86)	97 (96)	87 (78)	96 (90)	99 (97)	88 (93)	84 (94)
JQ723475	CEVA	Marijiniup7-2	Australia	93 (94)	97 (95)	97 (96)	97 (94)	–	92 (86)	97 (96)	87 (78)	96 (90)	99 (97)	88 (93)	84 (94)
AM158908	NYSV	Zhangzhou-1	China	90 (97)	99 (100)	86 (93)	98 (99)	99 (100)	99 (100)	83 (96)	77 (90)	87 (97)	95 (98)	92 (96)	95 (97)
NC_011541	NYSV	Zhangzhou-2	China	90 (97)	99 (100)	86 (93)	98 (99)	99 (100)	99 (100)	83 (96)	77 (90)	87 (97)	95 (98)	92 (96)	95 (97)
JQ911732	NYSV	ZZ-2	China	59 (78)	60 (82)	63 (83)	60 (66)	56 (83)	52 (63)	70 (88)	61 (71)	68 (82)	69 (86)	67 (84)	71 (82)
JQ395042	NYSV	Marijiniup 3	Australia	58 (78)	35 (42)	67 (84)	54 (59)	53 (81)	69 (84)	70 (88)	60 (83)	65 (85)	72 (90)	69 (85)	71 (81)
JQ326210	NLSYV	Zhangzhou	China	55 (74)	33 (38)	65 (81)	55 (66)	51 (77)	71 (78)	65 (86)	64 (77)	71 (87)	71 (85)	63 (80)	68 (76)
KC691259	NLSYV	Marijiniup8	Australia	54 (75)	32 (36)	64 (81)	56 (66)	49 (69)	63 (78)	67 (85)	68 (81)	67 (86)	66 (84)	64 (79)	68 (75)
JX156421	NLSYV	Marijiniup9	Australia	54 (75)	35 (39)	67 (81)	56 (66)	49 (69)	73 (78)	65 (86)	66 (79)	69 (87)	68 (85)	65 (79)	68 (75)
AJ316084	ScaMV	–	China	43 (63)	25 (22)	56 (65)	41 (50)	44 (64)	60 (67)	53 (72)	45 (54)	61 (71)	57 (71)	58 (71)	58 (69)
NC_003399	ScaMV	–	China	43 (63)	25 (22)	56 (65)	41 (50)	44 (64)	60 (67)	53 (72)	45 (54)	61 (71)	57 (71)	58 (71)	58 (69)
AB701735	TuMV	TIGD	–	42 (59)	15 (15)	58 (66)	25 (36)	41 (60)	56 (76)	55 (71)	48 (50)	53 (67)	60 (73)	62 (73)	64 (67)
AB989658	TuMV	NZ403B	New Zealand	39 (60)	21 (23)	55 (66)	30 (36)	40 (58)	64 (78)	55 (71)	54 (50)	54 (67)	59 (73)	59 (74)	60 (66)
NC_002509	TuMV	–	–	41 (59)	20 (19)	53 (66)	31 (34)	40 (58)	64 (76)	55 (70)	50 (58)	56 (69)	57 (71)	55 (73)	65 (67)
NC_000947	JYMV	–	–	40 (57)	19 (19)	53 (63)	40 (38)	39 (62)	58 (67)	55 (68)	52 (43)	55 (65)	50 (60)	55 (69)	56 (64)
NC_014790	PWV	PWV-MU2	Australia	25 (42)	13 (13)	35 (45)	19 (20)	10 (40)	23 (32)	44 (54)	40 (35)	37 (47)	37 (51)	44 (57)	50 (59)

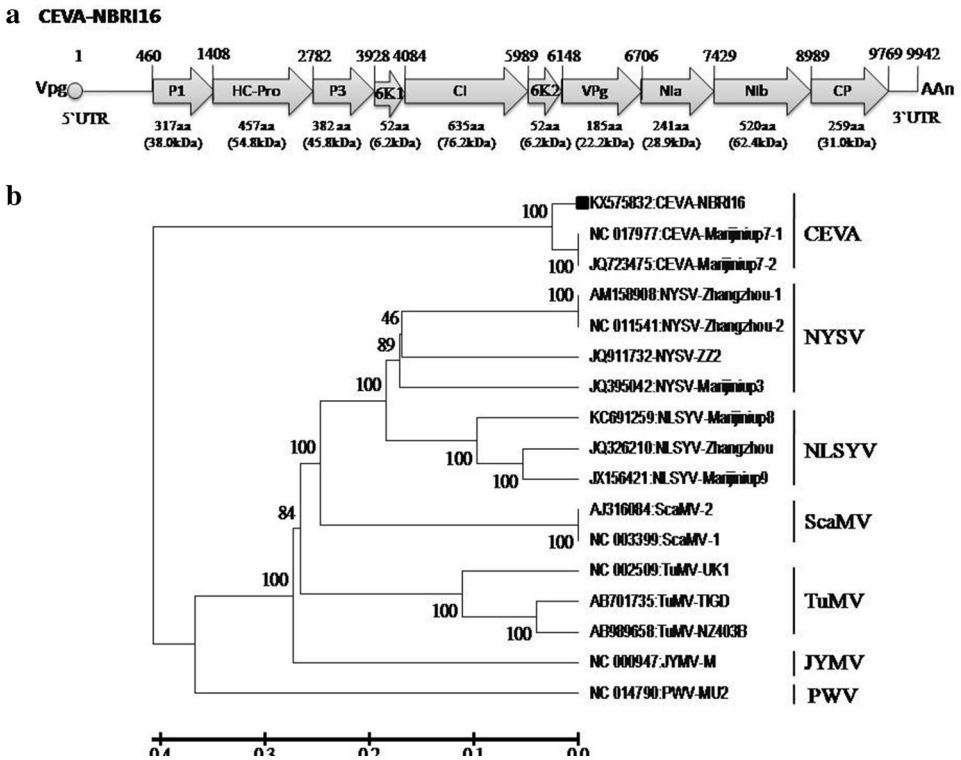

Fig. 2 **a** Schematic representation of full length genome of narcissus isolate CEVA-NBRI16. Arrow represents the orientation of ORFs present in virus genome. The numbers above the ORF indicate their starting site, while the length (amino acids) and predicted molecular weight (in kDa) of ORF are shown below. **b** Phylogenetic tree showing closest relationship of CEVA-NBRI16 isolates with CEVA-Marijiniup7-1 and Marijiniup7-2. Tree was constructed employing MEGA v6.1 tool using Maximum-likelihood method based on the Tamura–Nei model with 1000 bootstrap replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site and the percentage of trees in which the associated taxa clustered together is shown next to the branches. Bar at the bottom represents the nucleotide substitutions per site

hybridized with probes prepared by random primer labeling method (Fienberg and Vogelstein 1983); and pre-hybridization, hybridization, and washing steps were performed according to the standard methods (Sambrook et al. 1989) and exposed to X-ray films to observe hybridization signals. As a consequence, the presence of CEVA in 16 out of 18 samples showing positive signals with 88.8% disease incidence was observed (Electronic supplementary material 1).

Furthermore, a randomly chosen sample was used for full-length genome amplification taking the advantage of available degenerate primer pairs targeting different conserved motifs of potyvirus genome following the strategy as described earlier (Chen et al. 2002). The set of primer pairs: Pot-I/Pot -II (Gibbs and Mackenzie 1997); CI-F (Ha et al. 2008)/NIb-Pot-3 (Yakoubi et al. 2008); HP-F (Lucinda et al. 2010)/CI-R (Ha et al. 2008); 5′RACE and HP-R (Lucinda et al. 2010) were used. The 3′UTR to partial nuclear inclusion B (Nlb) region of ~1.6 kb was amplified using Pot-I/Pot-II primers which target the polyadenylated 3′ end and -GNNS- motif in Nlb region of CEVA isolate, respectively. The CI-F/NIb-Pot-3 primers were used which amplified the expected size ~3.0 kb band spanning the GxVGSGKST motif in cylindrical inclusion (CI) protein and targeted Nlb region. Furthermore, from partial CI to HC region of ~3.0 kb was amplified using HP-F/CI-R primers. For amplification of remaining 5′ end to HC region, RACE kit was employed which yielded ~2.0 kb DNA fragment.

All amplified DNA fragments were gel-purified and cloned in pGEM-T vector (Promega Corporation, Madison, USA). The positive transformants were screened by colony PCR and sequenced (three such positive clones for each insert). Sequences were edited and assembled using BIOEDIT tool (http://www.mbio.ncsu.edu/bioedit/bioedit.html) to eliminate sequence ambiguity. The consensus sequence for full-length genome was determined and submitted to GenBank under the accessions KX575832 (CEVA-NBRI16).

The open reading frames (ORFs) encoded by the genome were analyzed by ORF Finder (www.ncbi.nlm.nih.gov/projects/gorf/) and their putative proteins were translated by *ExPasy* tool (http://web.expasy.org/translate/). The sequences were compared with those of publically available sequences (Table 1) in NCBI by BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification. The nucleotide and

amino acid identity of ORFs of selected potyvirus isolates (Table 2) was obtained by *DiAlign* tool (http://www.genomatix.de/cgi-bin/dialign/dialign.pl). The results of sequence analysis showed that the genome of CEVA-NBRI16 isolate is 9942 nucleotides long and contain 5`UTR, a single large predicted ORF (nucleotide positions 460-9769) encoding large polyprotein (387.12 kDa) of 3100 amino acids and 3`UTR (Fig. 2a). The polyprotein further yields the predicted ten mature proteins identified as P1, HC-Pro, P3, 6K1, CI, 6K2, VPg (viral protein genome-linked), Nla-Pro (nuclear inclusion protein a protease), Nlb, and CP having the amino acid number/molecular weight (kDa) of 317/38.0, 457/54.8, 382/45.8, 52/6.2, 635/76.2, 52/6.2, 185/22.2, 241/28.9, 520/62.4, and 259/31.0, respectively (Fig. 2a). The putative proteolytic cleavage sites in NBRI16 isolate are Y/S, G/G, Q/H, Q/S, Q/S, Q/G, E/N, Q/S, and Q/S (Electronic supplementary material 2), and concurred with *Cyrtanthus elatus virus A* (CEVA)-Marijiniup7-1 (NC_017977) and CEVA-Marijiniup7-2 (JQ723475) isolates (Wylie and Jones 2012). The complete nucleotide and deduced large polyprotein sequence of NBRI16 isolate when compared to previously reported potyviruses (Table 1) shared only 93 and 94% identities, respectively, with the only available full-length sequences of CEVA-Marijiniup7-1 and CEVA-Marijiniup7-2 isolates (Table 2). The pairwise sequence comparison at the level of nucleotides and deduced amino acids of all the 10 ORFs revealed 84–97% and 78–97% identities, respectively, with the Marijiniup7-1 and Marijiniup7-2 isolates.

Phylogenetic relationship of NBRI16 isolate under study was inferred with other narcissus infecting full-length potyvirus sequences (Table 1) using maximum-likelihood method and the phylogram was obtained using Tamura-Nei model in MEGA v6.1 program (Tamura et al. 2013). Phylogeny revealed close relationship of NBRI16 isolate with CEVA and grouped it with CEVA-Marijiniup7-1 and Marijiniup7-2 (Fig. 2b) forming a discrete cluster. Phylogeny revealed its distant relationship with narcissus infecting *Narcissus yellow stripe virus* (NYSV) and *Narcissus late season yellows virus* (NLSYV) isolates reported from Australia (Wylie et al. 2010).

Conclusion

This study reports the first full-length genomic sequence of CEVA isolate of *N. tazetta* from India. The information on complete genome will allow the characterization and identification of other CEVA isolates and assist to study the occurring genetic diversity. Since *N. tazetta* is prone to infection with other virus also (Chen et al. 2003; Pearson et al. 2009), CEVA may not be the sole agent causing the chlorotic stripe disease and required further advanced unbiased studies like Illumina sequencing (Wylie and Jones 2011) to fish out the other potyvirus species. The vegetative propagation of CEVA-infected bulbs along with untrained culture practices may be the reason for viral disease dissemination and may exacerbate the cultivation problem in narcissus and other vegetatively propagating plant.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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