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SHORT REPORTS

Complete genome sequence analysis of *Narcissus yellow stripe virus* infecting *Narcissus tazetta* in India

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Abstract
The complete genome sequence of *Narcissus yellow stripe potyvirus* (NYSV) isolated from *Narcissus tazetta* cv. Paperwhite exhibiting leaf chlorotic stripe symptoms was determined for the first time from India. The viral genome sequence contained 9650 nucleotides that encode a large polyprotein (372.36 kDa) of 3103 amino acids. The comparison of the NYSV genome sequences with corresponding sequences of other potyviruses revealed 90–97% identities and closest phylogenetic relationships with NYSV-Zhangzhou-1 and -ZZ-2 isolates infecting *N. tazetta* reported from China. Therefore, the NYSV isolate understudy was considered as a new member of NYSV and designated as NYSV–NAR2.

Keywords *Narcissus tazetta* · Potyvirus · Complete genome · Sequence analyses · *Narcissus yellow stripe virus*

Introduction

Narcissus tazetta L., a member of *Amaryllidaceae* family, is an ornamental plant grown in garden beds and pots for its attractive blooms. It is used as a cut flower for horticulture industry and in perfumery for its sweet fragrance. Cultivation of *N. tazetta* under field/polyhouse conditions has been reported to be affected by a number of RNA viruses of the genera *Carlavirus*, *Maculavirus*, *Nepovirus*, *Potexvirus* and *Potyvirus* (Brunt 1995; Wylie and Jones 2012; Ohshima et al. 2016; Raj et al. 2018). Amongst them *Potyviruses* are most prevalent and found to be more infectious to narcissus which resulted in drastic reduction in its quality and quantity of blooms (Brunt 1995; Aminuddin et al. 1999; Wylie and Jones 2012; Raj et al. 2018).

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Potyviruses are flexuous filamentous particles of 680–900 nm in length and 11–12 nm in width. They belong to the family *Potyviridae* that contain a single stranded positive sense RNA of approximately ten thousand base pair long. Various potyviruses such as *Narcissus yellow stripe virus* (NYSV), *Narcissus late season yellow virus* (NLSYV), *Narcissus degenerate virus* (NDV), *Cyrtanthus elatus virus* A (CyEVA) and *Ornithogalum mosaic virus* (OrMV) have been reported to infect narcissus (Chen et al. 2006; Ohshima et al. 2016). Previously the full genome sequences of NYSV and NLSYV infecting narcissus were published from China and Australia respectively (Chen et al. 2006; Wylie and Jones 2012).

In India, an uncharacterized potyvirus, Lycoris potyvirus and *Cyrtanthus elatus virus*-A (CyEVA) associated with leaf yellow stripe disease of *N. tazeeta* have been investigated (Aminuddin et al. 1999; Yadav and Khan 2008; Kumar et al. 2015). Further, the full length genome sequence of an Indian isolate of CyEVA infecting *N. tazeeta* has also been published recently (Raj et al. 2018). In present study, the complete genome sequence of NYSV infecting *N. tazeeta* cv. Paperwhite plants is being reported first time from India.

Materials and methods

The leaf samples of *N. tazetta* cv. Paperwhite exhibiting chlorotic leaf stripes were collected from the cultivated field at NBRI, Lucknow. Partial purification of virus was

done and transmission electron microscopy (TEM) was carried out as described earlier (Kaur et al. 2015). The partially purified virus was inoculated on *Chenopodium album*, *Datura innoxia*, and *Narcissus tazetta* plants at 3–4 leaf stage. After inoculation local and systemic symptoms on inoculated plants were recorded up to 35 days post inoculation (dpi).

For virus detection, the total RNA was extracted from 100 mg leaf tissue of infected narcissus using TRI reagent (Sigma Aldrich, Missouri, USA) and reverse transcription-PCR was performed using potyvirus degenerate primers (Pot-I/Pot-II, Gibbs and Mackenzie 1997). For full length genome amplification, the four set of primers: Pot-I/Pot-II, CI-F/NIb-Pot-3(Ha et al. 2008), HP-F/CI-R (Yakoubi et al. 2008) and 5′RACE/HP-R (Lucinda et al. 2010) yielding the expected size amplicons of ~ 1.5, 3.0, 3.0 and 2.0 kb, respectively were used following the strategy described as earlier (Raj et al. 2018). For amplification of 5′ end, the First Choice RLM-RACE kit was employed. The size of DNA fragment was assessed on 1% agarose gel with comparison of 1 kb DNA ladder. Amplicons were gel eluted using Wizard® SV gel and PCR clean-up system (Promega, USA). The eluted products were ligated into pGEM-T vector and transformed into *E. coli* (DH5α) cells. The positive transformants were screened by colony PCR and clones were sequenced. The obtained sequence data were edited and assembled using BIOEDIT tool (http://www.mbio.ncsu.edu/bioedit/bioedit.html) to eliminate any sequence ambiguity. The consensus sequence for full length genome was determined and submitted to GenBank.

The NCBI tool open reading frames (ORF) finder (www.ncbi.nlm.nih.gov/projects/gorf/) was used to analyze the ORFs encoded by the genome and their putative proteins were translated using *ExPasy* tool (http://web.expasy.org/translate/). The complete analyzed sequence of the potyvirus isolate was compared with respective sequences of other potyviruses available in NCBI using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for potyvirus identification. *DiAlign* tool (http://www.genomatix.de/cgi-bin/dialign/dialign.pl) was used to obtain the nucleotide and amino acid identity of ORFs of selected potyvirus isolates. The phylogenetic analysis of full genome sequence was also performed by Molecular Evolutionary Genetic Analysis (MEGA) v6.1 tool using Maximum Likelihood algorithm at 1000 boostrap value (Tamura et al. 2013).

Further, the presence of NYSV in infected *N. tazetta* samples were also confirmed by nucleic acid spot hybridization (NASH) assay performed as described earlier (Raj et al. 2018) using the probe prepared from cloned NYSV (KM066972). The 2 µg RNA of uninfected *N. tazetta* plant was used as negative control whereas 200 ng cloned DNA of NYSV (KM066972) was used as positive controls. Blotted membranes were hybridized with probe prepared from cloned NYSV (KM066972) by random primer labelling method and pre-hybridization, hybridization and washing steps were performed following the standard method as described by Raj et al. (2018). The hybridization signals were observed after exposure to phosphor imaging screen in Typhoon Imaging Phosphor imager (GE Healthcare Life Sciences, USA).

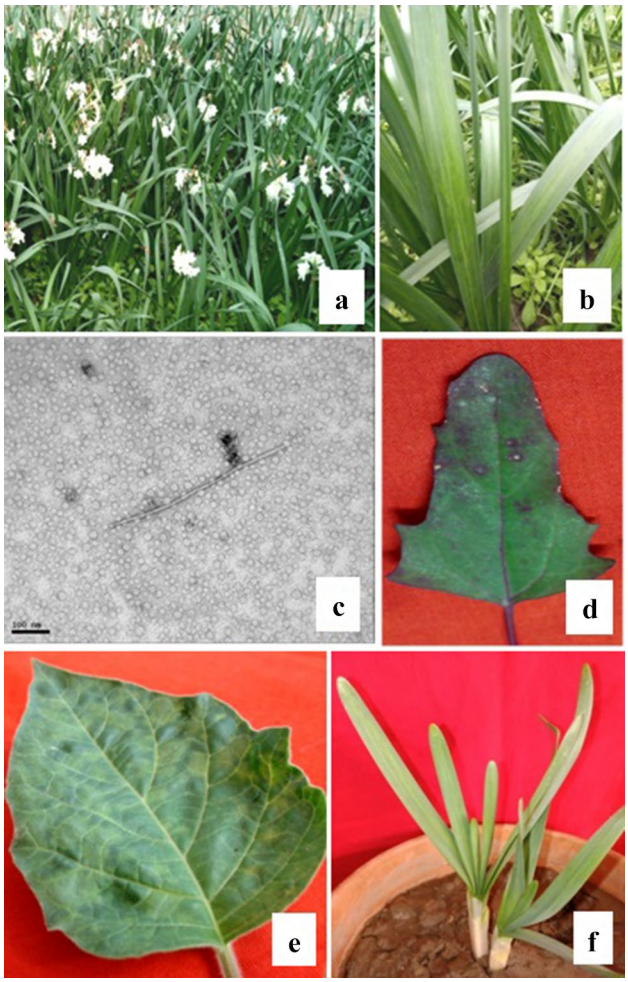

Fig. 1 **a** Field view of *N. tazetta* plants and a close view of diseased *N. tazetta* showing yellow stripes on leaves (**b**). TEM of partially purified preparation of infected *N. tazetta* showing a typical flexuous rod shaped virus particle of 680 nm × 11 nm in size, bar 100 nm, (**c**). Partially purified virus inoculated plants showing symptoms: necrotic local lesions on *C. album* at 10 dpi (**d**), systemic mosaic on *D. innoxia* at 30 dpi (**e**) and systemic yellow stripes on *N. tazetta* at 35 dpi (**f**)

Results and discussion

During survey in 2014, the *N. tazetta* plants showing chlorotic stripe disease symptoms with 99% disease incidence were observed in a cultivated field at NBRI, Lucknow. Virus

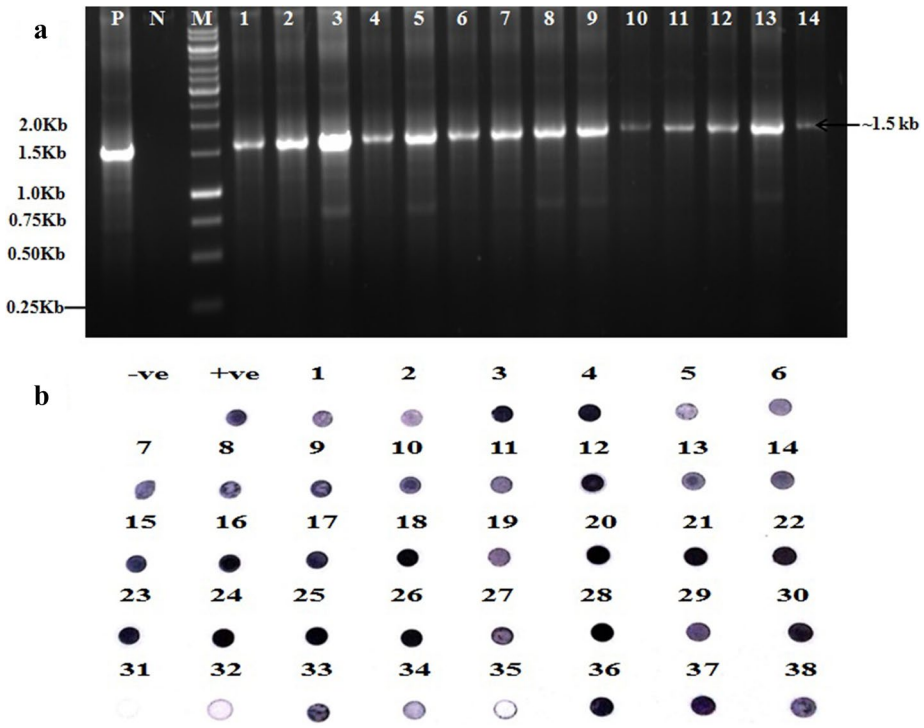

Fig. 2 **a** RT-PCR using potyvirus degenerate primers (Pot-I/Pot-II, Gibbs and Mackenzie 1997) showing ~ 1.5 kb band in all 14 symptomatic samples of *N. tazetta* (lanes 1–14) similar as in a positive control (P) but not in healthy *N. tazetta* plants (N). *M* = 1.0 kb DNA ladder marker. **b** Nucleic acid spot hybridization test using a probe developed from cloned coat protein gene of NYSV (KM066972) showing positive signals of hybridization with the probe in 37 *N. tazetta* leaf samples similar as a positive control (*P* cloned DNA of NYSV) but not in a negative control (*N* healthy *N. tazetta*)

infected plants showed chlorotic stripes on leaves and stunting symptoms (Fig. 1a, b). More or less the similar disease symptoms were described earlier on narcissus plants infected by NYSV, OrMV, NLSYV and CyEVA potyviruses (Chen et al. 2006; Yadav and Khan 2015; Wylie and Jones 2012; Wylie et al. 2014; Raj et al. 2018).

The initial virus detection was done by TEM from the partially purified virus preparation, where the typical flexuous rod-shaped virus particles of 680 nm × 11 nm were observed (Fig. 1c) that indicated the presence of potyviruses as reported earlier (Kaur et al. 2015). Further the purified virus was inoculated on some host plants viz., *C. album*, *D. innoxia*, and *N. tazetta*. The virus inoculated *C. album* plant developed local necrotic lesions at 10 days post inoculation (dpi) while *D. innoxia* developed systemic mosaic at 30 dpi, and *N. tazetta* developed yellow stripe at 35 dpi (Fig. 1d–f).

The presence of potyvirus was confirmed by RT-PCR using degenerate primers of potyvirus Pot-I/Pot-II (Gibbs and Mackenzie 1997). The RT-PCR resulted in successful amplification of expected size ~ 1.5 kb band in all 14 symptomatic narcissus samples (Fig. 2a). Sequence analysis of randomly selected two cloned PCR products: accession numbers JQ686724 (NAR-1) and KM066972 (NAR-2) suggested the presence of NYSV potyvirus in *N. tazetta*. The presence of NYSV in other samples of *N. tazetta* showing leaf stripe symptoms in field were also confirmed by NASH assay using the probe prepared from cloned NYSV (KM066972). The result revealed presence of NYSV in 37 out of 38 samples that showed positive signals of hybridization with the probe. Among them 35 sample showed the strong signals similar to that of a positive control while two samples showed weak signals (Fig. 2b) confirming ~ 99% disease incidence in field.

Further, the sample NAR-2 representing partial genome sequence of NYSV was selected for full length genome amplification by RT-PCR and RACE using the available potyvirus degenerate primers targeting the different conserved motifs of genome (Gibbs and Mackenzie 1997; Ha et al. 2008; Yakoubi et al. 2008; Lucinda et al. 2010). The Pot-I/Pot-II primers amplified the ~ 1.5 kb long region of polyadenylated 3′ end of 3′UTR and -GNNS- motif of partial nuclear inclusion B (Nlb) region (Fig. 3a-i) whereas, the other CI-F/NIb-Pot-3 primers amplified the GxVGSGKST motif in cylindrical inclusion (CI) protein and partial Nlb region of ~ 3.0 kb in size (Fig. 3a-ii). The region from partial CI to HC region of ~ 3.0 kb was amplified using HP-F/CI-R primers (Fig. 3a-iii). Finally RACE kit was used for amplification of remaining 5′ end to HC region of ~ 2.0 kb in size (Fig. 3a-iv). The amplified products were cloned, sequenced and assembled sequence data as full length genome was deposited in NCBI GenBank database under the accession number KU516386 (NYSV-NAR2).

The full-length genome of NAR2 isolate (KU516386) was of 9650 nucleotides long excluding the polyA tail. The genome organisation was found similar to a typical member of genus potyvirus (Fig. 3b). The translated product of the ORF contained large polyprotein of 372.36 kDa in size with 3103 amino acids. This polyprotein further yields ten well known proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg,

NIa-Pro, Nib and CP) along with a newly identified PIPO protein (Fig. 3b). These 11 proteins have amino acids/molecular weight of 317/38.0, 458/54.9, 55/6.2, 354/42.4, 644/77.3, 53/6.4, 191/22.9, 243/29.2, 513/61.6, 277/33.2 and 68/8.2, respectively (Fig. 3b). Further, the NYSV-NAR2 isolate contained some putative proteolytic cleavage sites as Y/S, G/G, Q/T, Q/S, Q/S, E/A, E/S, Q/M and Q/M (Fig. 3b) identified from alignments with full length polyprotein sequences of available potyviruses in NCBI database. The proteolytic cleavage sites in NYSV-NAR2 isolate were similar as in case of NYSV isolates (Wylie et al. 2014) with exception of Q/M at the NIb/CP junction instead of Q/S. The NLSYV-Zhangzhou, Marijiniup8 and Marijiniup9 isolates revealed differences at two cleavage sites, one at P1/HC-Pro junction where they possess F/S or F/T instead of Y/S amino acid combination and second at P3/6K1 junction having Q/V or Q/A instead of Q/T (Data not shown). Conserved potyvirus motifs like FRNK and IGN in the central region of the HC-Pro (Shiboleth et al. 2007; Cronin et al. 1995), GDD in the Nlb, and DAG motif in the CP region (Peng et al. 1998) are also present in NYSV-NAR2 isolate.

The full length genome data of the NYSV-NAR2 isolate was also compared with respective sequences of other potyviruses available in NCBI (Table 1) by BLASTn tool for virus identification. During BLASTn analysis, the NYSV-NAR2 isolate (KU516386) shared highest 93% identities with complete nucleotide sequence of NYSV of Chinese narcissus (AM158908, Shuang et al. 2012). It also shared 82% identities with two Narcissus virus-1 isolates of Japan (LC31498, LC31499) and 76% identities with NYSV-ZZ2 isolate of China (JQ911732), two NYSV isolates of Japan (LC31493, LC31495) and 74% identities with two isolates of NLSYV–Marijiniup8 (KC691259, Wylie et al. 2014) of Australia and NLSYV isolate Zhangzhou (JQ326210) of China.

The Genomatix tool was used to identify ORFs from nucleotide and amino acid sequence of the selected potyvirus isolates (Table 2). The pairwise sequence comparisons of NYSV-NAR2 isolate (KU516386) with other potyvirus isolates considered for study at complete genome nucleotides and deduced amino acids of polyprotein gene revealed highest 90% and 97% identities, respectively with NYSV-Zhangzhou-1 and ZZ-2 isolates reported from China

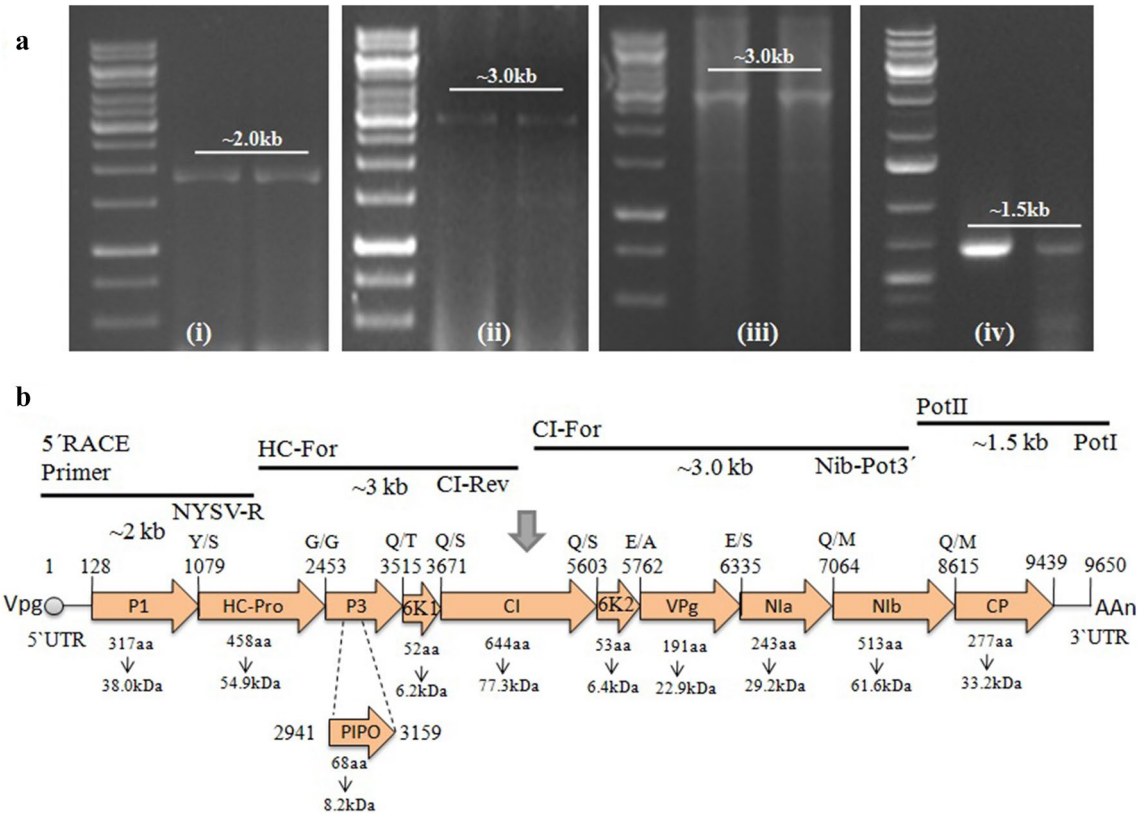

Fig. 3 **a** RT-PCR products of full length genome of NYSV using various potyvirus specific pair of primers: (i) 5′ RACE Inner primer/NYSV-R, (ii) HPFor/CIRev (iii) CIFor/NIbPot-3′ and (iv) Pot I/Pot II showing ~ 2.0 kb, 3.0 kb, 3.0 kb and 1.5 kb bands, respectively in NYSV infected narcissus samples (lanes 1 and 2). *M* = 1 kb DNA ladder marker. **b** The genome organization of NYSV-NAR2 isolate (KU516386). Arrow indicates the size, location and orientation of ORFs in the potyvirus genome understudy. Below the arrow, the number of amino acids and predicted molecular weight (in kDa) is shown. The polyadenylated tail is shown by AAn and VPg abbreviates for viral protein genome-linked

Table 1 Details of virus isolates of NYSV and other potyvirus isolates used in present and previous studies

Virus	Isolate	Host	Location	Genome length (nt)	GenBank Accession	Source
NYSV	NAR2	*Narcissus tazetta*	India	9650	KU516386	This study
NYSV	NY-OI1	*Narcissus tazzeta*	Japan	9626	LC314391	Ohshima et al. (2016)
NYSV	NY-KM1O	*Narcissus tazzeta*	Japan	9637	LC314392	Ohshima et al. (2016)
NYSV	NY-KM1P	*Narcissus tazzeta*	Japan	9639	LC314393	Ohshima et al. (2016)
NYSV	NY-CB5	*Narcissus tazzeta*	Japan	9630	LC314394	Ohshima et al. (2016)
NYSV	NY-EH173	*Narcissus tazzeta*	Japan	9630	LU314395	Ohshima et al. (2016)
NYSV	NY-HG19	*Narcissus tazzeta*	Japan	9629	LU314396	Ohshima et al. (2016)
NYSV	NY-HG27	*Narcissus tazzeta*	Japan	9629	LU314397	Ohshima et al. (2016)
NYSV	Zhangzhou-1	*Narcissus tazetta*	China	9650	AM158908	Chen et al. (2006)
NYSV	Zhangzhou-2	*Narcissus tazetta*	China	9650	NC_011541	Chen et al. (2006)
NYSV	ZZ2	*Narcissus tazetta*	China	9654	JQ911732	Shuang et al. (2012)
NYSV	Marijiniup3	*Narcissus* spp.	Australia	9647	JQ395042	Wylie and Jones (2012)
NLSYV	Zhangzhou	*Narcissus tazetta*	China	9651	JQ326210	Jenner et al. (2000)
NLSYV	Marijiniup8	*Narcissus* spp.	Australia	9687	KC691259	Wylie et al. (2014)
NLSYV	Marijiniup9	*Narcissus* spp.	Australia	9577	JX156421	Wylie et al. (2014)
CyEVA	NBRI16	*Narcissus tazetta*	India	9942	KX575832	Raj et al. (2018)
CyEVA	Marijiniup7-1	*Cyrtanthus elatus*	Australia	9908	NC_017977	Wylie and Jones (2012)
ScaMV	-1	*Allium chinense*	China	9324	NC_003399	Brunt (1995)
ScaMV	-2	*Allium chinense*	China	9324	AJ316084	Chen et al. (2006)
TuMV	TIGD	*Tigridia* spp.	Germany	9798	AB701735	Nguyen et al. (2013)
TuMV	NZ403B	*Lepidium oleraceum*	New Zealand	9796	AB989658	Yasaka et al. (2015)
JYMV	M	*Dioscorea japonica*	Japan	9760	NC_000947	Chen et al. (2006)
PWV	MU2	*Passiflora caerulea*	Australia	9682	NC_014790	Wylie et al. (2014)

Virus acronyms: NYSV = *Narcissus yellow stripe virus*, JYMV = *Japanese yam mosaic virus*, NLSYV = *Narcissus late season yellows virus*, CyEVA = *Cyrtanthus elatus virus*-A, ScaMV = *Scallion mosaic virus*, TuMV = *Turnip mosaic virus*, PWV = *Passion fruit woodiness virus*

infecting *N. tazetta* cv. Chinensis (Table 2). While the identities were 58–74% and 77–85% at nt of complete genome and at aa of polyprotein gene, respectively of other NYSV isolates considered for the study. It also showed 54-55% (nt) and 75% (aa) identities with Zhangzhou, Marijiniup8 and Marijiniup9 isolates of NLSYV reported from China and Australia, respectively (Table 2).

The phylogeny analysis of NYSV-NAR2 isolate with eleven full genome sequences of NYSV isolates (Zhangzhou-1, Zhangzhou-2, ZZ2, NY-OI1, NY-KM1O, NY-KM1P, NY-CB5, NY-EH173, NY-HG19, NY-HG27 and M3) along with other potyvirus sequences (Table 1) was performed. During the analysis, NYSV-NAR2 isolate clearly grouped with NYSV members and showed close phylogenetic relationships with them (Fig. 4). The clustering also revealed a close homology of NYSV members with NLSYV reported on *N. tazetta* (Wylie et al. 2010), whereas distant relationships with other potyviruses. The results of BLASTn analysis (90% identities) and close phylogenetic relationship with NYSV members was at par with recommendations of International Committee on Taxonomy of Viruses (ICTV, Adams et al. 2005), hence the NYSV-NAR2 isolate (KU516386) was considered as a new member of NYSV.

It is suggested by Wylie and Jones (2012) that the vegetative propagation of narcissus through bulbs and its international trading favours accumulation and spreading of potyviruses from one place to other, therefore, the nucleic acid hybridization test and RT-PCR utilizing NYSV probe and potyvirus specific primers, respectively may help in diagnosis of NYSV and other potyviruses in narcissus bulbs to check their dissemination from one to other field/country.

Conclusion

The present study reports the complete genome sequence of 9650 nucleotides of *Narcissus yellow stripe virus* (NYSV-NAR2, KU516386) for the first time from India as per best of our knowledge. The viral genome sequence of NYSV-NAR2 isolate shared highest 90 and 97% identities at nucleotides and deduced amino acid levels, respectively with NYSV-Zhangzhou-1 and ZZ-2 isolates reported from

Table 2 Percent (%) identities of full length genome of NAR-2 isolate (KU516386) with respective sequences of other potyviruses at nucleotides and amino acid levels obtained using *DiAlign* tool

GenBank accession	Virus	Isolate	Location	Percent identity										
				Complete genome (poly-peptide)	Open reading frames									
					P1	HC-Pro	P3	6K1	CI	6K2	VPg	NIa	NIb	CP
AM158908	NYSV	Zhangzhou-1	China	90 (97)	100 (100)	86 (93)	100 (99)	100 (100)	83 (96)	77 (94)	87 (97)	100 (100)	92 (96)	95 (96)
NC_011541	NYSV	Zhangzhou-2	China	90 (97)	100 (100)	86 (93)	100 (99)	100 (100)	83 (96)	77 (94)	87 (97)	100 (100)	92 (96)	95 (96)
LU314396	NYSV	NY-HG19	Japan	74 (85)	36 (42)	77 (88)	57 (66)	64 (87)	88 (96)	96 (100)	92 (99)	82 (98)	86 (93)	90 (97)
LU314397	NYSV	NY-HG27	Japan	72 (84)	37 (42)	77 (88)	54 (67)	65 (87)	86 (95)	96 (94)	90 (97)	83 (98)	85 (92)	89 (97)
LC314391	NYSV	NY-OI1	Japan	73 (84)	41 (42)	70 (87)	59 (68)	66 (84)	75 (94)	77 (94)	86 (94)	98 (99)	90 (96)	94 (96)
LC314393	NYSV	NY-KM1P	Japan	60 (78)	36 (42)	67 (84)	56 (63)	70 (81)	69 (90)	62 (64)	67 (82)	72 (90)	71 (85)	73 (85)
LC314394	NYSV	NY-CB5	Japan	59 (78)	30 (41)	66 (89)	60 (65)	67 (78)	68 (92)	64 (70)	72 (81)	69 (86)	67 (84)	72 (88)
JQ911732	NYSV	ZZ-2	China	59 (78)	29 (38)	63 (85)	60 (65)	52 (71)	70 (91)	61 (61)	68 (79)	69 (85)	67 (84)	71 (88)
LU314395	NYSV	NY-EH173	Japan	58 (78)	33 (40)	67 (90)	65 (65)	59 (75)	69 (91)	66 (70)	70 (81)	71 (86)	68 (84)	73 (88)
LC314392	NYSV	NY-KM1O	Japan	58 (77)	39 (41)	66 (85)	53 (63)	66 (81)	69 (90)	58 (58)	65 (82)	73 (91)	69 (84)	71 (85)
JQ395042	NYSV	Marijiniup 3	Australia	58 (78)	35 (42)	67 (84)	54 (59)	69 (84)	70 (88)	60 (70)	65 (82)	72 (90)	69 (85)	71 (81)
JQ326210	NLSYV	Zhangzhou	China	55 (75)	33 (38)	65 (83)	55 (64)	71 (84)	65 (88)	64 (64)	71 (83)	71 (87)	63 (80)	68 (81)
KC691259	NLSYV	Marijiniup8	Australia	54 (75)	32 (36)	64 (83)	56 (64)	63 (78)	67 (84)	68 (70)	67 (82)	66 (88)	64 (79)	68 (81)
JX156421	NLSYV	Marijiniup9	Australia	54 (75)	35 (39)	67 (83)	56 (65)	73 (84)	65 (73)	66 (64)	69 (84)	68 (87)	65 (79)	68 (80)
AJ316084	ScaMV	–	China	43 (63)	25 (22)	56 (66)	41 (47)	60 (65)	53 (73)	45 (47)	61 (70)	57 (70)	58 (71)	58 (78)
NC_003399	ScaMV	–	China	43 (63)	25 (22)	56 (66)	41 (47)	60 (65)	53 (73)	45 (47)	61 (70)	57 (70)	58 (71)	58 (78)
AB701735	TuMV	TIGD	–	42 (59)	15 (15)	58 (68)	25 (36)	56 (75)	55 (74)	48 (29)	53 (65)	60 (72)	62 (73)	64 (76)
NC_000947	JYMV	–	–	40 (57)	19 (19)	53 (72)	40 (36)	58 (65)	55 (70)	52 (35)	55 (66)	50 (54)	57 (69)	56 (72)
AB989658	TuMV	NZ403B	New Zealand	39 (60)	21 (23)	55 (67)	30 (37)	64 (78)	55 (74)	54 (29)	54 (64)	59 (72)	59 (74)	60 (75)
NC_014790	PWV	PWV-MU2	Australia	25 (42)	13 (13)	35 (47)	19 (22)	23 (40)	44 (55)	40 (29)	37 (44)	37 (45)	44 (57)	50 (65)
NC_017977	CyEVA	Marijiniup7-1	Australia	21 (38)	16 (15)	33 (41)	16 (17)	42 (43)	34 (52)	35 (23)	34 (37)	43 (39)	43 (54)	48 (58)
KX575832	CyEVA	NBRI16	India	20 (36)	10 (15)	32 (39)	15 (14)	37 (40)	32 (50)	18 (23)	33 (43)	41 (39)	46 (55)	44 (59)

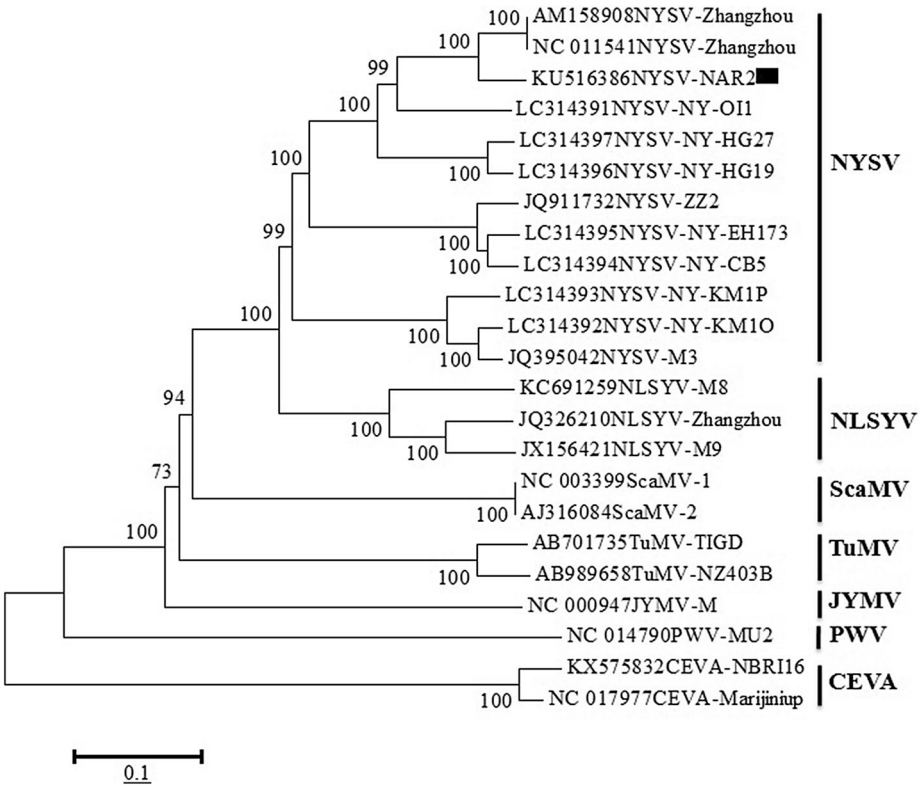

Fig. 4 Phylogenetic tree representing the relationships of NYSV-NAR2 isolate (KU516386) with other potyviruses. The phylogenetic tree was constructed using MEGA v6.1 tool (Tamura et al. 2013) and using maximum likelihood algorithm at 1000 bootstrap value

China infecting *N. tazetta*. It also showed closest phylogenetic relationships with full-length genome sequences of two Zhangzhou isolates of NYSV while distant relationships with other potyvirus isolates, therefore, the virus isolated form *N. tazetta* has been considered as a new member of NYSV and designated as NYSV-NAR2. The information on complete genome of NYSV-NAR2 available from present study will also help in designing the efficient management strategy through genetic engineering.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of Interest.

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