Molecular characterization of *Turnip mosaic potyvirus* (*TuMV*)-infecting radish (*Raphanus sativus* L.) crop in India

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Abstract Nine isolates of *Turnip mosaic potyvirus* (*TuMV*)-infecting radish collected from different regions of Northern India were characterized. All isolates except for New Delhi and Rajasthan isolates resulted positive for *TuMV* in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). RNA was isolated from leaves of infected plants and used in reverse transcriptase-polymerase chain reaction (RT-PCR) with *TuMV* coat protein (CP) gene-specific primers. Viral amplicons of expected 1000 bp size were obtained, which were further subjected to cloning and sequencing. *CP* gene of all the seven isolates was 867 bp long, encoding 288 amino acid residues. Percent homology of *CP* gene of all the Indian isolates among themselves and with other *TuMV* isolates retrieved from NCBI was in the range of 87–99 and 92–100% at nucleotide and amino acid levels, respectively. Phylogenetic analysis based upon *CP* gene nucleotide and amino acid sequences with other *TuMV* isolates reported from across the globe using unweighted pair group method with arithmetic mean (UPGMA) inferred classification of test isolates into basal-BR group due to their occurrence nearest to the *TuMV* isolates belonging to the basal-BR group. Information generated about the characteristic features of *TuMV* and geographical distribution of particular virus genotype-infecting radish crop will provide a platform for formulating disease resistance strategies.

Keywords *TuMV* · *Raphanus sativus* · DAS-ELISA · *CP* gene · Molecular characterization

Introduction

Radish (*Raphanus sativus* L.) is an important edible root vegetable crop grown worldwide, especially in Asia (Li et al. 2017). The napiform taproot is the most popular part of radish for eating and leafy portion is used as a vegetable. Due to a number of health benefits, it has been used by people with different gastrointestinal, hepatic, urinary, respiratory and cardiovascular disorders. However, radish is prone to numerous fungal, bacterial and viral diseases. Among them, *Turnip mosaic potyvirus* (*TuMV*), a member of the genus *Potyvirus* in the family *Potyviridae*, is one of the most devastating yield constraints of radish (Li et al. 2017). Members of this genus are characterized by long (700–900 nm) flexuous particles with a positive-sense single stranded poly A-tailed ribonucleic acid (RNA) molecule of approximately 10 kb size. *TuMV*-infected radish plants exhibit typical symptoms including stunting, mosaic, chlorosis, leaf distortion, mottling as well as malformed flowers, stalks and fruits, in addition to production of brown spots on the roots leading to reduction in plant vigor, root and seed weight.

Studies of the molecular evolutionary history of viruses help to provide an understanding of important features of their biology such as changes in virulence and geographical ranges and their emergence as new epidemic that is essential for designing strategies for controlling viruses. To control and minimize losses from *TuMV*, there is a need for an effective and rapid diagnostic technique to identify this virus in plant material and to assess the impact of virus infection on commercially grown crop like radish. At

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present, field diagnosis of *TuMV* infection is based on visual symptoms, which are highly variable depending on the growth stage at which the plant is infected. A plethora of new and non-characterized *potyviruses* isolated from diverse geographical locations necessitates the development of an easy, rapid and efficient method for their accurate identification and characterization.

Consequently, DNA-based diagnostic approaches using PCR amplification and DNA sequencing technologies have expedited the detection, identification and classification of *potyviruses* (Zhu et al. 2016). Many attempts had been made in the past to analyze *TuMV* variation at the nucleotide and amino acid levels (Nyalugwe et al. 2015; Valouzi et al. 2017; Yasaka et al. 2017). Previous studies based on phylogenetic analysis using different virus isolates collected from around the world revealed four main *TuMV* genogroups called basal-*Brassica* (basal-B), basal-*Brassica/Raphanus* (basal-BR), Asian-*Brassica/Raphanus* (Asian-BR) and world-*Brassica* (world-B) (Li et al. 2017). The *CP* gene is a highly variable region in *potyvirus* genome and has been widely accepted for classification of isolates in this genus. Molecular characterization of a virus is a pre-requisite for selection of control strategies based on expression of viral genes in a host. Therefore, the present investigation had been planned with the objectives of serological detection of *TuMV*-infecting radish and its further molecular characterization using molecular biology and in silico tools.

Materials and methods

Plant material and serological detection of *TuMV*

Extensive surveys were conducted in different radish growing regions of northern India including Himachal Pradesh (Solan, Shimla, Kinnaur & Mandi), Chandigarh, Punjab (Ludhiana), Haryana (Karnal), New Delhi (West Patel Nagar) and Rajasthan (Bharatpur) in the cropping season to ascertain the distribution and incidence of *TuMV* in this crop. All the test samples were subjected to DAS-ELISA (with ELISA kit containing the polyclonal anti-serum recognizing all *TuMV* isolates) for detection of *TuMV* (Clark and Adams 1977).

cDNA synthesis and RT-PCR

Total plant RNA was isolated from 50 mg of healthy and infected radish leaf tissues of different isolates using plant RNA extraction kit (Real Biotech Corporation, Taiwan). For reverse transcription reaction, 2 µl of total RNA of each isolate was mixed with 5 µl of 5X RT buffer, 1 µl of 30 mM dNTP mix, 1.2 µl (20 pmol) of reverse primer (5'-

TATTTCCCATAAGCGAGAATAC-3'), 1 µl (10 units) RNase inhibitor, 1 µl (50 units) of M-MuLVRT (Genei) and Milli Q water to make a final volume of 25 µl. The reaction mixture was incubated at 37 °C for an hour. PCR was carried out in an automated thermocycler (ABI, USA). For cDNA amplification, total reaction mixture of 25 µl was prepared for each isolate which comprised of 2.5 µl of cDNA product, 0.6 µl (10 pmol) of forward primer (5'-CAAGCAATCTTTGAGGATTATG-3'), 0.6 µl (10 pmol) of reverse primer (5'-TATTTCCCATAAGCGAGAATAC-3'), 2.5 µl of 10× Taq DNA polymerase buffer, 1 µl of 10 mM dNTP mix, 0.5 µl (1.5 units) of Taq DNA polymerase (Genei, Bangaluru) and 17.30 µl of Milli Q water. PCR program consisted of initial denaturation step at 94 °C for 4 min, 35 cycles of denaturation at 94 °C for 20 s, annealing at 53.5 °C for 30 s and extension at 72 °C for 1 min, followed by final primer extension at 72 °C for 5 min. PCR-amplified products were electrophoretically separated on 0.8% agarose gel containing 0.01% ethidium bromide, prepared in 1× TAE (Tris–Acetic acid–EDTA). After electrophoresis, the amplification products were visualized in a gel documentation system (IG/LHR, Syn-gene, UK). After this step, specific DNA fragments (*CP* gene amplicons) were eluted using QIAquick gel extraction kit (Qiagen, Germany) as per the manufacturer's protocol.

Cloning, sequencing and phylogenetic analysis

The pPrime cloning vector was used for cloning of PCR products. *E. coli* strain JM 107 cells were made competent and transformed with ligated vector following heat shock method. These putative transformed cells were then allowed to grow on Luria–Bertani agar medium supplemented with 50 mg/ml kanamycin. The positive transformants (forming white colonies) were inoculated in 5 ml of LB medium containing kanamycin (50 µg/ml) in sterile capped culture tubes and incubated overnight at 37 °C at 180 rpm in a shaking incubator. The recombinant DNA from these bacterial colonies was isolated using HiYield™ Plasmid mini kit (Real Biotech Corporation, Taiwan) as per the manufacturer's instructions (Brinboim and Doly 1979). Restriction digestion was done with restriction enzyme Bam HI and Hind III (Bio Labs, England) to release the cloned insert of coat protein gene of seven *TuMV* isolates (IND1–IND7) from putative recombinant plasmids followed by their sequencing at Xcleris Lab, Ahmedabad, India.

Analysis of the raw sequence data was done using Chromas Pro (version 1.5) of Technelysium Pvt. Ltd., Australia. All the seven *TuMV* coat protein gene sequences (IND1–IND7) of Indian isolates were further analyzed using BLASTN and BLASTP to know their homology among themselves and with other sequences of *TuMV*

Table 1 List of *TuMV* isolates used in the present investigation for bioinformatics analysis

S. no.	Accession no. (nucleotide)	Description	Isolate/ strains	Original host	Location	Accession no. (protein)
1	AF434720	<i>TuMV</i> isolate BEL 1 coat protein gene, partial cds	BEL1	<i>Rorippa nasturtium-aquaticum</i>	Belgium	AAN63599
2	AY395796	<i>TuMV</i> coat protein gene, partial cds	ESC8	<i>Impatiens balsamina</i>	USA	AAQ96730
3	AF434726	<i>TuMV</i> isolate PV 377 coat protein gene, partial cds	PV377	<i>Alliaria officinalis</i>	Italy	AAN63605
4	AF434725	<i>TuMV</i> isolate PRT 1 coat protein gene, partial cds	PRT1	<i>Brassica oleracea</i>	Madeira, Portugal	AAN63604
5	AF434724	<i>TuMV</i> isolate JPN 1 coat protein gene, partial cds	JPN1	<i>Raphanus sativus</i>	Japan	AAN63603
6	AF434722	<i>TuMV</i> isolate DEU 2 coat protein gene, partial cds	DEU2	<i>R. sativus</i>	Germany	AAN63601
7	AF434721	<i>TuMV</i> isolate CHN 1 coat protein gene, partial cds	CHN1	<i>Brassica</i> sp.	Taiwan	AAN63600
8	AF320127	<i>TuMV</i> polyprotein gene, coat protein region, partial cds	ZCH	<i>B. napus</i>	Zhejiang, China	AAG41203
9	AY495713	<i>TuMV</i> coat protein gene, partial cds	WCH	<i>R. sativus</i>	Weifang, China	AAR87497
10	AF316361	<i>TuMV Crepidiastrum denticulatum</i> coat protein gene, partial cds	CDT	<i>Crepidiastrum denticulatum</i>	South Korea	AAK01628
11	AF316360	<i>TuMV Rorippa islandica</i> coat protein gene, partial cds	RSD	<i>Rorippa islandica</i>	South Korea	AAK01627
12	AF316359	<i>TuMV Rorippa indica</i> coat protein gene, partial cds	RID	<i>R. indica</i>	South Korea	AAK01626
13	AY941820	<i>TuMV</i> isolate Weifang coat protein gene, partial cds	WFLB2	<i>R. sativus</i>	Shandong, China	AAX46324
14	AF539407	<i>TuMV</i> -SD1 coat protein mRNA, partial cds	TuMV-SD1	<i>B. rapa</i> ssp. <i>pekinensis</i>	Shandong, China	AAO17364
15	AF539408	<i>TuMV</i> -SD2 coat protein (CP) mRNA, partial cds	TuMV-SD2	<i>B. rapa</i> ssp. <i>pekinensis</i>	Shandong, China	AAO17365
16	AF539409	<i>TuMV</i> -SD3 coat protein (CP) mRNA, partial cds	TuMV-SD3	<i>B. rapa</i> ssp. <i>pekinensis</i>	Shandong, China	AAO17366
17	AF539410	<i>TuMV</i> -SD4 coat protein mRNA, partial cds	TuMV-SD4	<i>R. sativus</i>	Shandong, China	AAO17367
18	AF539411	<i>TuMV</i> -SD5 coat protein mRNA, partial cds	TuMV-SD5	<i>R. sativus</i>	Shandong, China	AAO17368
19	AF539412	<i>TuMV</i> -SD6 coat protein mRNA, partial cds	TuMV-SD6	<i>R. sativus</i>	Shandong, China	AAO17369
20	AF434723	<i>TuMV</i> isolate ITA 6 coat protein gene, partial cds	ITA 6	<i>Matthiola incana</i>	Italy	AAN63602
21	DQ228764	<i>TuMV</i> isolate BJ1 coat protein mRNA, partial cds	BJ1	<i>B. oleracea</i>	China	ABB58730
22	EF061745	<i>TuMV</i> isolate ESP 1 coat protein gene, partial cds	ESP 1	<i>Eruca vesicaria</i> subsp. <i>sativa</i>	Spain	ABN71586
23	EF061746	<i>TuMV</i> isolate ESP 2 coat protein gene, partial cds	ESP 2	<i>Sisymbrium orientale</i>	Spain	ABN71587
24	EF061747	<i>TuMV</i> isolate ESP 3 coat protein gene, partial cds	ESP3	<i>B. cretica</i>	Spain	ABN71588
25	EF061748	<i>TuMV</i> isolate ESP 4 coat protein gene, partial cds	ESP4	<i>B. napus</i>	Spain	ABN71589
26	EF061749	<i>TuMV</i> isolate ESP 5 coat protein gene, partial cds	ESP5	<i>B. juncea</i>	Spain	ABN71590
27	DQ228765	<i>TuMV</i> isolate BJ2 coat protein mRNA, partial cds	BJ2	<i>B. juncea</i>	China	ABB58731

Table 1 continued

S. no.	Accession no. (nucleotide)	Description	Isolate/ strains	Original host	Location	Accession no. (protein)
28	Y09144	<i>TuMV</i> mRNA for coat protein, partial	CZE1	<i>B. oleracea</i>	Czech Republic	–
29	X81140	<i>TuMV</i> mRNA for coat protein	CDN1	<i>B. napus</i>	Canada	–
30	X65978	<i>TuMV</i> gene for coat protein	UK1	–	United Kingdom	CAA46793
31	JQ246074	<i>TuMV</i> isolate IND 1 coat protein gene, partial cds	IND1	<i>R. sativus</i>	Chandigarh, India	AFE55681
32	JQ246075	<i>TuMV</i> isolate IND 2 coat protein gene, partial cds	IND2	<i>R. sativus</i>	Haryana, India	AFE55682
33	JQ246076	<i>TuMV</i> isolate IND 3 coat protein gene, partial cds	IND3	<i>R. sativus</i>	Punjab, India	AFE55683
34	JQ246077	<i>TuMV</i> isolate IND 4 coat protein gene, partial cds	IND4	<i>R. sativus</i>	Mandi, India	AFE55684
35	JQ246078	<i>TuMV</i> isolate IND 5 coat protein gene, partial cds	IND5	<i>R. sativus</i>	Solan, India	AFE55685
36	JQ246079	<i>TuMV</i> isolate IND 6 coat protein gene, partial cds	IND6	<i>R. sativus</i>	Shimla, India	AFE55686
37	JQ246080	<i>TuMV</i> isolate IND 7 coat protein gene, partial cds	IND7	<i>R. sativus</i>	Kinnaur, India	AFE55687

available at National Centre for Biotechnology Information (NCBI) (Table 1). Multiple sequence alignment (MSA) of nucleotide and amino acid sequences were generated between the *TuMV* coat protein gene sequences (IND1–IND7) obtained for seven test Indian isolates with other *TuMV* isolates retrieved from NCBI database. MSA was performed using CLUSTAL W program (Thompson et al. 1994) available at Network Protein Sequence Analysis (NPS@) web server of PBIL (Pole BioInformatique Lyonnais) Lyon, France. For the computational phylogenetic studies, Molecular Evolutionary Genetics Analysis [(MEGA version 4.0; (Tamura et al. 2007)] was used and phylogenetic trees were generated by UPGMA.

Results and discussion

Serological detection of *TuMV*

In serological detection by DAS-ELISA, virus isolates from Himachal Pradesh (Solan, Kinnaur, Shimla, Mandi), Chandigarh, Punjab (Ludhiana) and Haryana (Karnal) reacted positively with the *TuMV* antisera, recording very high optical density (O.D.) values ranging from 0.612 to 1.625. The values of O.D. for New Delhi (West Patel Nagar) and Rajasthan (Bharatpur) isolates were less than twice the O.D. value of healthy counterparts, thereby clearly indicating absence of the particular test virus in these isolates. There are numerous reports in which DAS-ELISA had been used to confirm the presence of *TuMV* in

the infected plant samples (Nyalugwe et al. 2015; Valouzi et al. 2017).

RT-PCR, cloning, sequencing and in silico analysis

PCR amplicons of approx. 1000 bp of *CP* gene of *TuMV* were obtained in all the seven test samples, whereas healthy plant sample and negative control showed no amplification during RT-PCR followed by PCR. These results were found in coherence with findings of Sanchez et al. (2007), who reported approximately similar size (~ 986 bp) amplicon for *CP* gene of five *TuMV* isolates from Spain. After cloning and sequencing, the sequences of these *CP* gene amplicons was found to be 986 nucleotide long, comprised of 54 bp of the 3' end of the nuclear inclusion b (NIb) gene, the whole coat protein gene (*CP*) gene and 65 bp of the 3' untranslated region (UTR). During further analysis, the sequences of only whole *CP* gene of seven *TuMV* isolates (867 bp) were taken into consideration. Analysis of sequences showed that *CP* gene of all the seven Indian isolates encoded a single open reading frame (ORF) of 288 amino acids, with a molecular weight of 33.1 kDa (IND1, IND2, IND5, IND6 and IND7), 33.04 kDa (IND3) and 33.14 kDa (IND4).

Nucleotide and amino acid sequences of coat protein (*CP*) gene of all the seven Indian isolates of *TuMV* were compared among themselves and with 37 nucleotide and 35 amino acid sequences of *CP* gene of *TuMV* obtained from NCBI database using BLASTN and BLASTP software. Identity among different nucleotide test sequences

Table 2 Percent homology of nucleotide query sequences of coat protein gene of seven Indian isolates of *TuMV* with other exotic isolates of *TuMV* using BLASTN analysis

S. no.	Accession no. (nucleotide)	Virus isolates/ strains	Percent homology (%)						
			IND1 (JQ246074)	IND2 (JQ246075)	IND3 (JQ246076)	IND4 (JQ246077)	IND5 (JQ246078)	IND6 (JQ246079)	IND7 (JQ246080)
1	AF434720	BEL1	90	90	89	89	90	90	90
2	AY395796	ESC8	88	88	88	88	88	89	89
3	AF434726	PV377	88	88	87	87	88	88	88
4	AF434725	PRT1	91	90	90	91	90	90	90
5	AF434724	JPN1	98	97	97	97	97	97	97
6	AF434722	DEU2	91	90	90	90	90	90	90
7	AF434721	CHN1	93	93	92	93	93	93	92
8	AF320127	ZCH	90	90	90	90	90	90	90
9	AY495713	WCH	98	98	97	97	98	98	97
10	AF316361	CDT	91	91	90	90	91	91	90
11	AF316360	RSD	91	91	91	91	91	91	91
12	AF316359	RID	91	91	91	91	91	91	91
13	AY941820	WFLB2	94	94	93	93	93	94	93
14	AF539407	<i>TuMV</i> -SD1	91	91	90	90	91	91	90
15	AF539408	<i>TuMV</i> -SD2	91	91	90	90	91	91	90
16	AF539409	<i>TuMV</i> -SD3	91	91	90	90	91	91	90
17	AF539410	<i>TuMV</i> -SD4	90	90	90	90	90	90	90
18	AF539411	<i>TuMV</i> -SD5	90	90	89	90	90	90	90
19	AF539412	<i>TuMV</i> -SD6	91	90	90	90	90	91	90
20	AF434723	ITA 6	88	88	88	88	88	88	89
21	DQ228764	BJ1	91	91	90	91	91	91	90
22	EF061745	ESP 1	89	89	88	88	89	89	89
23	EF061746	ESP 2	89	89	88	88	89	89	89
24	EF061747	ESP3	88	88	88	88	89	89	89
25	EF061748	ESP4	88	89	88	88	89	89	89
26	EF061749	ESP5	88	88	88	88	88	89	88
27	DQ228765	BJ2	90	90	90	90	90	90	90
28	Y09144	CZE1	89	89	89	89	90	90	89
29	X81140	CDN1	91	91	90	91	91	91	90
30	X65978	UK1	91	91	90	90	91	91	90
31	JQ246074	IND1	–	99	99	98	99	99	99
32	JQ246075	IND2	99	–	99	99	99	99	99
33	JQ246076	IND3	99	99	–	98	99	99	99
34	JQ246077	IND4	98	99	98	–	98	98	98
35	JQ246078	IND5	99	99	99	98	–	98	99
36	JQ246079	IND6	99	99	99	98	99	–	99
37	JQ246080	IND7	99	99	99	98	99	99	–

and with other sequences of *CP* gene of *TuMV* was observed within a range of 87–99% (Table 2). These sequences were submitted to GenBank NCBI with accession no. JQ246074 to JQ246080. In BLASTP analysis, identity observed was within the range of 92–100% among different amino acid sequences of *CP* gene (Table 3).

Phylogenetic analysis

The determination and alignment of the nucleotide and/or deduced amino acid sequences have been widely applied to analyze the genetic diversity of *TuMV* (Nyalugwe et al. 2015; Farzadfar and Pourrahim 2017). Nucleotide and

Table 3 Percent homology of amino acid query sequences of coat protein gene of seven Indian isolates of *TuMV* with other exotic isolates of *TuMV* using BLASTP analysis

S. no.	Accession no. (protein)	Virus isolates/ strains	Percent homology (%)						
			IND1 (AFE55681)	IND2 (AFE55682)	IND3 (AFE55683)	IND4 (AFE55684)	IND5 (AFE55685)	IND6 (AFE55686)	IND7 (AFE55687)
1	AAN63599	BEL1	94	94	93	93	94	94	94
2	AAQ96730	ESC8	95	95	94	94	95	95	95
3	AAN63605	PV377	94	94	93	93	94	94	94
4	AAN63604	PRT1	95	95	94	94	95	95	95
5	AAN63603	JPN1	99	99	98	98	99	99	99
6	AAN63601	DEU2	96	96	95	95	96	96	96
7	AAN63600	CHN1	97	97	96	96	97	97	97
8	AAG41203	ZCH	96	96	95	95	96	96	96
9	AAR87497	WCH	98	98	97	97	98	98	98
10	AAK01628	CDT	97	97	95	96	97	97	97
11	AAK01627	RSD	97	97	96	96	97	97	97
12	AAK01626	RID	97	97	96	96	97	97	97
13	AAX46324	WFLB2	98	98	97	97	98	98	98
14	AAO17364	<i>TuMV</i> -SD1	96	96	95	95	96	96	96
15	AAO17365	<i>TuMV</i> -SD2	96	96	95	95	96	96	96
16	AAO17366	<i>TuMV</i> -SD3	95	95	94	94	95	95	95
17	AAO17367	<i>TuMV</i> -SD4	96	96	95	95	96	96	96
18	AAO17368	<i>TuMV</i> -SD5	96	96	95	95	96	96	96
19	AAO17369	<i>TuMV</i> -SD6	96	96	95	95	96	96	96
20	AAN63602	ITA 6	95	95	94	95	95	95	95
21	ABB58730	BJ1	95	95	94	95	95	95	95
22	ABN71586	ESP 1	94	94	93	94	94	94	94
23	ABN71587	ESP 2	95	95	94	94	95	95	95
24	ABN71588	ESP3	94	94	93	94	94	94	94
25	ABN71589	ESP4	93	93	92	93	93	93	93
26	ABN71590	ESP5	94	94	93	93	94	94	94
27	ABB58731	BJ2	95	95	94	95	95	95	95
28	CAA46793	UK1	97	97	95	96	97	97	97
29	AFE55681	IND1	–	100	99	99	100	100	100
30	AFE55682	IND2	100	–	99	99	100	100	100
31	AFE55683	IND3	99	99	–	98	99	99	99
32	AFE55684	IND4	99	99	98	–	99	99	99
33	AFE55685	IND5	100	100	99	99	–	100	100
34	AFE55686	IND6	100	100	99	99	100	–	100
35	AFE55687	IND7	100	100	99	99	100	100	–

deduced amino acid sequences of *CP* gene of Indian isolates were compared and evaluated for their phylogenetic relationship with other *TuMV CP* gene sequences already present in the NCBI database using UPGMA. Four distinct clusters with appreciable bootstrap support were observed for *CP* nucleotide sequences of *TuMV* (Fig. 1). One minor cluster having five sequences of *TuMV* isolates: ESP5 (EF061749), ESP4 (EF061748), PV377 (AF434726), ESC8

(AY395796) and ITA6 (AF434723) diverged early in the evolution. It was followed by another cluster supported with high bootstrap value of 100 comprised of *TuMV* isolate ESP2 (EF061746) and other two isolates ESP1 (EF061745) and ESP3 (EF061747) which shared common descent. UPGMA is a distance-based clustering algorithm for phylogenetic analysis that derived a bootstrap value of 55 for sequence BEL1 (AF434720) which was present on a

Fig. 1 Phylogenetic relationship of seven test Indian isolates (JQ246074-JQ246080) with other isolates of *TuMV* from all over the world based on coat protein gene nucleotide sequences using UPGMA

separate branch. Two major clusters at bootstrap of 55 divided 28 sequences into two halves. Seven test *CP* gene sequences (JQ246074 to JQ246080) were clustered with three other sequences from China (AY941820, AY495713) and Japan (AF434724). Indian isolate IND4 (JQ246077) was in close proximity to isolate WCH (AY495713) from Weifang, China. Other Indian isolates were placed slightly at a distance from the isolate WCH and these were more closely related among themselves supported with high

bootstrap value of 95. Most recently diverged cluster was having 18 nucleotide sequences (AF316360 to AF434725) of *TuMV CP* gene from different parts of the world.

All the amino acid sequences of the *CP* gene of *TuMV* isolates when plotted into an evolutionary tree through UPGMA presented four distinct clusters in the phylogenetic tree (Fig. 2). The sequence of isolate from Belgium (AAN63599) evolved independently with a low bootstrap value. The earliest diverging clade consisted of five

Fig. 2 Phylogenetic relationship of seven test Indian isolates (AFE55681-AFE55687) with other isolates of *TuMV* from all over the world based on coat protein gene amino acid sequences using UPGMA

sequences; whereas, the second clade consisted of three sequences dominated by Spain isolates (ABN71588, ABN71586 and ABN71587). *CP* test sequences (AFE55681 to AFE55687) of *TuMV* isolates were present in the third clade consisting of 11 sequences. Isolate IND3 (AFE55683) was in close proximity to Japanese isolate JPN1 (AAN63603). Similarly, other Indian test isolates (AFE55681, AFE55682, AFE55686, AFE55685 and AFE55687) were closely related sharing most recent

common ancestry with a bootstrap support of 83. The fourth clade was mainly dominated by isolates from China and South Korea having 15 sequences of *TuMV* coat protein gene from all over the world.

At nucleotide level, all the seven Indian isolates JQ246074 to JQ246080 of *CP* gene of *TuMV* were clustered along with isolates WFLB2 (AY941820), WCH (AY495713) from China and JPN1 (AF434724) from Japan. On the other hand, at amino acid level, all the seven

Indian isolates AFE55681 to AFE55687 of *CP* gene of *TuMV* were present in one clade along with isolates WFLB2 (AAX46324), WCH (AAR87497) from China, CHN1 (AAN63600) from Taiwan and JPN1 (AAN63603) from Japan. Therefore, all the seven Indian isolates were closer to isolates WFLB2, WCH, JPN1 and CHN1. All of these isolates were obtained from *Raphanus sativus* L. except for isolate CHN1 which was obtained from *Brassica napus*. All the seven Indian isolates were inferred as a member of basal-BR group on the basis of current phylogenetic analysis. The basal-BR type isolates are found to infect both *Brassica* and *Raphanus* plants systemically with mosaic on uninoculated leaves. Further, it also indicates that the Indian isolates had evolved in China or via China or in nearby countries of East Asia. Similar results have been obtained by Tian et al. (2007), which showed that six Chinese *TuMV* isolates HZLB1, HZLB2, HBLB, FZLB, TAYC and WFLB2 belonged to the basal-BR group. Based upon complete genome sequence analysis of *TuMV* isolates from China, Wang et al. (2009) also concluded that the Basal-BR group is one of the four lineages of *TuMV* and is a new emergent lineage in East Asia. Yasaka et al. (2017) also studied the timescale of emergence and spread of *TuMV*-radish using whole-genome sequences in Turkey and neighboring countries. In a similar study, serological and molecular characterization of *TuMV*-infecting wild plants had been carried out in Iran (Valouzi et al. 2017).

Conclusion

In this investigation, the *CP* gene of all the seven Indian isolates was observed to share 87–99 and 92–100% nucleotide and amino acid identity, respectively, among themselves and with other isolates of *CP* gene of *TuMV* from across the world. All the seven Indian isolates were inferred as a member of basal-BR group on the basis of current phylogenetic analysis. This knowledge will further assist in making rational decision for making the disease resistance efforts and identify isolates to be chosen for cultivar screening for resistance breeding. Further, present findings will provide a platform for using *CP* gene of *TuMV* for inducing coat protein-mediated cross protection in the genetically engineered plants which can be effective against *TuMV* over various geographical areas in future.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

- Brinboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res* 7:1513–1518
- Clark MF, Adams AN (1977) Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J Gen Virol* 34:475–483
- Farzadfar S, Pourrahim R (2017) First report of *Turnip mosaic virus* infection of *Alliaria petiolata* in Iran. *Plant Dis* 101(8):1558. <https://doi.org/10.1094/PDIS-01-17-0141-PDN>
- Li X, Zhu T, Yin X, Zhang C, Chen J, Tian Y, Liu J (2017) The genetic structure of *Turnip mosaic virus* population reveals the rapid expansion of a new emergent lineage in China. *Plant Sci*. <https://doi.org/10.20944/preprints201705.0109.v1>
- Nyalugwe EP, Jones RAC, Barbet MJ, Kehoe MA (2015) Biological and molecular variation amongst Australian *Turnip mosaic virus* isolates. *Plant Pathol* 64:1215–1223
- Sanchez F, Rodriguez MM, Tourino A, Fresno J, Gomez CC, Jenner CE, Walsh JA, Ponz F (2007) Identification of new isolates of *Turnip mosaic virus* that cluster with less common viral strains. *Arch Virol* 152:1061–1068
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl Acid Res* 22:4673–4680
- Tian YP, Zhu XP, Liu JL, Yu XQ, Du J, Kreuze J, Li XD (2007) Molecular characterization of the 3' terminal region of *Turnip mosaic virus* isolates from eastern China. *J Phytopathol* 155:333–341
- Valouzi H, Golnaraghi A, Abedini-Aminabad L, Diyanat M (2017) Serological and molecular identification of *Turnip mosaic virus* in some wild plants in Iran. *Australas Plant Dis Notes* 12:3. <https://doi.org/10.1007/s13314-016-0225-2>
- Wang HY, Liu JL, Gao R, Chen J, Shao YH, Li XD (2009) Complete genomic sequence analysis of *Turnip mosaic virus* basal-BR isolates from China. *Virus Genes* 38:421–428
- Yasaka R, Fukagawa H, Ikematsu M, Soda H, Korkmaz S, Golnaraghi A, Katis N, Ho SYW, Gibbs AJ, Ohshima K (2017) The timescale of emergence and spread of *Turnip mosaic potyvirus*. *Sci Rep* 7:4240. <https://doi.org/10.1038/s41598-017-01934-7>
- Zhu F, Sun Y, Wang Y, Pan H, Wang F, Zhang X, Zhang Y, Liu J (2016) Molecular characterization of the complete genome of three basal-BR isolates of *Turnip mosaic virus* infecting *Raphanus sativus* in China. *Int J Mol Sci* 17:888. <https://doi.org/10.3390/ijms17060888>