First report of ToCV and Detection of other Viruses in Field-grown Tomatoes in North-western Region of India

POONAM CHAUDHARY

Guru Nanak Dev University

Amritpreet Kaur

Guru Nanak Dev University

Balwinder Singh

Khalsa College Charitable Society

Surender Kumar

CSIR-IHBT: Institute of Himalayan Bioresource Technology CSIR

Vipin Hallan

CSIR-IHBT: Institute of Himalayan Bioresource Technology CSIR

Avinash Kaur Nagpal (✉ avnagpal@yahoo.co.in)

Guru Nanak Dev University

Research Article

Keywords: DAS-ELISA, RT-PCR, symptomatic tomato samples, ToCV, phylogenetic analysis

Posted Date: March 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1357114/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Tomato crop is known to be infected by large number of viruses across the globe causing severe losses in its yield. Accurate information on the distribution and incidence of different viruses is essential to implement virus control strategies. This study provides information on prevalence and distribution of different viruses infecting tomato crop in North-western region of India.

Methods and Results: Leaf samples of 76 symptomatic tomato and 30 symptomatic and asymptomatic plants of *Chenopodium sp.* (weed) were collected from eight villages. DAS-ELISA and/or RT-PCR were used to detect occurrence of nineteen viruses and one viroid in tomatoes. Nine viruses viz. *Cucumber mosaic virus*, *Groundnut bud necrosis virus*, *Potato virus M*, *Potato virus S*, *Potato virus X*, *Potato virus Y*, *Tomato chlorosis virus*, *Tomato leaf curl New Delhi virus* and *Tomato mosaic virus* were detected in 58 of 76 tomato samples. Detection of viruses was confirmed by cloning of specific amplicons followed by sequencing and submission of sequences to the GenBank database. None of the targeted pathogens were found in collected weed samples. *Tomato leaf curl New Delhi virus* (ToLCNDV) was the most prevalent virus (64.47%) followed by *Potato virus Y* (PVY) (23.68%). Double, triple, quadruple and quintuple infections were also noticed. Phylogenetic analysis of nucleotide sequences was also carried out.

Conclusions: Nine viruses infecting tomato crop from North-western region of India were detected. ToLCNDV was most prevalent with highest incidence. To the best of our knowledge, this is the first report of ToCV on tomato from India.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a world-famous vegetable crop for its edible fruits and profits in the market. India ranks second in the global trade of tomato and approximately 781,000 hectares of land come under cultivation of tomato crop, which contributes about 19.01 million tonnes of tomatoes to the world production [1]. Despite its economic importance, production of this valuable crop is susceptible to physiological, biotic and abiotic stresses [2]. Tomato plants are prone to many bacterial, fungal, and viral diseases, but incidence of viral diseases has smashed the production of tomatoes severely [3–5]. Plant viruses are obligatory parasites that exploit host's cellular machinery for their own reproduction causing significant loss in the yield and quality of crops across the globe [5]. More than 130 viruses are known to infect tomatoes with many of them causing severe disease symptoms leading to severe losses in crop production [6]. The transmission of viruses has been aided by different vectors all across the planet [7]. Anthropogenic activities and presence of weed species also have a significant role in transmission of viral diseases making their management difficult if not impossible.

Among plant viruses which have been known to infect tomato plants, *Cucumber mosaic virus* (CMV) infects more than 1200 species covering over 100 families of monocots and dicots [8]. This virus can infect a wide variety of hosts and can be spread by seeds, aphids, and mechanical transmission. The host range of *Potato virus M* (PVM) in contrast to CMV is restricted to Solanaceae family. It is sap-transmissible to a finite range of species and is vectored by aphids including *Myzus persicae* and *Macrosiphum euphorbiae*. The two strains known for *Potato virus S* (PVS) are ordinary strain PVS⁰ and Andean strain PVS^A [9, 10]. Like PVM, *Potato virus S* (PVS) also has a limited host range. PVS is mainly associated with Solanaceae and Chenopodiaceae families where it is transmitted through sap and in some cases vectored by aphids. *Potato virus X* (PVX) is primarily transmitted mechanically and its natural hosts are mainly confined to family solanaceae (such as tomato, eggplant, potato, and cape gooseberry), though some plants in other families, such as Amaranthaceae and Chenopodiaceae, are susceptible [11]. *Potato virus Y* (PVY) is known to infect a wide range of plant species, especially belonging to family solanaceae and aphids are known to transmit this virus in a nonpersistent manner [12]. It can also be transmitted mechanically [13]. *Tomato chlorosis virus* (ToCV) was first noticed in greenhouse-grown tomatoes in Florida, USA and is known to infect nearly 85 species of dicots [14]. ToCV is transmitted by five species of whiteflies from two genera viz. *Bemisia* and *Trialeurodes* [15]. *Tomato mosaic virus* (ToMV) is found to be seed-borne and readily spreads from infected plants to healthy ones. It is known to be a very stable virus which can survive upto many years in plant debris, seeds and soil [16]. *Tomato leaf curl New Delhi virus* (ToLCNDV) is an economically important begomovirus that has been linked to severe damage to tomato crop and it is more common in northern India. It is transmitted by *Bemisia tabaci* in a circulative and persistent manner [17]. *Groundnut bud necrosis virus* (GBNV) is also known to cause severe disease symptoms in tomatoes resulting in yield loss and in extreme situations; it may result in 100% loss of the crop. It is transmitted by thrips [18].

In order to implement efficient control strategies against viruses, complete knowledge of viruses and their transmission is required. However, to the best our knowledge, not much information is available on viral diseases of tomato in Punjab and neighbouring states of India. Also, there is very less data on the occurrence of multiple virus infections on tomato crop. Therefore, the present study was planned to investigate the prevalence and distribution of different viruses and a viroid infecting tomato crop in North-western region of India during 2015–2017. Samples of a potential weed host, *Chenopodium sp.* surrounding tomato fields that could act as reservoir for these viruses were also analysed.

2. Materials And Methods

2.1. Survey and sample collection

Field inspections of three major tomato producing districts of Punjab (Amritsar in Majha zone, Kapurthala in Doaba zone and Patiala in Malwa zone) and a district (Una) of Himachal Pradesh were carried out during growing seasons between 2015 and 2017 in eight different villages (Table 1). Infected leaf samples showed multiple virus like symptoms such as chlorosis, bunchy appearance, shortened internodes, blossom drop, stunted plant growth, leaf crumpling, necrosis, wilting and bronzing, leaf curling, leaf rolling, leaf purpling, mosaic and mottling in various combinations (Fig. 1). Total 76 symptomatic tomato leaf samples and 10 symptomatic and 20 asymptomatic weed samples (*Chenopodium sp.*) were collected in liquid nitrogen, transported to laboratory and stored in Deep Freezer at -80°C for further serological and molecular diagnosis.

Table 1
Sample codes for symptomatic tomato leaf samples collected from various villages of Punjab and Himachal Pradesh (H.P), India.

State	District	Village	Latitude	Longitude	Sample Code
Punjab	Amritsar	Jodhey	31°34'44.8968N	75°19'58.3284E	B (1–10)
		Chhapaa Ram Singh	31°38'6.756N	74°58'58.5444E	C (1–5)
	Kapurthala	Daula	31°7'8.2968N	75°11'52.5048E	D (1–25)
		Pati Sardar Nawi Baksh	31°19'36.1128N	75°15'21.6E	P (1–3)
		Muradpur	31°18'46.0512N	75°16'34.0536E	M (1–5)
	Patiala	Kartarpur	30°19'3.5184N	76°28'50.3904E	KP (1–11)
		Poonian Jattan	30°19'12.6588	76°29'58.74E	PJ (1–4)
H.P	Una	Khanpur	31°21'33.8904N	76°19'22.5804E	K (1–13)

On the basis of the observed symptoms, symptomatic leaf samples were analysed for 20 virus and virus like pathogens (Table 2). Antisera for DAS-ELISA were available for eight viruses i.e. CMV, PLRV, PVX, PVY, TBSV, ToMV, TSWV and TYLCV; hence presence of these viruses was investigated using ELISA kits. Once a sample was tested positive for DAS-ELISA, it was further confirmed by PCR or RT-PCR. Due to lack of ELISA kits for the remaining viruses, the serological analysis could not be performed for them. However, RT-PCR method was utilised for all nineteen viruses and a viroid as mentioned in Table 2 and the results were validated by nucleotide sequencing.

2.2. Preparation of samples for DAS-ELISA

Briefly, diluted specific antibody was adsorbed to surface of ELISA plate and incubated at 30°C for 4 h. Leaves were homogenized in extraction buffer, added to the plate followed by overnight incubation at 4–6°C. Diluted enzyme-labelled antibody was added and incubated at 30°C for 5 h. Enzyme substrate pNPP was added and colour reaction was recorded at 405 nm. Positive and negative controls of respective viruses were included in all ELISA tests and values three times higher than that of the negative control were considered as positive for that particular virus.

2.3. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Eighteen RNA viruses (AMV, CMV, GBNV, INSV, PLRV, PMTV, PVA, PVM, PVS, PVX, PVY, TAV, TBSV, TICV, ToCV, ToMV, TRV and TSWV) along with a viroid, *Potato Spindle Tuber Viroid* (PSTVd), were diagnosed by RT-PCR. Sequences coding for coat proteins were used as primers for all RNA viruses (except ToMV) and the viroid, PSTVd. Whereas for ToMV, sequences coding for replicase gene were used as primers (Table 2). Total plant RNA was isolated using the Genei kit. The reaction mixture for first strand synthesis consisted of 1 µg of RNA, 5 µl of M-MLV RT reaction buffer (5X) (USB, Cleveland, Ohio, USA), 1 µl (10 pmol/µl) of reverse primer of respective virus, 1.25 µl dNTP mix (10 mM), 0.5 µl (200U/µl) M-MLV reverse transcriptase (USB) and 0.5 µl RNase inhibitor (USB, USA). The reaction tubes were incubated at 42°C for 30 min followed by heat inactivation of reverse transcriptase at 95°C for 5 min. The first strand cDNA (1 µl) was utilized for PCR immediately, while the rest was kept at -20°C.

Polymerase Chain Reaction (PCR) was carried out in 0.2 ml tubes in a GeneAmp Thermal cycler 9700 system (Applied Biosystems, USA) using detection primers for the amplification of desired sequences. The reaction mix (25 µl) consisted of 2.5 µl Taq DNA polymerase buffer 10X (Genei, Bangalore, India), 0.50 µl dNTP mix (10 mM), 0.50 µl each of forward and reverse primers of tested virus (10 pmol/ µl), 1 µl cDNA, and 0.25 µl Taq DNA polymerase (Genei, Bangalore, India). Denaturation was executed at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 sec, primer binding respective temperatures ranging from 50–58 °C for 35 sec, extension at 72°C for 35 sec and final elongation step at 72°C for 10 min. The amplified PCR products were partitioned in 1.5% agarose gel at 85 V for approximately 45 mins, stained with ethidium bromide and observed under UV transilluminator.

2.4. DNA extraction and PCR

For the detection of ToLCNDV (a begomovirus), total DNA was extracted by CTAB method [35]. PCR reaction (20 µl) consisted of 2.5 µl Taq DNA polymerase buffer 10X (Genei, Bangalore, India), 0.50 µl dNTP mix (10 mM), 1.0 µl each of forward and reverse primers for DNA-A segment (10 pmol/ µl), 1 µl DNA, and 0.25 µl Taq DNA polymerase (Genei, Bangalore, India). Denaturation was executed at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 sec, primer binding at 58°C for 35 sec, extension at 72°C for 50 sec and final elongation step at 72°C for 10 min. The amplified PCR products were fractioned in 1.5% agarose gel at 85 V for approximately 45 min, stained with ethidium bromide and observed under UV transilluminator.

2.5. Cloning, sequencing and sequence analysis

Positive amplicons for each virus were excised and purified from the gel using GenElute Gel Extraction kit (Sigma Aldrich, USA) and cloned into pGEM-T easy vector (Promega, Madison, Wisconsin, USA). Purification of recombinant plasmids was carried out using GenElute Plasmid Miniprep kit (Sigma–Aldrich, USA). Both the DNA strands were sequenced in an automated DNA sequencer (ABI PRISM®3130xl Genetic Analyzer) using ABI prism Big Dye™ Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, USA).

2.6. Phylogenetic analysis

The extent of relationship between genera, species and strains, divergence among geographical isolates and the emergence and evolution of plant viruses are all represented by phylogenetic analysis [36]. To understand the evolutionary relationship, nucleotide sequences of amplified gene products from different isolates and already available sequences of respective viruses downloaded from the nucleotide database of NCBI were aligned by the MUSCLE tool present in MEGA-X [37]. The analysis included both full-length and partial coat protein (CP) and replicase gene sequences from the database (Table 3). The phylogenetic tree was constructed using the Neighbor-joining method with 1000 bootstraps present under the phylogeny option in MEGA-X software. The tree was refined using Interactive Tree Of Life (iTOL) v5 [38].

Table 3
Virus sequences used for phylogenetic analysis

S.No.	Abbreviation	Virus Name [Acronym] (Genome)	Isolate name	Country (Region/City/State/Province) If available	Sequence length (nucleotides)	Host species	GenBank Accession number
1.	CMV-In1*	Cucumber mosaic virus [CMV] (RNA)	K3 Khanpur	India (Una-Khanpur)	534	<i>Solanum lycopersicum</i>	MH038
2.	CMV-In2*		B7 Jodhey	India (Amritsar-Jodhey)	534	<i>Solanum lycopersicum</i>	MZ322
3.	CMV-Au		SW-11	Australia	2249	<i>Cymbidium sp.</i>	KM434
4.	CMV-Ba		Bangladesh:Barisal:2014	Bangladesh (Barisal)	529	<i>Solanum melongena</i>	KM516
5.	CMV-Br		Para	Brazil	2207	<i>Arachis pintoi</i>	MK387
6.	CMV-Ch		PE	China	2216	-	AF268
7.	CMV-Ec		Salvador	Ecuador (El Salvador)	2187	<i>Capsicum annum</i>	MW29
8.	CMV-Eg		HM3	Egypt	2277	<i>Solanum lycopersicum</i>	KX014
9.	CMV-Ge		Salzlandkreis-2_18	Germany	2159	Legume	MN399
10.	CMV-Id		B2	Indonesia	2219	<i>Musa sapientum</i>	AB046
11.	CMV-Ir		IRN-Khs1	Iran	2176	<i>Raphanus sativus</i>	LC066
12.	CMV-It		Vir56	Italy	2414	<i>Thevetia nereifolia</i>	DQ006
13.	CMV-Ja		CMV-MT	Japan (Fukuoka)	2201	<i>Solanum lycopersicum</i>	AB189
14.	CMV-Ma		-	Malaysia (Mardi Teluk Telong)	2219	<i>Cucumis sativus</i>	JN054
15.	CMV-Po		Del	Poland	1124	<i>Delphinium sp.</i>	EU191
16.	CMV-Sk		RB3	South Korea	2213	<i>Capsicum annum</i>	MT661
17.	CMV-SI		PK1	Slovakia	2190	<i>Papaver somniferum</i>	MN799
18.	CMV-Sp		PV-0474	Spain	2219	<i>Cucumis melo</i>	KX525
19.	CMV-Tu		NG1	Turkey (Demre)	486	<i>Capsicum annum</i>	KY973
20.	CMV-Ug		Ug92-RNA3	Uganda	2212	<i>Xanthosoma sp.</i>	MG027
21.	CMV-Vi		VN-Bavi	Vietnam (HàTây)	1913	<i>Nicotiana tabacum</i>	AM048
22.	GNV-In1*	Groundnut bud necrosis virus [GBNV] (RNA)	K3 Khanpur	India (Una-Khanpur)	831	<i>Solanum lycopersicum</i>	MZ643
23.	GNV-In2		Tomato/Benthamangala/ UHSASB26	India (Bethamangala)	898	<i>Solanum lycopersicum</i>	MN739
24.	GNV-In3		-	India (Coimbatore)	831	<i>Solanum lycopersicum</i>	AY618

^c Complete genome sequence reported but for phylogenetic analysis only sequence for coat protein gene was used

^d Complete genome sequence reported but for phylogenetic analysis only sequence for replicase gene was used

* Present study isolates

S.No.	Abbreviation	Virus Name [Acronym] (Genome)	Isolate name	Country (Region/City/State/Province) If available	Sequence length (nucleotides)	Host species	GenBank Accession number
25.	GNV-In4	Potato virus M [PVM] (RNA)	Thir-Tom	India (Thirumalapura)	831	<i>Solanum lycopersicum</i>	MK557
26.	GNV-In5		Pune	India (Pune)	831	<i>Dolichus lablab</i>	AY882
27.	GNV-In6		MDU	India (Madurai)	833	<i>Solanum lycopersicum</i>	MK032
28.	GNV-In7		Bangalore	India (Bangalore)	831	<i>Solanum lycopersicum</i>	AY173
29.	GNV-In8		Arvikendra	India (Pune)	831	<i>Solanum lycopersicum</i>	AY882
30.	GNV-In9		Capsicum/Budamanahalli/UHSASB185	India (Budamanahalli)	898	<i>Capsicum annuum</i>	MN731
31.	GNV-In10		Chilli/Boreddigari/Palli/ UHSASB15	India (Boreddigari Palli)	898	<i>Capsicum annuum</i>	MN731
32.	GNV-In11		To-Kol	India	831	<i>Solanum lycopersicum</i>	EU373
33.	GNV-In12		To-Ujj.7	India	831	<i>Solanum lycopersicum</i>	EU373
34.	GNV-In13		Tomato/UAS/Dharwad/ UHSASB193	India (Dharwad)	898	<i>Solanum lycopersicum</i>	MN731
35.	GNV-In14		Tomato/Garag/UHSASB196	India (Garag)	898	<i>Solanum lycopersicum</i>	MN731
36.	GNV-In15		Anantapur	India (Anantapur)	831	<i>Arachis hypogaea</i>	FJ3559
37.	GNV-In16		To-Khe	India	831	<i>Solanum lycopersicum</i>	EU373
38.	PVM-In1*		KP7 Kartarpur	India (Patiala-Kartarpur)	524	<i>Solanum lycopersicum</i>	MG557
39.	PVM-In2*		B9 Jodhey	India (Amritsar-Jodhey)	524	<i>Solanum lycopersicum</i>	MG557
40.	PVM-In3*		M1 Muradpur	India (Kapurthala- Muradpur)	524	<i>Solanum lycopersicum</i>	MG557
41.	PVM-Ch		YN-1-8	China	1294	<i>Solanum muricatum</i>	KF561
42.	PVM-Cr		VIRUBRA 4/007	Czech Republic	8474 ^c	<i>Solanum tuberosum</i>	HM854
43.	PVM-Ge		DSMZ PV0273	Germany	8523 ^c	-	EU604
44.	PVM-Ir		PVM-352	Iran	8523 ^c	<i>Solanum tuberosum</i>	JX678
45.	PVM-It		-	Italy	1389	<i>Solanum lycopersicum</i>	X8511
46.	PVM-La		-	Latvia (<i>Priekule</i>)	1007	<i>Solanum tuberosum</i>	GQ496
47.	PVM-Ru		FE-Queen_anne10	Russia (Far East Federal Region)	915	<i>Solanum tuberosum</i>	LC511
48.	PVM-SI		T40	Slovakia	8523 ^c	<i>Solanum lycopersicum</i>	MH558

^c Complete genome sequence reported but for phylogenetic analysis only sequence for coat protein gene was used

^d Complete genome sequence reported but for phylogenetic analysis only sequence for replicase gene was used

* Present study isolates

S.No.	Abbreviation	Virus Name [Acronym] (Genome)	Isolate name	Country (Region/City/State/Province) If available	Sequence length (nucleotides)	Host species	GenBank Accession number
49.	PVM-Ta		TZ:PVM12U:11	Tanzania (Mbeya)	917	<i>Solanum tuberosum</i>	KC866
50.	PVS-In1*	Potato virus S	K9 Khanpur	India (Una-Khanpur)	729	<i>Solanum lycopersicum</i>	MG55
51.	PVS-In2*	[PVS] (RNA)	KP7 Patiala	India (Patiala-Kartarpur)	729	<i>Solanum lycopersicum</i>	MG55
52.	PVS-Ba		PVS175	Bangladesh	8480 ^c	<i>Solanum tuberosum</i>	MF503
53.	PVS-Ch		HB24	China	8453 ^c	<i>Solanum tuberosum</i>	KU896
54.	PVS-Ge		BA-365-4	Germany	8437 ^c	<i>Solanum tuberosum</i>	MF418
55.	PVS-Hu		FabiolaC	Hungary	885	<i>Solanum tuberosum</i> cv. <i>Fabiola</i>	LN794
56.	PVS-Ir		Os-11-PVS	Iran	885	<i>Solanum tuberosum</i>	MH159
57.	PVS-Ne		-	Netherlands	885	<i>Solanum tuberosum</i>	GU319
58.	PVS-Ru		VF-RedScarlet1	Russia (Volga Federal region)	885	<i>Solanum tuberosum</i>	LC511
59.	PVS-Sy		PVS3-5	Syria (Aleppo)	1289	<i>Solanum tuberosum</i>	AB364
60.	PVX-In1*	Potato virus X	K9 Khanpur	India (Una-Khanpur)	562	<i>Solanum lycopersicum</i>	MH038
61.	PVX-Au	[PVX] (RNA)	QLD1974	Australia	714	<i>Solanum tuberosum</i>	GU384
62.	PVX-Ba		Bogra	Bangladesh	714	<i>Solanum tuberosum</i>	MK587
63.	PVX-Ch		Meixian2	China (Meixian)	714	<i>Actinidia sp.</i>	MH457
64.	PVX-In2		FAI-13	India (Faizabad)	714	<i>Solanum tuberosum</i>	KR605
65.	PVX-Ir		Iran	Iran	6435 ^c	<i>Pisum sativum</i>	FJ4613
66.	PVX-Ja		-	Japan (Gunma)	6435 ^c	-	AB196
67.	PVX-Pe		Jin170B	Peru (Huancayo)	6436 ^c	<i>Solanum tuberosum</i> cv. <i>Yungay</i>	MT752
68.	PVX-Sp		PVX.a	Spain	6435 ^c	<i>Nicotiana benthamiana</i>	MT799
69.	PVY-In1*	Potato virus Y	K8 Khanpur	India (Una- Khanpur)	266	<i>Solanum lycopersicum</i>	MZ322
70.	PVY-In2*	[PVY] (RNA)	KP7 Patiala	India (Patiala-Kartarpur)	266	<i>Solanum lycopersicum</i>	MZ322
71.	PVY-In3*		B7 Jodhey	India (Amritsar-Jodhey)	218	<i>Solanum lycopersicum</i>	MZ322
72.	PVY-Br		1027	Brazil	801	<i>Nicotiana sp.</i>	KJ741
73.	PVY-Co		70SE	Columbia (Antioquia)	798	<i>Solanum lycopersicum</i>	MN119

^c Complete genome sequence reported but for phylogenetic analysis only sequence for coat protein gene was used

^d Complete genome sequence reported but for phylogenetic analysis only sequence for replicase gene was used

* Present study isolates

S.No.	Abbreviation	Virus Name [Acronym] (Genome)	Isolate name	Country (Region/City/State/Province) If available	Sequence length (nucleotides)	Host species	GenBank Accession number
74.	PVY-Eg	Tomato chlorosis virus [ToCV] (RNA)	zalat-YB2	Egypt	420	<i>Solanum tuberosum</i> cv. <i>Sponda</i>	MT242
75.	PVY-Mc		KUA7-2013	Macedonia	653	<i>Capsicum annuum</i>	MF000
76.	PVY-Pa1		S22	Pakistan	801	<i>Solanum tuberosum</i>	LC627
77.	PVY-Pa2		4C-Ghannian	Pakistan (Ghannian)	807	<i>Nicotiana tabacum</i>	MG460
78.	PVY-Pa3		-	Pakistan	756	<i>Solanum tuberosum</i>	KX255
79.	PVY-Pa4		-	Pakistan (Lahore)	645	-	MK130
80.	PVY-Pa5		PK	Pakistan	795	<i>Solanum tuberosum</i>	JQ518
81.	TCV-In*	Tomato chlorosis virus [ToCV] (RNA)	K4 Khanpur	India (Una-Khanpur)	774	<i>Solanum lycopersicum</i>	MN517
82.	TCV-Br		ToC-Br2	Brazil (<i>São Paulo</i>)	8242	<i>Solanum lycopersicum</i>	JQ952
83.	TCV-Ch		HBHS	China (Hengshui)	774	<i>Solanum lycopersicum</i>	KP217
84.	TCV-Fr		Fr	France (<i>Bouches-du-Rhône</i>)	3783	<i>Solanum lycopersicum</i>	EU625
85.	TCV-Gr		To-II1857	Greece (Ileia)	774	<i>Solanum lycopersicum</i>	HG380
86.	TCV-Ja		Kuma1	Japan (Kumamoto)	774	<i>Solanum lycopersicum</i>	LC342
87.	TCV-Sa		ToCR-186	South Africa	8242	<i>Solanum lycopersicum</i>	KY471
88.	TCV-Sk		JJ	South Korea	8242	<i>Solanum lycopersicum</i>	KP137
89.	TCV-Sp		PI-1-2	Spain	8241	<i>Solanum lycopersicum</i>	KJ200
90.	TCV-Tu		FETHIYE72	Turkey	774	<i>Solanum lycopersicum</i>	MF576
91.	TCV-Uk		-	United Kingdom	8241	<i>Solanum lycopersicum</i>	KY810
92.	TCV-US		Florida	USA	8247	<i>Solanum lycopersicum</i>	AY903
93.	TMV-In*	Tomato mosaic virus [ToMV] (RNA)	B10 Jodhey	India (Amritsar-Jodhey)	317	<i>Solanum lycopersicum</i>	MH038
94.	TMV-Au		Queensland	Australia (Queensland)	6383 ^d	<i>Solanum lycopersicum</i>	AF332
95.	TMV-Ch		XJT-1	China (Xinjiang)	6383 ^d	<i>Solanum lycopersicum</i>	FN985
96.	TMV-Ja		-	Japan (Ibaraki)	6383 ^d	<i>Nicotiana tabacum</i>	AB355
97.	TMV-Ka		K1	Kazakhstan	6383 ^d	-	AJ243
98.	TMV-Sk		1 ISC-2020-02	South Korea	6356 ^d	<i>Capsicum annuum</i>	LC623

^c Complete genome sequence reported but for phylogenetic analysis only sequence for coat protein gene was used

^d Complete genome sequence reported but for phylogenetic analysis only sequence for replicase gene was used

* Present study isolates

S.No.	Abbreviation	Virus Name [Acronym] (Genome)	Isolate name	Country (Region/City/State/Province) If available	Sequence length (nucleotides)	Host species	GenBank Accession number
99.	TMV-SI		SL-1	Slovakia	6383 ^d	<i>Solanum lycopersicum</i>	KY912
100.	TMV-Ug		ToMV-Ug	Uganda	6383 ^d	<i>Solanum lycopersicum</i>	MG456
101.	TMV-Us		99 – 1	USA (Florida)	6383 ^d	<i>Jasminum multiflorum</i>	KR537
102.	TMV-Zi		Mutoko	Zimbabwe (Mutoko)	6383 ^d	<i>Solanum lycopersicum</i>	KX711
103.	TLC-In1*	Tomato leaf curl New Delhi virus [ToLCNDV] (DNA)	K12 Khanpur	India (Una-Khanpur)	771	<i>Solanum lycopersicum</i>	MZ643
104.	TLC-Ba1		ToLCNDV-IN[BD:Jam:01:40:Tom:10]	Bangladesh (Jamalpur)	2740 ^c	<i>Solanum lycopersicum</i>	KM383
105.	TLC-Ba2		ToLCNDV-IN[BD:Jam:02:44:Tom:10]	Bangladesh (Jamalpur)	2740 ^c	<i>Solanum lycopersicum</i>	KM383
106.	TLC -In2		ToLCNDV-CKTD:2017	India (Chitrakoot)	2740 ^c	<i>Solanum lycopersicum</i>	MF807
107.	TLC -In3		2B	India (Varanasi)	2740 ^c	<i>Solanum lycopersicum</i>	KF537
108.	TLC-In4		-	India (Varanasi)	771	<i>Cucurbita pepo</i>	GQ225
109.	TLC-In5		DH01	India (Dhaulpur)	771	<i>Capsicum annum</i>	GU831
110.	TLC-In6		India:UP:Bahraich:Tomato1:2011	India (Bahraich)	771	<i>Solanum lycopersicum</i>	KC207
111.	TLC-In7		Gorakhpur	India (Gorakhpur)	771	<i>Luffa cylindrical</i>	EU439
112.	TLC-In8		New Delhi	India (New Delhi)	2739 ^c	<i>Solanum lycopersicum</i>	U1501
113.	TLC-In9		2	India (New Delhi)	2739 ^c	<i>Cucurbita pepo</i>	AM286
114.	TLC-In10		Ludhiana	India (Ludhiana)	769	<i>Cucumis sativus</i>	MH883
115.	TLC-In11		Himachal	India (Himachal Pradesh)	771	-	DQ029
116.	TLC-In12		-	India (Jabalpur)	771	-	AY691
117.	TLC -Pa1	m54073_190913_041431_27459837_ccs	Pakistan (Sindh province)	2739 ^c	<i>Solanum lycopersicum</i>	MW42	
118.	TLC-Pa2	m54073_190913_041431_16647093_ccs	Pakistan (Sindh province)	2739 ^c	<i>Solanum lycopersicum</i>	MW42	
119.	TLC -Pa3	m54073_190913_041431_14483568_ccs	Pakistan (Sindh province)	2739 ^c	<i>Solanum lycopersicum</i>	MW42	
° Complete genome sequence reported but for phylogenetic analysis only sequence for coat protein gene was used							
^d Complete genome sequence reported but for phylogenetic analysis only sequence for replicase gene was used							
* Present study isolates							

3. Results

3.1. Survey and sample collection

The symptomatic plants were screened, selected and photographed. Leaf samples of these plants were packed in labelled aluminium foil and stored in liquid nitrogen. Infected tomato plants under natural conditions, show many symptoms such as chlorosis, bunchy appearance, shortened internodes, blossom drop, stunted plant growth, leaf crumpling, necrosis, wilting and bronzing, leaf curling, leaf rolling, leaf purpling, mosaic and mottling in various combinations

(Fig. 1). Symptoms caused by abiotic agents such as nutritional deficiencies, weedicides, insecticides, or insect manifestation were also taken into consideration while surveying the fields.

3.2. Serological and Molecular detection

Of the nineteen viruses tested (by DAS-ELISA and/or RT-PCR), 8 RNA viruses viz. CMV, GBNV, PVM, PVS, PVX, PVY, ToCV, ToMV and a DNA virus, TYLCV were detected in four districts. Amplicons of the expected size were obtained for CMV (534 bp), GBNV (831 bp), PVM (524 bp), PVS (729 bp), PVX (562 bp), PVY (218 bp), ToCV (774 bp), ToMV (317 bp) and TYLCV (827 bp) (Fig. 2). These findings reveal that the commercially grown tomatoes in North-western region of India are infected with viruses. One primer set for *Potato Spindle Tuber Viroid* (PSTVd) was also used which gave negative results for all the samples confirming its absence. Chi-square analysis (data not shown) among the present viruses showed no detectable association making all the viruses as independent variables ($\alpha = 0.05$).

Double (CMV/GBNV, CMV/TYLCV and PVM/PVY), triple (CMV/PVX/TYLCV, PVM/PVY/TYLCV, PVM/ToMV/TYLCV, and PVY/ToMV/TYLCV), quadruple (CMV/PVY/TYLCV/ToMV, CMV/PVX/TYLCV/ToMV, CMV/PVX/PVY/ToMV) and CMV/PVY/ToCV/TYLCV and quintuple (CMV/PVM/PVX/PVY/TYLCV, PVM/PVS/PVY/ToMV/TYLCV and CMV/PVS/PVX/PVY/ToMV) infections were also observed.

TYLCV was the most prevalent virus in all the four districts surveyed infecting 58 out of 76 samples (64.47%). PVY ranked second (23.68%) followed by CMV (17.11%). CMV was more prominent in Una district of Himachal Pradesh than any of the three districts of Punjab. PVY was more prevalent in Una and Patiala districts. All plants were tested negative in serological or molecular tests against TSWV, AMV, PVA, PLRV, PMTV, TAV, TBSV, INSV, TICV and TRV (data not shown).

To partially characterize the viruses, PCR-amplified products obtained with virus/viroid-specific primers were cloned and sequenced. The sequences obtained were assembled and submitted to GenBank (accession numbers MH038048 and MZ322850 for CMV, MZ643265 for GBNV, MG551665, MG551666 and MG551667 for PVM, MG551668 and MG551669 for PVS, MH038050 for PVX, MZ322851, MZ322852 & MZ322853 for PVY, MN511714 for ToCV, and MH038047 for ToMV). Antibodies against *Tomato yellow leaf curl virus* (TYLCV) were procured for performing DAS-ELISA. Later, degenerate primer pair for Begomovirus DNA-A segment was used for RT-PCR which amplified the PCR product of size 827 bp. Sequence of this RT-PCR product has been submitted to GenBank with accession no. MZ643266 and revealed 97.67% identity with the Indian isolate of TYLCV i.e. Tomato Leaf Curl New Delhi Virus (ToLCNDV; accession no. MF807949.1).

Four isolates from each district appeared negative in ELISA for PVX, but RT-PCR using PVX-specific primers confirmed its presence and the sequence was submitted to GenBank (MH038050) that exhibited 99.64% sequence identity with PVX sequence isolated from potato from Bangladesh (MK587458.1). In addition to PVX, the comparative analysis of sequence of ToCV coat protein gene (GenBank accession number MN511714) with other *Criniviruses* available in the GenBank revealed highest sequence identity (99.48%) with ToCV (KP137101.1) from South Korea and ToCV (EU625350.1) from France. Furthermore, GBNV isolate of this study (MZ643265) shared 98.80% nucleotide similarity with Indian GBNV isolate Tomato/Bethamangala/UHSASB26 (GenBank Accession no. MN735625.1).

3.4. Phylogenetic analysis

Phylogenetic tree was constructed using nucleotide sequences of amplified gene products from different isolates of the present study and already available sequences of respective viruses downloaded from the nucleotide database of NCBI. For better representation of tree, the acronyms of different isolates were abbreviated and different types of information of each of these isolates like isolate name, country name, sequence length, host species and GenBank accession number are presented in Table 3. The phylogenetic tree (Fig. 3) shows that sequences of each specific virus are clustering together and form individual clades. The tree is represented by two large (L1 and L2) clades, which are further divided into two subclades (L1A and L1B; L2A and L2B) and four small (S1, S2, S3 and S4) clades. Tree also shows clustering of 12 individual and two pairs of nucleotide sequences belonging to *Groundnut bud necrosis virus* (GNV) isolates from India.

L1A represents 10 individual nucleotide sequences belonging to *Tomato mosaic virus* (TMV) isolates. TMV-In* (isolate: B10 Jodhey) from Amritsar, India is in close association with the sequence TMV-Ug (isolate: ToMV-Ug) detected from Uganda. L1B clade shows clustering of 12 sequences of *Tomato chlorosis virus* (TCV) with two small clusters of 6 sequences each. In one cluster, TCV sequences belonging to South Africa (TCV-Sa; isolate: ToCR-186), India (TCV-In*; isolate: K4 Khanpur; this study), South Korea (TCV-Sk; isolate: JJ), United Kingdom (TCV-Uk), Spain (TCV-Sp; isolate: Pl-1-2) and France (TCV-Fr; isolate: Fr) are clustering whereas, in another cluster, TCV sequences belonging to Turkey (TCV-Tu; isolate: FETHIYE72), USA (TCV-US; isolate: Florida), China (TCV-Ch; isolate: HBHS), Japan (TCV-Ja; isolate: Kuma1), Brazil (TCV-Br; isolate: ToC-Br2) and Greece (TCV-Gr; isolate: To-Il1857) are grouped together.

In L2A, two PVS sequences viz. PVS-In1* (isolate: K9 Khanpur) and PVS-In2* (isolate: KP7 Patiala) detected from this study are in close association with the sequence, PVS-Ba, reported from Bangladesh. These three sequences and a sequence from China (isolate: HB24) form cluster with six individual sequences from Russia, Syria, Hungary, Germany, Netherlands and Iran. Clade L2B represents clustering of 12 sequences belonging to PVM isolates.

Clade S1 represents 9 individual nucleotide sequences belonging to PVX isolates. Eight PVX sequences retrieved from NCBI along with PVX-In1* (isolate: K9 Khanpur) detected in this study have a common origin. Clade S2 is represented by 10 gene sequences belonging to PVY isolates. Out of these PVY sequences, PVY-In3* (isolate: B7 Jodhey) detected in this study is closely related to an isolate identified from Macedonia (isolate: KUA7-2013) and an isolate from Pakistan (isolate PK). Clade S3 shows clustering of 17 *Tomato leaf curl New Delhi virus* (TLC) nucleotide sequences. An isolate from this study (TLC-In1*; isolate: K12 Khanpur) is closely related to an isolate from Bahraich, Uttar Pradesh, India (TLC-In6; isolate: India: UP: Bahraich: Tomato1: 2011). Small clade S4 is represented by 21 nucleotide sequences belonging to CMV isolates. All sequences have a close association with each other. CMV isolates (CMV-In1*; isolate: K3 Khanpur and CMV-In2*; isolate: B7 Jodhey) from the present study are in close association with each other and with an isolate from Vietnam (CMV-Vi; isolate: VN-Bavi) and another from Poland (isolate: Del).

To the best of our knowledge, this is the first report of ToCV on tomato in India.

4. Discussion

Detection and prevalence of viruses infecting field-grown tomato in North-western region of India were studied through serological and molecular approaches. Although visual assessment of symptoms is not accurate way of determining incidence of a viral disease, yet this method is widely used by many plant pathologists in monitoring the prevalence, incidence and intensity of a disease. Symptoms can also vary depending on virus strain, mixed infections, cultivar, light intensity, time of infection during the year, stage of plant growth, temperature and/or the presence of unknown pathogens [39].

Analysis of leaf samples collected from symptomatic tomato plants during surveys conducted for the present study (2015 to 2017) revealed occurrence of 8 RNA viruses viz. CMV, GBNV, PVM, PVS, PVX, PVY, ToCV, ToMV and a DNA virus viz. ToLCNDV. The surveyed fields were found to have same cropping cycles which mostly included solanaceous crops one after the other. In our earlier study, we reported natural infection of CMV, PVM and PVY on a weed species, *Solanum nigrum*, commonly found in tomato fields of the study area [40]. However, none of the plants of another weed species (*Chenopodium* sp.) analysed in the present study showed occurrence of any of the above viruses

It was noticed that potato crop was harvested prior to tomato crop. Keeping this in mind, symptomatic plant samples were tested against eight potato viruses also, out of which four potato viruses (PVM, PVS, PVX and PVY) were detected. Out of these isolates, PVY positive isolate (B7-Asr) shared 100% sequence identity with another isolate of PVY from Egypt (MT242381.1) whereas PVM isolate (B9-Asr) shared 97.33% similarity with other isolates from Japan (LC511893.1) and New Delhi (KJ462137.1). K9-Una isolate of PVX showed 99.64% nucleotide similarity with an isolate from Bangladesh (MK587458.1). PVY isolates are divided into five strains viz. PVY^O, PVY^C, PVY^N, PVY^Z, and PVY^E [41]. PVY^O (causes systemic mottling) and PVY^N (causes veinal necrosis) are commonly found in potato fields, while PVY^C is relatively less prevalent [12]. PVY evolution has been aided by genomic recombination, which has resulted in the formation of novel genotypes/strains such as the recombinant PVY^{NTN}, PVY^{N-Wi}, and PVY^{NTN-NW} [42]. Lorenzen et al. [43] described multiplex assay utilising eight PVY primers capable of discriminating various strains of PVY (PVY^O, PVY^N, PVY^{N:O} (Wilga) and PVY^{NTN}). The primer combinations include PVY n2258 + o2439c for NTN, N:O strain, PVYo2172 + o2439c for O strain and PVYn2258 + n2650c for N strain. In our study, three different PCR assays were run to check the presence of target strains for Potato virus Y. Isolates of Una and Patiala gave amplification of 266 bp for strain O (GenBank Accession numbers MZ322852 and MZ322853) (Fig. 2H) and isolate of Amritsar confirmed band of 181 bp for strain NTN, N:O (Fig. 2G) whereas no bands were seen corresponding to strain N.

CMV has been found on several crops including cucumber, tomato, chilli, potato, brinjal, banana, pepper, vanilla, gherkin, gerbera, snake gourd, bottle gourd, etc. from India [44]. Rybicki [45] listed CMV among the top ten economically significant plant viruses. During visual symptom based surveys conducted in various fields, typical shoestring symptoms on tomato leaves were observed. CMV isolate K3 shared 97.94% nucleotide sequence identity with other isolates from Ecuador (MW291545) followed by 97.20% sequence similarity with isolates from Uganda (MG021462.2). Bud necrosis disease in tomatoes is caused by *Groundnut bud necrosis virus* (GBNV), the most common tospovirus in India. *Groundnut bud necrosis virus* was named in 1992 after it was discovered through serology that the bud necrosis disease of groundnut was caused by a tospovirus other than TSWV [46]. In the present study, only one sample from dist. Una was confirmed for GBNV by RT-PCR (Fig. 2B); nucleotide sequence of which was submitted to NCBI.

ToCV causes a deadly disease that primarily affects tomatoes, but it has also been seen infecting a variety of other commercially significant vegetables and wild plants [14]. ToCV was first discovered in Florida (USA) in the mid-1990s and since then, it has spread to thirty five nations and territories, making it a typical example of being an emergent plant pathogen [14]. Chlorotic regions are usually polygonal in shape in the early stages of infection, and are confined by main veins. Interveinal yellow regions can produce reddish-brown necrotic specks in advanced stages. Lower leaves are curled, thicker, and brittle, with a crisp texture when touched [15]. In our study, whiteflies were observed in open-tomato fields. Only one sample responded positively in RT-PCR assay. Sequencing the cloned RT-PCR product finally confirmed the presence of ToCV in India (774 bp) (Fig. 2I). To the best of our knowledge, this is the first time that ToCV and CMV/PVY/ ToCV/ToLCNDV mixed infections are being reported from India. ToMV isolate (B10-Asr) shared 99.68% sequence identity at nucleotide level with an isolate of ToMV from Vietnam (MH393621.1).

Besides tomatoes, many other economically significant crops are being attacked by plant viruses, particularly begomoviruses, in Asia, which is one of the reasons causing frustration among small-scale farmers. Despite the fact that farmers are confused by plant viral diseases, only few research articles have addressed this issue, particularly in underdeveloped nations. ToLCNDV-infected tomato plants show typical symptoms such as upward or downward curling and crinkling of leaves, yellow mottling, vein clearing, leaf blistering, and leaf puckering. Plants become stunted with shortened internodes and poor fruit set as the infection progresses, resulting in full crop loss [17]. Leaf curling was noticed to be the most common symptom in infected plants during field surveys, and it was later proven that it was caused by a *Begomovirus*. Although, in fields, mixed infections involving two or more plant viruses were prevalent, and they could have interacted in a variety of ways, complicating things further. Unrelated viruses that infect the same host cells are known to produce synergistic interactions. The mechanism underlying the synergistic relationships is unknown, but it is possible that a variety of viral and/or host products are involved.

A combined phylogenetic tree was constructed using nucleotide sequences (RNA & DNA) of different isolates of all the nine viral species analysed in the present study along with sequences obtained from NCBI (Fig. 3). Phylogenetic analysis of the viruses studied in the present investigation revealed that different isolates belonging to each specific virus clustered together irrespective of the geographical locations from where they have been detected. The tree consists of two large clades (L1 and L2) each of which is further comprised of two subclades (A and B). In clade L1, the coat protein sequence of *Tomato chlorosis virus* (TCV) is clustering with replicase gene sequence of *Tomato mosaic virus* (TMV), which hints toward their common ancestral origin. In clade L2, the coat protein sequences of *Potato virus M* (PVM) and *Potato virus S* (PVS) revealed some striking similarity, as these two RNA viruses belong to the same family. Phylogenetic analysis confirmed that CMV isolates (K3 Khanpur and B7 Jodhey) from this study were clustering together and were closely related to an isolate of Vietnam (VN-Bavi) identified from *Nicotiana tabacum*; *Groundnut bud necrosis virus* isolate (GNV-In1*; K3 Khanpur) was closely related to an

Indian isolate (Tomato/UAS/Dharwad/UHSASB193); two PVY isolates, K8 Khanpur and KP7 Patiala, were clustering together whereas the third PVY isolate (PVY-In3*; B7 Jodhey) was closely related to a Macedonian isolate (KUA7-2013) and another isolate from Pakistan (PK); *Tomato chlorosis virus* isolate (TCV-In*; K4 Khanpur) was closely related to a South African isolate (ToCR-186); *Tomato leaf curl New Delhi virus* isolate (TLC-In1*; K12 Khanpur) was closely related to an Indian isolate (India:UP:Bahraich:Tomato1:2011) reported from Bahraich and identified from tomato; and TMV (B10 Jodhey) was closely related to an isolate from Uganda (ToMV-Ug). TMV isolates from India, Uganda, Zimbabwe, Slovakia, South Korea and Kazakhstan were forming one cluster which was further divided into a subcluster comprising TMV sequences from China, Japan, Australia and United States. The results of this study may be useful for further molecular evolutionary research on tomato or potato viruses in India.

4. Conclusion

Tomatoes are a valuable cash crop in many countries across the world. In recent years, increasing incidences of virus infection are being reported affecting quality and yield of tomatoes. To prevent virus spread and yield reduction, it is necessary to track occurrence and distribution of major viruses infecting tomato crop. In order to reduce the incidence of tomato viruses, weed sanitation programs could be put into action. Moreover, monitoring of weeds is required to upgrade our knowledge of the weeds serving as alternate hosts or primary sources of inoculum for virus spread in the region. Majority of plant viruses rely on vectors for transmission and dissemination. Population of these insect vectors should be checked and insecticides or pesticides should be used to reduce their spread. Biological control methods and integrated control are two new techniques. To avoid introduction of new economically important diseases into Punjab, constant monitoring and vigilance are needed in tomato fields. Thus, the data reported here, is crucial in laying the groundwork for viral epidemic management in field-grown tomatoes in North-western region of India. This is the first report of ToCV infection in tomato from India. The research also revealed mixed infections of various viruses infecting tomato that can cause severe losses in India. Efforts to find efficient control techniques to mitigate severe harm caused by these viruses to Indian vegetable production are urgently needed.

Declarations

Funding: We are grateful to Council of Scientific and Industrial Research, New Delhi (F.No. 38 (1399)/14/EMR-II); Department of Science and Technology, New Delhi; University Grants Commission, New Delhi for providing the financial assistance under MRP; DST-FIST; UPE, CPEPA and DRS schemes.

Author contributions: Conceptualization: PC, VH and AKN. Methodology: PC and SK. Phylogenetic analysis: AK and AKN. Formal analysis and investigation: BS and SK. Writing-original draft preparation: PC and AKN. Manuscript review and editing: BS, VH and AKN. Funding acquisition: AKN. Resources: VH and AKN. Supervision: BS, VH and AKN. All authors have read and agreed to the final version of the manuscript.

Conflict of interest: The authors declare that there is no conflict of interest and also competing interests.

Consent for publication: We hereby declare that we participated in this research and the paper's development. We have read its final version and give consent for the publication.

Research involving human participants and/or animals: This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. FAOSTAT (2019) Food Agriculture and Organization (FAOSTAT). Retrieved from <http://www.fao.org/faostat/en/#data/QC>
2. Mothapo MC, Dube T, Abdel-Rahman E, Sibanda M (2021) Progress in the use of geospatial and remote sensing technologies in the assessment and monitoring of tomato crop diseases. *Geocarto Int* 1–21. <https://doi.org/10.1080/10106049.2021.1899303>
3. Ni L, Punja ZK (2019) Management of fungal diseases on cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) crops in greenhouses using *Bacillus subtilis*. *Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol*. Springer, Cham, pp 1–28
4. Petrov N, Stoyanova M, Gaur RK (2021) Ecological methods to control viral damages in tomatoes. *Plant Virus-Host Interaction*. Academic Press, United States, pp 469–488
5. Ong SN, Taheri S, Othman RY, Teo CH (2020) Viral disease of tomato crops (*Solanum lycopersicum* L.): an overview. *J Plant Dis Prot* 127:725–739. <https://doi.org/10.1007/s41348-020-00330-0>
6. Hanssen IM, Lapidot M, Thomma BP (2010) Emerging viral diseases of tomato crops. *Mol Plant Microbe Interact*. <https://doi.org/10.1094/MPMI-23-5-0539>. 23:539 – 48
7. Gadhave KR, Gautam S, Rasmussen DA, Srinivasan R (2020) Aphid transmission of potyvirus: The largest plant-infecting RNA virus genus. *Viruses* 12:773. <https://doi.org/10.3390/v12070773>
8. Edwardson JR, Christie RG (2018) CRC handbook of viruses infecting legumes. CRC Press, United States
9. Foster GD, Mills PR (1992) The 3'-nucleotide sequence of an ordinary strain of potato virus S. *Virus Genes* 6:213–220. <https://doi.org/10.1007/BF01702560>
10. Cox BA, Jones RA (2010) Genetic variability in the coat protein gene of Potato virus S isolates and distinguishing its biologically distinct strains. *Arch Virol* 155:1163–1169. <https://doi.org/10.1007/s00705-010-0680-6>
11. Kubaa RA, Choueiri E, De Stradis A, Jreijiri F, Saponari M, Cillo F (2021) Occurrence and Distribution of Major Viruses Infecting Eggplant in Lebanon and Molecular Characterization of a Local Potato Virus X Isolate. *Agriculture* 11:1–16. <https://doi.org/10.3390/agriculture11020126>

12. Blanco-Urgoiti B, Dopazo J, Ponz F (1998) Potato virus Y group C isolates are a homogeneous pathotype but two different genetic strains. *J Gen Virol* 79:2037–2042. <https://doi.org/10.1099/0022-1317-79-8-2037>
13. Karasev AV, Gray SM (2013) Genetic diversity of Potato virus Y complex. *Am J Potato Res* 90:7–13. <https://doi.org/10.1007/s12230-012-9287-7>
14. Fiallo-Olivé E, Navas-Castillo J (2019) Tomato chlorosis virus, an emergent plant virus still expanding its geographical and host ranges. *Mol Plant Pathol* 20:1307–1320. <https://doi.org/10.1111/mpp.12847>
15. Wisler GC, Li RH, Liu HY, Lowry DS, Duffus JE (1998) Tomato chlorosis virus: a new whitefly-transmitted, phloem-limited, bipartite closterovirus of tomato. *Phytopathology* 88:402–409. <https://doi.org/10.1094/PHYTO.1998.88.5.402>
16. Dombrovsky A, Smith E (2017) Seed transmission of Tobamoviruses: Aspects of global disease distribution. In: Jimenez-Lopez JC (ed) *Advances in Seed Biology*, Books on Demand, pp233-260
17. Chakraborty S (2009) Tomato leaf curl viruses from India. In: Mahy BW, Van Regenmortel MH (eds) *Desk encyclopedia of plant and fungal virology*. Elsevier, United Kingdom, pp 339–347
18. Kunkalikar SR, Poojari S, Arun BM, Rajagopalan PA, Chen TC, Yeh SD, Naidu RA, Zehr UB, Ravi KS (2011) Importance and genetic diversity of vegetable-infecting tospoviruses in India. *Phytopathology* 101:367–376. <https://doi.org/10.1094/PHYTO-02-10-0046>
19. Navarre DA, Shakya R, Holden J, Crosslin JM (2009) LC-MS analysis of phenolic compounds in tubers showing zebra chip symptoms. *Am J Potato Res* 86:88–95. <https://doi.org/10.1007/s12230-008-9060-0>
20. De Blas C, Borja* MJ, Saiz M, Romero J (1994) Broad spectrum detection of cucumber mosaic virus (CMV) using the polymerase chain reaction. *J Phytopathol* 141:323–329. <https://doi.org/10.1111/j.1439-0434.1994.tb01476.x>
21. Tanina K, Inoue K, Date H, Okuda M, Hanada K, Nasu H, Kasuyama S (2001) Necrotic spot disease of cineraria caused by Impatiens necrotic spot virus (INSV). *Plant Pathol J* 67:42–45. <https://doi.org/10.3186/jjphytopath.67.42>
22. Singh RP (1999) A solvent-free, rapid and simple virus RNA-release method for potato leafroll virus detection in aphids and plants by reverse transcription polymerase chain reaction. *J Virol Methods* 83:27–33. [https://doi.org/10.1016/S0166-0934\(99\)00102-0](https://doi.org/10.1016/S0166-0934(99)00102-0)
23. Crosslin JM, Hamlin LL (2011) Standardized RT-PCR conditions for detection and identification of eleven viruses of potato and Potato spindle tuber viroid. *Am J Potato Res* 88:333–338. <https://doi.org/10.1007/s12230-011-9198-z>
24. Verhoeven J TJ, Jansen CC, Willems TM, Kox LF, Owens RA, Roenhorst JW (2004) Natural infections of tomato by Citrus exocortis viroid, Columnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. *Eur J Plant Pathol* 110:823–831. <https://doi.org/10.1007/s10658-004-2493-5>
25. Rajamäki M, Merits A, Rabenstein F, Andrejeva J, Paulin L, Kekarainen T, Kreuze JF, Forster RL, Valkonen JPT (1998) Biological, serological, and molecular differences among isolates of potato A potyvirus. *Phytopathology* 88:311–321. <https://doi.org/10.1094/PHYTO.1998.88.4.311>
26. Nie X, Singh RP (2001) A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers. *J Virol Methods* 91:37–49. [https://doi.org/10.1016/S0166-0934\(00\)00242-1](https://doi.org/10.1016/S0166-0934(00)00242-1)
27. Zhang J, Wang R, Song J, Luo Z, Yang J, Lin F (2013) One-step Multiplex RT-PCR for Simultaneous Detection of Four Viruses in Tobacco. *J Phytopathol* 161:92–97. <https://doi.org/10.1111/jph.12032>
28. Verma N, Kumar K, Kulshrestha S, Raikhy G, Hallan V, Ram R, Zaidi AA, Garg ID (2009) Molecular studies on Tomato aspermy virus isolates infecting chrysanthemums. *Arch Phytopathol Pflanzenschutz* 42:99–111. <https://doi.org/10.1080/03235400600951779>
29. Kim MK, Kwak HR, Jeong SG, Ko SJ, Lee SH, Park JW, Kim KH, Choi HS, Cha BJ (2007) First report on Tomato bushy stunt virus infecting tomato in Korea. *Plant Pathol J* 23:143–150. <https://doi.org/10.5423/PPJ.2007.23.3.143>
30. Vaira AM, Accotto GP, Vecchiati M, Bragaloni M (2002) Tomato infectious chlorosis virus causes leaf yellowing and reddening of tomato in Italy. *Phytoparasitica* 30:290–294. <http://dx.doi.org/10.1007/BF03039998>
31. Jacquemond M, Verdin E, Dalmon A, Guilbaud L, Gognalons P (2009) Serological and molecular detection of Tomato chlorosis virus and Tomato infectious chlorosis virus in tomato. *Plant Pathol* 58:210–220. <https://doi.org/10.1111/j.1365-3059.2008.01959.x>
32. Kumar S, Udaya Shankar AC, Nayaka SC, Lund OS, Prakash HS (2011) Detection of Tobacco mosaic virus and Tomato mosaic virus in pepper and tomato by multiplex RT-PCR. *Lett Appl Microbiol* 53:359–363. <https://doi.org/10.1111/j.1472-765X.2011.03117.x>
33. Robinson DJ (1992) Detection of tobacco rattle virus by reverse transcription and polymerase chain reaction. *J Virol Methods* 40:57–66. [https://doi.org/10.1016/0166-0934\(92\)90007-Z](https://doi.org/10.1016/0166-0934(92)90007-Z)
34. Zaffalon V, Mukherjee SK, Reddy VS, Thompson JR, Tepfer M (2012) A survey of geminiviruses and associated satellite DNAs in the cotton-growing areas of northwestern India. *Arch Virol* 157:483–495. <https://doi.org/10.1007/s00705-011-1201-y>
35. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. Cold spring harbor laboratory press, New York
36. Gibbs A, Ohshima K (2010) Potyviruses and the digital revolution. *Annu Rev Phytopathol* 48:205–223. <https://doi.org/10.1146/annurev-phyto-073009-114404>
37. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
38. Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 49:W293–W296. <https://doi.org/10.1093/nar/gkab301>
39. Blancard D (2019) *Tomato diseases: identification, biology and control: a colour handbook*. CRC Press, United States
40. Chaudhary P, Kumari R, Singh B, Hallan V, Nagpal AK (2019) First report of potato virus M, potato virus Y and cucumber mosaic virus infection in Solanum nigrum in India. *J Plant Pathol* 101:419–419. <https://doi.org/10.1007/s42161-018-0194-8>

41. Singh RP, Valkonen JP, Gray SM, Boonham N, Jones RA, Kerlan C, Schubert J (2008) Discussion paper: The naming of Potato virus Y strains infecting potato. Arch Virol 153:1–3. <https://doi.org/10.1007/s00705-007-1059-1>
42. Glais L, Tribodet M, Kerlan C (2002) Genomic variability in Potato potyvirus Y (PVY): evidence that PVY^{NW} and PVY^{NTN} variants are single to multiple recombinants between PVY^O and PVY^N isolates. Arch Virol 147:363–378. <https://doi.org/10.1007/s705-002-8325-0>
43. Lorenzen JH, Piche LM, Gudmestad NC, Meacham T, Shiel P (2006) A multiplex PCR assay to characterize Potato virus Y isolates and identify strain mixtures. Plant Dis 90:935–940. <https://doi.org/10.1094/PD-90-0935>
44. Nagendran K, Priyanka R, Aravintharaj R, Balaji CG, Prashant S, Basavaraj B, Mohankumar S, Karthikeyan G (2018) Characterization of Cucumber mosaic virus infecting snake gourd and bottle gourd in India. Physiol Mol Plant Pathol 103:102–106. <https://doi.org/10.1016/j.pmpp.2018.05.010>
45. Rybicki EP (2015) A Top Ten list for economically important plant viruses. Arch Virol 160:17–20. <https://doi.org/10.1007/s00705-014-2295-9>
46. Reddy DV, Ratna AS, Sudarshana MR, Poul F, Kumar IK (1992) Serological relationships and purification of bud necrosis virus, a tospovirus occurring in peanut (*Arachis hypogaea* L.) in India. Ann Appl Biol 120:279–286. <https://doi.org/10.1111/j.1744-7348.1992.tb03425.x>

Table

Table 2 is only available as a download in the Supplemental Files section.

Figures

Fig. 1. Visual symptoms of possible virus infection in different tomato plants (row wise; from left to right; **A:** chlorosis, **B:** bunchy appearance, **C:** shortened internodes, **D:** blossom drop, **E:** stunted plant growth, **F:** leaf crumpling, **G:** necrosis, **H:** wilting and bronzing, **I:** leaf curling, **J:** leaf rolling, **K:** leaf purpling, and **L:** mosaic and mottling)

Figure 1

See image above for figure legend

Fig.2. Representative photograph showing agarose gel electrophoresis of RT-PCR products obtained with primers; **A:** CMV (534 bp), **B:** GBNV (831 bp), **C:** PVM (524 bp), **D:** PVS (729 bp) **E:** PVX (562 bp), **F:** PVY (218 bp), **G:** PVY n2258/o2439c (181 bp), **H:** PVY o2172/o2439c (266 bp), **I:** ToCV (774 bp), **J:** ToMV (317 bp), and **K:** TYLCV (827 bp)

Figure 2

See image above for figure legend

Fig. 3. Phylogenetic tree showing the relationships of coat protein and replicase gene sequences of various virus isolates identified from Northwestern region of India with other GenBank sequences reported from different countries and also from other parts of India.

Figure 3

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table2.doc](#)