Int.J.Curr.Microbiol.App.Sci (2021) *10*(07): 484-502

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 07 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1007.054

First Report of Begomovirus causing Leaf Curl Disease in Medicinal Legume *Macrotyloma uniflorum* Lam. (Verdc.) in Northern Region of India

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Keywords

Horsegram, *Bemisia tabaci*, Pedilanthus leaf curl virus, GenBank

Article Info

Accepted:
20 June 2021
Available Online:
10 July 2021

A B S T R A C T

Horsegram is a drought hardy, medicinal, nutritional legume and capable to tolerate various biotic and abiotic stresses. The presence of begomovirus was found to be closely associated with the population build-up of its vector i.e. whitefly (*Bemisia tabaci*) in different accessions of horsegram in the field. The begomoviral associated disease was detected by using Polymerase chain reaction (PCR) with begomovirus specific degenerate primer. The amplified region of 545 bp were sequenced and submitted to GenBank Accession number MK637450.1. Sequence analysis revealed the highest identity 99.63% with *Pedilanthus leaf curl virus*. The symptoms were matching with the typical begomoviral symptoms like upward leaf curling and yellow patches with stunted growth respectively. A total of 48 accessions of horsegram were tested against of begomoviral leaf curl disease in a randomized complete block design with three replications check. The maximum average disease incidence of leaf curling found to be 54.44% (L1).100% germination of seeds was given by L4, L14, L29 and L30 accessions. To the best of our knowledge, this is the first report of leaf curl disease in horsegram by begomovirus in India.

Introduction

Horsegram is highly important nutritious legume crop of Indian sub-continent (Chahota *et al.*, 2017) with ethno-medicinal beliefs in India, which is usually known as Kulattha in Sanskrit, Kurti-kalai in Bengali, Kollu in Tamil, Ullavallu in Telgu, Muthira in Malyalam, Gahot in Kumaon and Garhwal region of Utrakhand (Bhartiya *et al.*, 2015) and Kulith in Marathi (Gadgil *et al.*, 2016). Horsegram is a protein rich, underutilized pulse crop of family Fabaceae (Prasad and Singh, 2015). It is highly nutritious food source among well-known legumes (Kaundal and kumar, 2020).

It has also been identified as a potential food source for the future by U.S. National Academy of Sciences. Since it has very low

water requirement, therefore it is widely grown in the semi-arid regions of India and also in other parts of world. It is generally referred as a protein rich poor man's crop that grows well under low rainfall conditions and marginal soil fertility (Kiranmai *et al.,* 2016). It is considered as an important medicinal pulse in Ayurveda (Bhardwaj and Yadav 2015, Prasad and Singh, 2015) and various pharmaceutical use (Rlds and Erhss 2017, Kaundal *et al.,* 2019).

Horsegram has been reported to be affected by a begomovirus i.e. *Horsegram yellow mosaic virus* and *Tobacco streak virus* (Muniyappa *et al.,* 1987, Barnabas *et al.,* 2010, Abubakar *et al.,* 2018, Goud *et al.,* 2013, Vermana and Jain, 2010).

In the present study, plants of horsegram showed the upward leaf curling, stunted growth, yellow patches on leaves and chlorosis symptoms of begomovirus infection. More yellow discoloration patches in yellow mosaic whereas upward leaf curling symptoms were shown in Leaf curl disease. Leaf curl like symptoms were different from previously reported viral diseases of horsegram. Therefore, the core coat protein (CP) region (AV1 gene) of DNA-A segment was analyzed for identification of the suspected begomovirus.

It is now well established that the CP gene can be used as an effected marker for identification and molecular characterization of begomoviruses (Mahaeshwari *et al.,* 2014).

Therefore, group specific degenerate primers (Wyatt and Brown, 1996) were used to amplify CP region and to obtain its sequence in the present isolate. Bioinformatics analysis of the obtained sequence confirmed its identity as begomovirus. To the best of our knowledge, this is the first report of leaf curl disease in horsegram by begomovirus in India.

Materials and Methods

Plant material

The horsegram accessions were procured from different geographical regions of India and cultivated at the Agriculture farm, DAV University, Jalandhar for germplasm multiplication (Table 1). Upward leaf curling, stunting, chlorosis symptoms of leaf curl disease and yellow coloration patches (increases with growth of leaves) were observed. The symptomatic and asymptomatic leaves were collected and stored at -80℃ in deep freezer for further studies.

DNA Isolation

Total genomic DNA was isolated by using CTAB method (Doyle and Doyle, 1990) from the stored infected leaves having the symptomatic symptoms.

Virus detection using Degenerate primers

DNA template for PCR was prepared from Leaves of infected horsegram plants. Amplification of begomovirus genome was carried out using a pair of degenerate primers designed for the amplification of the DNA A, DNA B genomic components and coat protein. The degenerate primer namelyAV494 and AC1048(Wyatt and Brown, 1996)was used for the detection of leaf curl disease in horsegram (Table 2). A master mix for final volume of 25 µl was prepared in 1.5 ml eppendorf tube by adding all PCR components. Then DNA template (1 µl) and 24µl master mix were dispensed into PCR tubes. Lastly the PCR tubes was transferred in Veriti Thermal Cycler (Applied Biosystems , USA) as follows : Initially, the initial denaturation was done on 94˚ C for 5 min followed by 30 cycles: 94˚ C for 2 min (Denaturation), 55˚ C for 1 min (Annealing) and 72˚ C for 1 min (Extension) and then final

extension with 72° C for 10 min. PCR hold was done at 4° C for infinite time. After the PCR amplification, the amplified PCR products were electrophoresed in agarose gel and eluted using Wizard SV Gel and PCR Clean-Up System (Promega, USA). Sequencing of the eluted product was done using 3730xl DNA Analyzer (Applied Biosystems, USA).

In silico analysis

Sequence obtained was first analyzed (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using Basic local alignment search tool (Altschul *et al.,* 1990). Among top 1000 BLASTn hits, the sequences of ICTV recognized begomovirus species were shortlisted based on the highest sequence identities. These sequences along with the outgroup sequence were used for multiple alignment and phylogenetic analysis using ClustalWprogramme (Thompson *et al.,* 1994). Mega X programme (https://www.megasoftware.net) (Kumar *et al.,* 2018) was used to construct phylogenetic tree using neighbor-joining method (Saitou and Nei,1987) with 1000 bootstrap replicates (Felsenstein, 1985) and the evolutionary distances were computed using the Maximum Composite Likelihood Method (Tamura *et al.,* 2004). The partial and complete CP gene sequence of the present isolate was deposited in NCBI GenBank database.

Screening of horsegram accessions for disease incidence

Forty eight accessions of horsegram were selected for carried out experiment during August, 2019 in field trial. All the selected accessions of horsegram were tested against leaf curling. The screening of begomoviral associated disease was laid out in a randomized complete block design with three replications check. The accessions were gown in five rows each of with spacing of 60 ×30 cm in RBD whereas 1 meter space was kept in between each accessions of horsegram to maintain easy path and time to time irrigation. Twenty four accessions of horsegram were grown in three replications and in-between each replication, 1m space was left for the irrigation to the plants. The first two rows were kept to prevent from bordering effect. The disease incidence was noticed after 60 days after appearance of first symptoms of leaf curling to different accessions of horsegram (Khan *et al.,* 2018).

Disease incidence = (Total infected plant⁄Total healthy plant) ×100

Results and Discussion

During field trial, upward curling, yellowing of leaves, along with stunting of the plant were recorded in horsegram growing in the agricultural farms of DAV University, Jalandhar. These were quite similar to the begomoviral symptoms (Fig.1).The screened samples were showing the disease basically transmitted by whitefly (*Bemisiatabaci*) in various accessions of horsegram in the field. A total of 48 accessions were collected from different geographical regions of India (Table 1) and used to screen for the begomoviral associated disease in the field through randomised block design with three replication each.A sharp bands of 570bp was amplified by using degenerate primers AV494 and AC1048 having leaf curl disease (Fig. 2). The purified amplified regions 570 bpamplicons with their respective symptoms are shown. Top BLASTn hits were belonging to the coat protein region of *Pedilanthus leaf curl virus isolate Petunia* (accession number MF135486) and a sequence identity of 99.63% was found. The sequencing results from the eluted PCR amplified product was submitted in Genbank with accession number MK637450.1. A total of 181 amino acids coded for partial coat protein from 545bp of submitted nucleotide sequences in Genbank.

Table.1 Collection of horsegram accessions from different geographical regions of India

Sr. No.	Accessions No.	Location	State	Seeds Colour	Locality Geographical Coordinates		Elevation in Meters
					Latitute (°N)	Longitude (°E)	
1.	L4	Bakarti, Hamirpur-1	Himachal Pradesh	Straw with blackish green tinge	31°38’24’’N	76°30’39’’E	707m
2.	L7	Nerchownk, Mandi-1	Himachal Pradesh	Straw with orange tinge	31°35’56’’N	76°53’38’’E	813m
3.	L9	Rampur, Shimla-1	Himachal Pradesh	Straw with blackish green tinge	31°26’16’’N	77°39’37’’E	2484m
4.	L11	Rampur, Shimla-2	Himachal Pradesh	Straw with black tinge	31°26’16’’N	77°39’37’’E	2484m
5.	L12	Sarahan, Sirmour	Himachal Pradesh	Straw with blackish green tinge	30°43’02’’N	77°08’04’’E	880m
6.	L13	Pehrwin, Bilaspur	Himachal Pradesh	Straw with greenish tinge	31°22’43’’N	76°44’29’’E	594m
7.	L14	Bharmour, Chamba	Himachal Pradesh	Straw with blackish green tinge	32°26’47’’N	76°32’16’’E	2046m
8.	L18	Dalhousi, Chamba-1	Himachal Pradesh	Straw with orange tinge	32°32’42’’N	75°50’08’’E	575m
9.	L23(A)	Gondpur, Una-1	Himachal Pradesh	Straw with black tinge	31°44’48’’N	76°01’49’’E	525m
10.	L20	Rampur, Shimla-3	Himachal Pradesh	Straw with blackish green tinge	31°26’16’’N	77°39’37’’E	2484m
11.	L22	Shoali, Shimla	Himachal Pradesh	Straw with blackish green tinge	31°04’23’’N	77°40’03’’E	2813m
12.	L23(B)	Gondpur, Una-2	Himachal Pradesh	Straw with blackish green tinge	31°44’48’’N	76°01’49’’E	525m
13.	L24	Shrog, Shimla	Himachal Pradesh	Straw with greenish tinge	31°11’27’’N	77°42’01’’E	1942m
14.	L25	Nauni, Solan	Himachal Pradesh	Straw with blackish green tinge	30°50’50’’N	77°09’37’’E	1158

15.	L26	Sarahan, Sirmour-1	Himachal Pradesh	Straw with blackish green tinge	30°40’23’’N	77°09’49’’E	872m
16.	L27	Sarahan, Sirmour-2	Himachal Pradesh	Straw with blackish green tinge	30°40’23’’N	77°09’49’’E	872m
17.	L28	Nerchownk, Mandi-2	Himachal Pradesh	Straw with orange tinge	31°26’16’’N	77°39’37’’E	2484m
18.	L29	Pandoga, Una	Himachal Pradesh	Straw with orange tinge	31°28’30’’N	76°07’58’’E	469m
19.	L36	Patiala-1	Punjab	Straw with greenish tinge	30°26’15’’N	76°14’02’’E	268m
20.	L37	Patiala-2	Punjab	Straw with greenish tinge	30°26’15’’N	76°14’02’’E	268m
21.	L38	Patiala-3	Punjab	Straw with orange tinge	30°26’15’’N	76°14’02’’E	268m
22.	L40	Patiala-4	Punjab	Straw with blackish green tinge	30°26’15’’N	76°14’02’’E	268m
23.	L44	Utrakhand	Utrakhand	Straw with black tinge	31°08’16’’N	75°48’54’’E	750m
24.	L49	Chhatisgarh-1	Chhatisgarh	Light straw	23°01’43’’N	75°10’14’’E	-191m
25.	L1	Usnarkalan, Hamirpur	Himachal Pradesh	Straw with black tinge	31°29’43’’N	76°30’22’’E	712m
26.	L3	Bakarti, Hamirpur-2	Himachal Pradesh	Straw with black tinge	31°38’24’’N	76°30’39’’E	707m
27.	L5	Dharmpur, Solan	Himachal Pradesh	Straw with orange tinge	30°51’35’’N	77°00’07’’E	1050m
28.	L6	Roru, Shimla	Himachal Pradesh	Straw with blackish green tinge	31°10’47’’N	77°43’20’’E	1724m
29.	L8	Baruhi, Una	Himachal Pradesh	Straw	31°35’46’’N	76°10’34’’E	440m
30.	L15	Amb, Una	Himachal Pradesh	Straw	31°39’24’’N	76°08’59’’E	447m
31.	L18	Dalhousi, Chamba-2	Himachal Pradesh	Light Straw	32°32’42’’N	75°50’08’’E	575m
32.	L19	Baldwara, Mandi	Himachal Pradesh	Black	31°33’42’’N	76°45’57’’E	796m
33.	L30	Sarahan, Sirmour-3	Himachal Pradesh	Straw with green tinge	30°40’23’’N	77°09’49’’E	872m
34.	L31	Patiala-5	Punjab	Straw with green tinge	30°26’15’’N	76°14’02’’E	268m
35.	L35	Patiala-6	Punjab	Straw with green tinge	30°26’15’’N	76°14’02’’E	268m

36.	L39	Patiala-7	Punjab	Straw with blackish green tinge	30°26'15''N	76°14'02''E	268m
37.	L41	Jammu	Jammu and Kashmir	Straw with orange tinge	34°47'04''N	73°04'08''E	2098m
38.	L42	Rajasthan-1	Rajasthan	Black	26°45'12''N	75°05'49''E	175m
39.	L43	Rajasthan-2	Rajasthan	Black	26°45'12''N	75°05'49''E	175m
40.	L45	Korba, Chhatisgarh	Chhatisgarh	Straw with green tinge	22°20'19''N	82°32'48''E	338m
41.	L46	Chhatisgarh-2	Chhatisgarh	Straw with green tinge	22°20'19''N	82°32'48''E	338m
42.	L48	Chhatisgarh-3	Chhatisgarh	Straw with green tinge	22°20'19''N	82°32'48''E	338m
43.	L50	Chhatisgarh-4	Chhatisgarh	Straw with green tinge	22°20'19''N	82°32'48''E	338m
44.	L2	Kiyar, Solan	Himachal Pradesh	Straw	30°52'53''N	77°03'41''E	1577m
45.	L10	Ghandhir, Bilaspur	Himachal Pradesh	Straw with green tinge	31°21'49''N	76°35'09''E	718m
46.	L17	Gwalpathar, Hamirpur	Himachal Pradesh	Straw with green tinge	31°40'47''N	76°21'20''E	578m
47.	L21	Sandal, Shimla	Himachal Pradesh	Black	31°04'34''N	77°07'36''E	1539m
48.	L34	Patiala-8	Punjab	Straw with green tinge	30°26'15''N	76°14'02''E	268m

Table.2 A degenerate primer used for the detection of begomoviral disease infecting horsegram

Primer Name	Nucleotide sequence (5'→3')	Target molecule	Product size	Annealing temperature	Reference
AV494	GCCYATRTAYAGRAAGCCMAG	Coat protein	600bp	56	**Wyatt and Brown 1996**
AC1048	GGRTTDGARGCATGHGTACATG				

Table.3 Leaf curl disease incidence (60 days) and Germination data of 48 accessions of Horsegram

Sample No.	Accession No.	Replications	Total seeds Sown	Total seeds germinated	Germination percentage	Total Healthy Plant	Leaf curl Infection after 60 days	Disease Incidence (%)	Average
1	L4	A1	50	50	100	34	0	0	0
		B11	50	50		20	0	0	
		C24	50	50		2	0	0	
2	L7	A2	50	35	72	20	0	0	0
		B12	50	35		12	0	0	
		C9	50	38		13	0	0	
3	L9	A3	50	38	74	20	2	10	14.44
		B13	50	35		2	0	0	
		C8	50	38		12	4	33.33	
4	L11	A4	50	45	82.66	40	2	5	13.41
		B14	50	36		14	4	28.57	
		C15	50	43		30	2	6.66	
5	L12	A5	50	36	72	19	5	26.31	12.47
		B15	50	38		4	0	0	
		C6	50	34		18	2	11.11	
6	L13	A6	50	50	96.66	50	5	10	19.23
		B16	50	45		28	9	32.14	
		C5	50	50		45	7	15.55	
7	L14	A7	50	50	100	50	0	0	0
		B17	50	50		38	0	0	
		C12	50	50		10	0	0	
8	L16	A8	50	35	72.66	15	2	13.33	19.59
		B18	50	36		22	10	45.45	
		C2	50	38		0	0	0	
9	L23(A)	A9	50	45	90	22	7	31.81	26.73
		B19	50	42		21	9	42.85	

		C3	50	48		18	1	5.55	
10	L20	A10	50	46	69.32	15	5	33.33	15.03
		B20	50	48		17	2	11.76	
		C7	50	47		2	0	0	
11	L22	A11	50	38	70.66	14	4	28.57	26.69
		B21	50	32		6	2	33.33	
		C18	50	36		11	2	18.18	
12	L23(B)	A12	50	38	76.66	3	1	33.33	26.47
		B22	50	35		23	6	26.08	
		C17	50	42		35	7	20	
13	L24	A13	50	45	68.66	3	1	33.33	22.22
		B23	50	48		0	0	0	
		C16	50	46		3	1	33.33	
14	L25	A14	50	38	70	15	4	26.66	33.33
		B24	50	35		5	2	40	
		C1	50	32		6	2	33.33	
15	L26	A15	50	42	80	15	3	20	40.39
		B10	50	38		12	7	58.33	
		C19	50	40		14	6	42.85	
16	L27	A16	50	35	63.32	3	1	33.33	11.11
		B9	50	32		5	0	0	
		C14	50	28		2	0	0	
17	L28	A17	50	45	95.32	37	8	0	0
		B8	50	48		21	7	0	
		C13	50	50		40	10	0	
18	L29	A18	50	50	100	10	2	20	12.22
		B7	50	50		12	1	8.33	
		C4	50	50		12	1	8.33	
19	L36	A19	50	38	77.32	18	6	33.33	18.53
		B6	50	42		25	2	8	
		C20	50	36		14	2	14.28	

20	L37	A20	50	48	88	27	6	22.22	26.55
		B5	50	36		11	5	45.45	
		C21	50	48		25	3	12	
21	L38	A21	50	35	78.66	0	0	0	21.11
		B4	50	45		30	9	30	
		C22	50	38		3	1	33.33	
22	L40	A22	50	35	72	4	2	50	53.33
		B3	50	38		10	6	60	
		C23	50	35		6	3	50	
23	L44	A23	50	45	83.32	10	4	40	35.55
		B2	50	42		15	7	46.66	
		C11	50	38		5	1	20	
24	L49	A24	50	48	93.32	16	2	12.5	19.8
		B1	50	50		26	7	26.92	
		C10	50	42		10	2	20	
25	L1	D1	50	38	69.32	10	3	30	54.44
		E11	50	35		12	4	33.33	
		F22	50	31		2	2	100	
26	L3	D2	50	35	72	6	2	33.33	22.22
		E12	50	38		3	1	33.33	
		F9	50	35		1	0	0	
27	L5	D3	50	45	83.32	33	12	36.36	28.78
		E13	50	38		8	2	25	
		F8	50	42		4	1	25	
28	L6	D4	50	50	89.32	20	8	40	37.73
		E14	50	38		8	3	37.5	
		F7	50	46		14	5	35.71	
29	L8	D5	50	50	95.32	31	5	16.12	21.48
		E15	50	48		3	1	33.33	
		F6	50	45		20	3	15	
30	L15	D6	50	45	90	34	2	5.88	27.26

		E16	50	48		2	1	50	
		F5	50	42		27	7	25.92	
31	L18	D7	50	35	75.32	5	1	20	46.66
		E17	50	36		2	2	100	
		F4	50	42		10	2	20	
32	L19	D8	50	38	83.32	6	4	66.66	43.93
		E18	50	45		22	7	31.81	
		F3	50	42		12	4	33.33	
33	L30	D9	50	50	100	30	5	16.66	19.52
		E19	50	50		15	2	13.33	
		F2	50	50		35	10	28.57	
34	L31	D10	50	42	85.32	25	8	32	17.94
		E20	50	38		13	2	15.38	
		F1	50	48		31	2	6.45	
35	L35	D11	50	35	64	10	5	50	33.33
		E21	50	33		2	1	50	
		F23	50	28		0	0	0	
36	L39	D12	50	25	42.66	0	0	0	
		E22	50	21		0	0	0	
		F24	50	18		0	0	0	
37	L41	D13	50	27	48.66	0	0	0	0
		E23	50	25		0	0	0	
		F21	50	21		0	0	0	
38	L42	D14	50	30	58	11	4	36.36	12.12
		E24	50	26		2	0	0	
		F20	50	31		0	0	0	
39	L43	D15	50	35	68	15	4	26.66	24.69
		E10	50	38		26	8	30.76	
		F19	50	29		12	2	16.66	
40	L45	D16	50	25	54	8	2	25	37.08
		E9	50	27		5	4	80	

		F18	50	29		32	2	6.25	
41	L46	D17	50	38	77.32	15	7	46.66	31.84
		E8	50	46		32	4	12.5	
		F14	50	32		11	4	36.36	
42	L48	D18	50	27	47.32	8	2	25	12.77
		E7	50	23		15	2	13.33	
		F17	50	21		0	0	0	
43	L50	D19	50	36	70	13	2	15.38	18.72
		E6	50	38		20	5	25	
		F16	50	31		19	3	15.78	
44	L2	D20	50	28	54	4	2	50	38.88
		E5	50	27		12	2	16.66	
		F15	50	26		4	2	50	
45	L10	D21	50	45	92.66	24	2	8.33	18.33
		E4	50	48		40	12	30	
		F13	50	46		42	7	16.66	
-46	L17	D22	50	25	46	0	0	0	0
		E3	50	23		0	0	0	
		F12	50	21		0	0	0	
-47	L21	D23	50	22	45.32	0	0	0	12.22
		E2	50	27		15	3	20	
		F11	50	19		6	1	16.66	
48	L34	D24	50	35	70.66	10	3	30	35.18
		E1	50	38		15	3	20	
		F10	50	33		18	10	55.55	

Fig.1 (A) Horsegram plants with asymptomatic leaves in field (B and C) Plants with leaf curling, chlorosis and stunted growth

Fig.2 PCR gel purified eluted amplified region of 570 bp with degenerate primers (AV494 and AC1048) from leaf curl like symptoms, whereas L stands for Ladder (100bp, Promega)

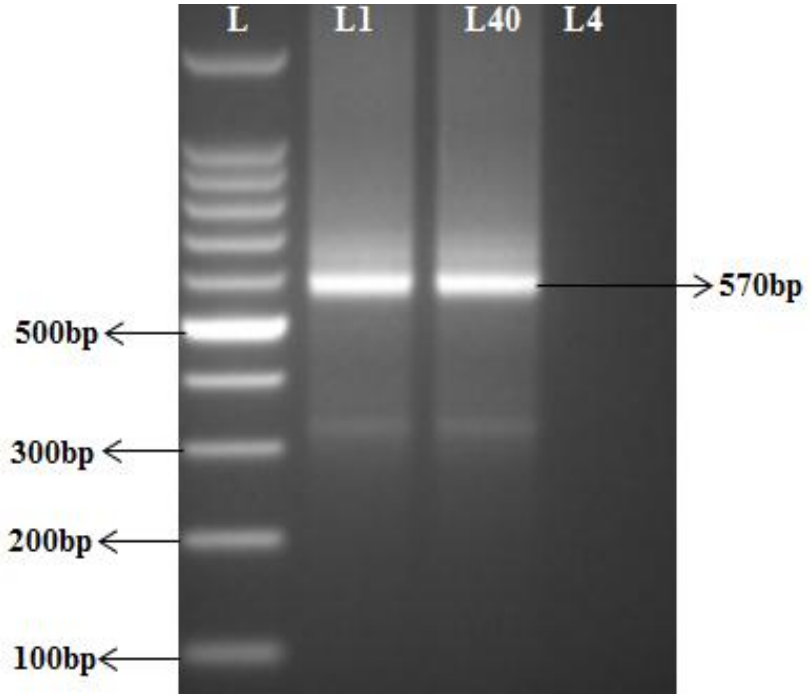
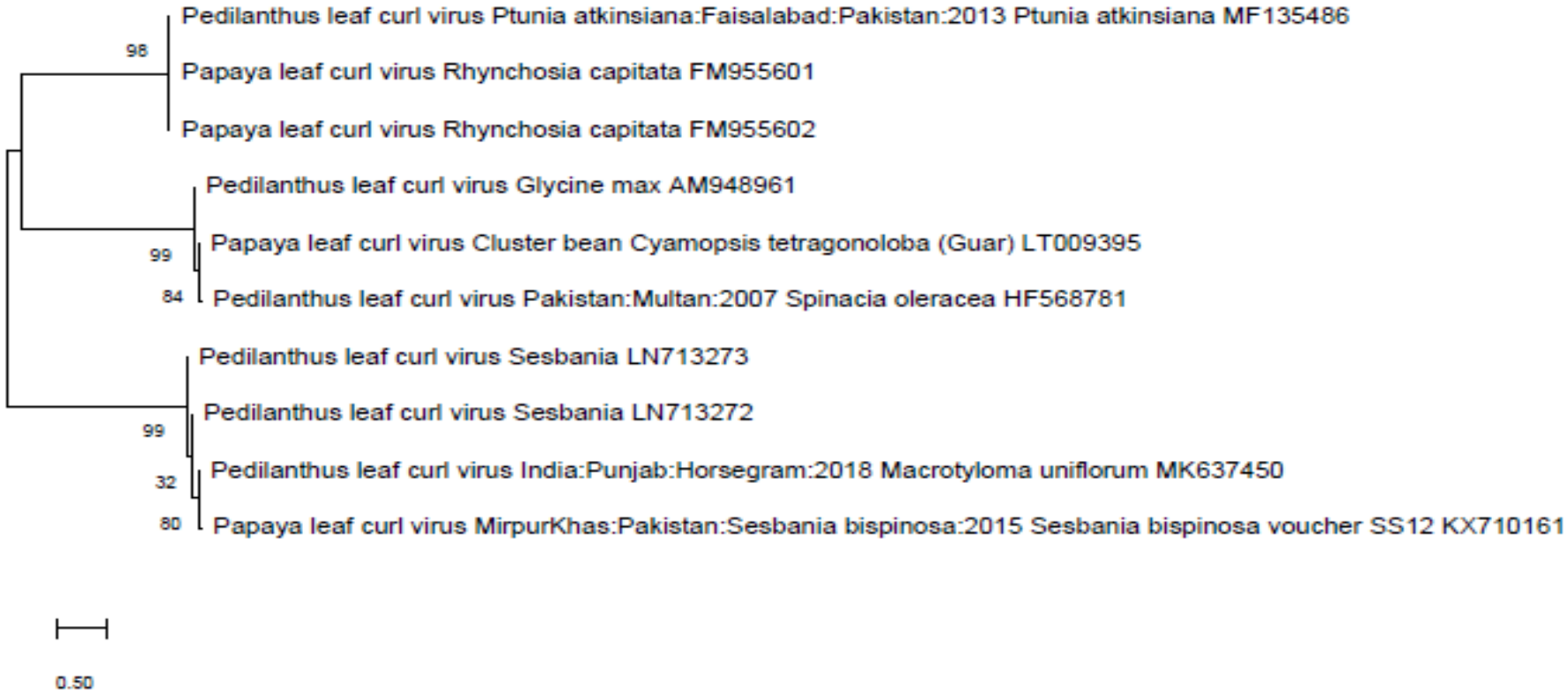


Fig.3 Phylogenetic tree analysis of amplified sequence of *Pedilanthus leaf curl virus* with the most identical sequences of various organisms with MEGA X (64-bit) software.



The phylogenetic tree based on the amplified nucleotide sequences of *Pedilanthus leaf curl virus* infecting horsegram along with various other selected and well described begomoviruses contracted using MEGA X (64-bit) software by Neighbour-joining method with bootstrap value 1000 (Fig.3).

The known begomoviruses infecting horsegram were clearly out-grouped. All these results confirm that the present isolate belongs to the genus *Begomovirus*. Cloning and sequencing of complete genome of the present isolates is being attempted in order to reveal its exact identity.

In field screening, the accessions namely L4, L14, L29 and L30 were showed the 100% germination. The germination data and screening of disease incidence (60 days) were taken after sowing of seeds of horsegram against leaf curl disease). The maximum average disease incidence of leaf curling found to be 54.44% (Table 3).

To the best of our knowledge, this is the first report of leaf curl disease caused by begomovirus in horsegram in India. In this study, *Pedilanthus leaf curl virus* from Northern region of India were isolated, identified and characterized. Yellow mosaic disease is commonly occurred and previously reported by different researcher in Southern region of India. This present study will open the opportunities for epidemiological studies and challenging the contagion of the virus. The relationship of these two identified viruses with the other known begomoviruses in different geographical regions is helpful to generate the most infectious stage for the commercial plants by sequencing studies. The nucleotide uniqueness and arrangement of the genes of this virus revealed that it belong to the genus *Begomovirus* of Family *Germiniviridae*. It is further used to check the whole genome of this virus by modern molecular techniques in further studies. The evaluation of disease resistance in legumes is a vital step in controlling plant diseases and host plant interaction. The identified resistant accessions of different legumes through field screening are helpful in the management against begomoviral associated diseases. The previous studies indicated that identification of resistant sources to YMV is a reliable option for controlling YMV disease. However, serious investigations are needed to conclude the resistance level in the germplasm lines and to further use in breeding programmes. Genotypic evaluations of similar types were previously documented by several workers (Peerajade *et al.,* 2004). In legumes, virus transmission rates or percent infection varied from host to host and this difference in transmission rates of virus symptoms on crops due to host biochemical compositions of *B. tabaci* (Colvin *et al.,* 2006 and Sharma *et al.,* 2008). The host range of yellow mosaic viruses of legume limited to Leguminaceae or Fabaceae species (Nene, 1972). Different chemical methods suggested for managing viral diseases are not economical because of very low yield potential of the crop. The only possible economical method for controlling viral diseases is the development of resistant varieties under natural conditions. To resolve this, there is a need of screening huge number of germplasm lines to identify the resistant source against viral diseases. In the present study, procured horsegram accessions have been assessed under natural field conditions to identify resistant sources and used in breeding programme for developing tolerant varieties.

Acknowledgements

Mr. Sumeeet Parkash Kaundal acknowledges to DST-SERB, New Delhi for providing funding to current research project. NBPGR, New Delhi is also duly acknowledged for providing the germplasm of Horsegram. He is also thankful to Dr. Poonam Suri, Chancellor,

DAV University and President, DAVCMC New Delhi for providing logistics support at DAV University, Jalandhar to conduct and complete the current research work.

Conflict of interest

Authors declare that there is no conflict of interest.

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How to cite this article:

Sumeet Parkash Kaundal and Rahul Kumar. 2021. First Report of Begomovirus causing Leaf Curl Disease in Medicinal Legume *Macrotyloma uniflorum* Lam. (Verdc.) in Northern Region of India. *Int.J.Curr.Microbiol.App.Sci.* 10(07): 484-502.
doi: https://doi.org/10.20546/ijcmas.2021.1007.054