

***In silico* characterization of Tomato Leaf Curl New Delhi Virus in Manipur: Evidence for its global expansion and future threat**

Journal Name: Phytoparasitica

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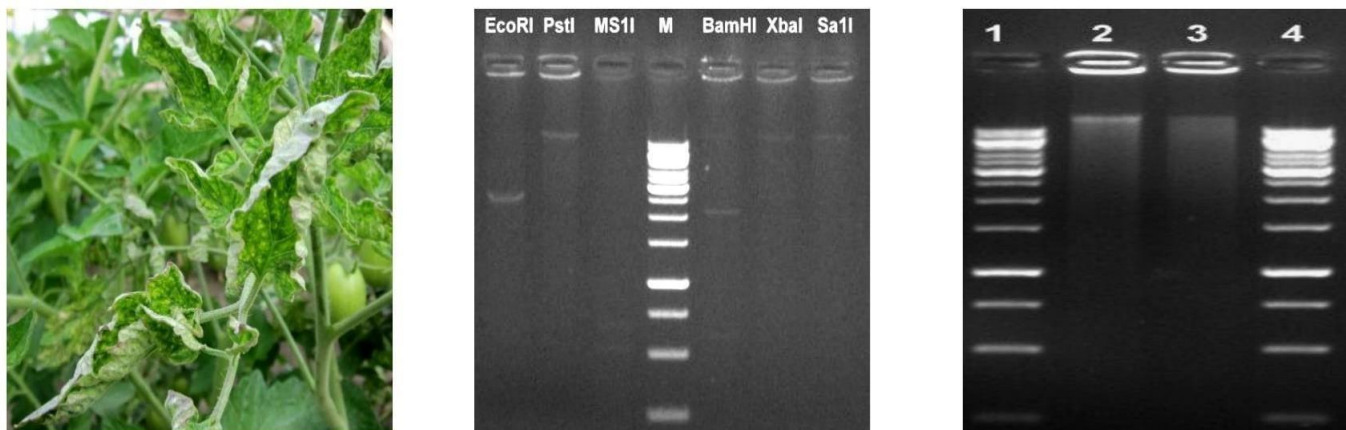
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Genome organization and characterization of ToLCNDV- Manipur isolate (MG649330)

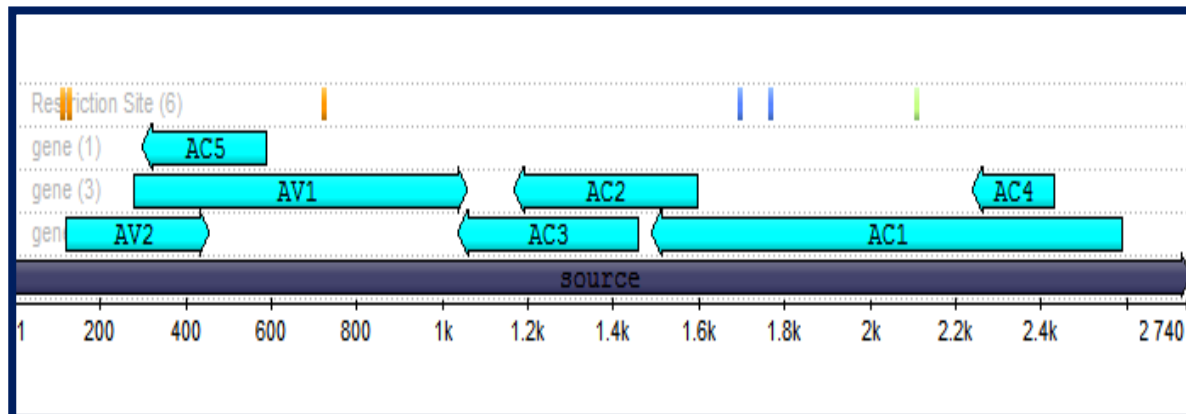


A. Visual Symptoms of ToLCNDV on tomato at Manipur.

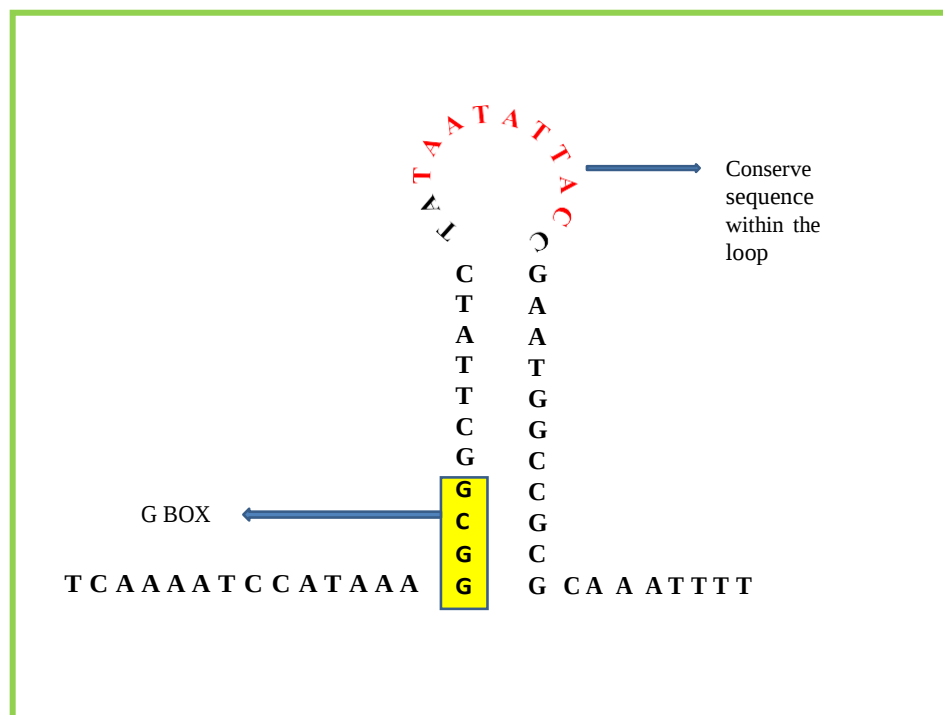
B. PCR amplification product of ToLCV- Manipur isolate infecting tomato using degenerated primer pair fragmented size of 840 bp amplicon. Lane 1 and 6: 1kb DNA ladder and Lane 2-5: amplicon of respective genome from 4 infected samples.

C. Amplified RCA products (Lane 2,3) and 1,4 : 1-kb DNA ladder marker.

D. Restriction fragment length polymorphism (RFLP) analysis of tomato leaf curl New Delhi virus (ToLCNDV)-infected samples from Manipur, performed by digestion of rolling circle amplification (RCA) products with the restriction enzymes *Eco RI*, *pstI*; *MSII*, *BamHI*, *XbaI*, *SalI*, revealed on a 1.5% agarose gel, M: 1-kb DNA ladder marker.



Circular genome organization of ToLCNDV- Manipur isolate infecting tomato constructed by UniPro UGENE version 1.12.2. B: Schematic representation of ToLCNDV-Manipur isolate with genomic position of ORFs of DNA A.



Conserved stem loop containing a common region (CR) show in red colour within intergenic region of ToLCNDV- Manipur isolate infecting tomato.

	F1 →	F2 →	TATA BOX ←	R1 ←
ToLCNDV-[BD:Jam]_KM383742	ACAAC-TTGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[BD:Jes]_KM383741	AAAAC-TTGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[BD:Joy]_KM383739	AAAAC-TTGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[IN:Pun]_KF551580	AGAACATTGTCGTTTCGATCCGGTGTCTCTCAACTTT-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[IN:Var]_KF537780	AAAAC-TTGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[PK:Che]_LN908936	AAAAC-ATGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[PK:Dor]_AF448058	AAAAC-TTGTCGTTTTCGATCCGGAGTCTCTCAACTTTGCTCTATGTAATT	GGTGTCTGGGGTCCCATATATAGGTAAGACACCAATATGGCA		
ToLCNDV-[IN:Cha]_KP178729	AAAAC-TTGTCGTTTCGATCCGGCGTCTCTCAACTTA-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAGACGCTAAATGGCA		
ToLCNDV-[PK:Fai]_FN435310	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTA-TCTATATGATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[PK:Lah]_AM258977	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTA-TCTATGTGATT	GGTGTATGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[IN:Jun]_KF515617	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTA-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCAATATGGCA		
ToLCNDV-[BD:Chi]_KM383744	AAAAC-TTGTCGTTTTCGATCCGGAGTCTCTCACCTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATACATTGACACTAAATGGCA		
ToLCNDV-[PK:Rak]_LN887908	AAAAC-TTGTCGTTTTCGAATCCGGCGTCCCTCAACTTA-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAGACCTAAATGGCA		
ToLCNDV-[PK:Isl]_AF448059	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTA-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[PK:Mul]_DQ116883	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTA-TCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		
ToLCNDV-[BD:Tha]_KM383736	AAAAC-CTGTCGTTTTCGATCCGGCGTCCCTCAACTTCCCTCTATGTAATT	GGCGTCTGGCGTCCCATATATAGGTAGACGCTAAATGGCA		
ToLCNDV-[IR:Zab]_KP641673	AACAC-TTGTCGTTTTCGATCCGGCGTCCCTCACCTTCCCTCTATGTAATT	AGTGTCTGGAGTCCCATATATAGGTAAGAAACCATATATGGCA		
ToLCNDV-[PK:Loh]_AM849548	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTCCCTCTATGTAATT	GGCGTCTGGCGTCCCATATATAGGTAGACGCTAAATGGCA		
ToLCNDV-[Tha]_AB330079	AAAAC-TTGTCGTTTTCGATCCGGCGTCCCTCAACTTCCCTCTATGTAATT	GGCGTCTGGCGTCCCATATATAGGTAGACGCTAAATGGCA		
ToLCNDV-[IN:Man]_MG649330	AAAAC-TTGTCGTTTCGATCCGGTGTCTCTCAACTTTCTCTATGTAATT	GGTGTCTGGAGTCCCATATATAGGTAAGACACCATATATGGCA		

Continued

						Stem	Loop	Stem				
						=====	-----	=====				
ToLCNDV-[BD:Jam]_KM383742	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCATAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[BD:Jes]_KM383741	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTAT				
ToLCNDV-[BD:Joy]_KM383739	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IN:Pun]_KF551580	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IN:Var]_KF537780	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Che]_LN908936	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Dor]_AF448058	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAGTCACTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IN:Cha]_KP178729	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAACCCACAT	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Fai]_FN435310	TTATTGTATATTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Lah]_AM258977	TTATTGTATATTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IN:Jun]_KF515617	TTATTGTATATTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[BD:Chi]_KM383744	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAACCCCTCAT	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Rak]_LN887908	TAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Isl]_AF448059	TTATTGTATATTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Mul]_DQ116883	TAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[BD:Tha]_KM383736	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAACCCCTCAT	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IR:Zab]_KP641673	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAATCCCTAA	AGCGGTCAATT	CTATAATACTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[PK:Loh]_AM849548	AAATTGTAATTTT	CAAAAGAAAATT	TACTTTAATT	CAAAATCCCTCAT	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[Tha]_AB330079	GAATTGTAATTTT	GAAAAGAAAATT	TACTTTAATT	CAAAACCCCTCAT	AGCGGCCATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				
ToLCNDV-[IN:Man]_MG649330	GATTTGTAATTTT	GGAAAAGAAAATT	TACTTTAATT	CAAAATCCATAA	AGCGGCTATT	CGTATAATATTA	CCGAATGGCCG	CGCAAAATTTT				

Alignment of intergenic region of DNA-A (ToLCNDV- MG649330) component of the ToLCNDV Manipur isolate characterized in this study and obtained from the EMBL database. Gap (-) were introduced into the sequence to optimize the alignment. The position of the stem loop structure (the two legs of which are highlighted in brick red), that contain the nanonucleotide sequence (TAATATTAC) highlighted in olive green, the TATA box of the Rep promoter highlighted in olivegreen and the predicted iterons (F1 and F2 for the virion-sense highlighted in yellow; R1 for complementary sense highlighted in light blue) are indicated

Confirmation Table		
Methods	# seqs detected in	Av. P-Val
RDP	1	6.924×10^{-09}
GENECONV	1	9.652×10^{-09}
BootScan	--	--
MaxChi	1	4.969×10^{-08}
Chimaera	1	4.519×10^{-07}
SiScan	1	1.188×10^{-12}
3Seq	1	2.033×10^{-12}
LARD	--	--
Phylpro	--	--

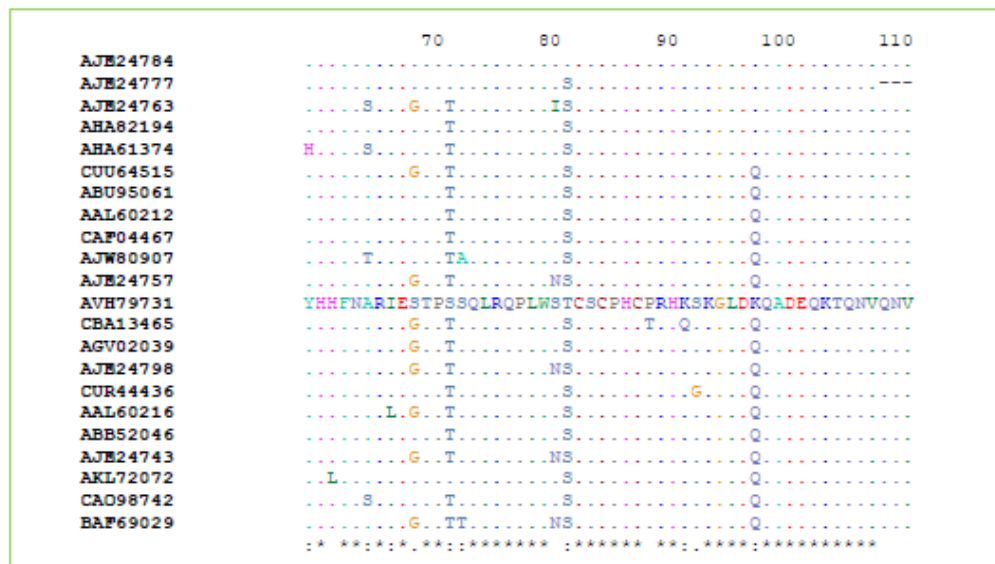
RECOMBINATION EVENT NUMBER 10
 Beginning breakpoint: 2619 in alignment (2608 without gaps)
 Beginning breakpoint 99% CI: 2570 - 31 in alignment (2560 - 23 without gaps)
 Ending breakpoint: 20 in alignment (12 without gaps)
 Ending breakpoint 99% CI: 2570 - 31 in alignment (2560 - 23 without gaps)
 Recombinant: MG649330_TLCNDV_Manipur_India
 Major Parent: Unknown (MK240306_TLCNDV_Pakistan)
 Minor Parent: MH577012.1 Tomato leaf curl New Delhi virus clone (92.3% similarity)
 Beginning breakpoint probability: 1.702289
 Ending breakpoint probability: 1189.125
 Region probability (MC uncorrected): 0.040530689426235
 Region probability (MC corrected): 1.70228895590187
 Region permutation probability: Undetermined

Integrated view of a recombination result of ToLCNDV Manipur isolates with similarity plot that showed a clear recombination event, detected by pairwise alignment of sliding windows and manually verified by similarity

CAO98743	GCEGPCKVQSFESRHDVSH
AKL72073	GCEGPCKVQSFESRHDVSH
AGV02040	GCEGPCKVQSFESRHDVSH
AJW80908	GCEGPCKVQSFESRHDVSH
ABB52041	GCEGPCKVQSFESRHDVSH
CAF04468	GCEGPCKVQSFESRHDVSH
CUU64516	GCEGPCKVQSFESRHDVSH
BAF69030	GCEGPCKVQSFESRHDVSH
AAL60217	GCEGPCKVQSFESRHDVSH
CUR44437	GCEGPCKVQSFESRHDVSH
CBA13466	GCEGPCKVQSFESRHDVSH
AHA61375	GCEGPCKVQSFESRHDVSH
AHA82195	GCEGPCKVQSFESRHDVSH
AJE24764	GCEGPCKVQSFESRHDVSH
AVH79732	GCEGPCKVQSFESRHDVSH
AJE24758	GCEGPCKVQSFESRHDVSH
AJE24799	GCEGPCKVQSFESRHDVSH
AJE24744	GCEGPCKVQSFESRHDVSH
AAL60213	GCEGPCKVQSFESRHDVSH
ABU95062	GCEGPCKVQSFESRHDVSH
AJE24785	GCEGPCKVQSFESRHDVSH
AJE24778	GCEGPCKVQSFESRHDVSH
	*****.****.***** *

(67-85 aa)

Conserved Zinc finger motif within coat protein gene (AV1) of ToLCNDV-Manipur isolate infecting tomato and other isolates. Within the alignment ‘*’ indicate the same residues and ‘.’ indicate similar residues among the sequences



Multiple amino acid alignment of movement protein (AV2)gene of ToLCNDV- Manipur isolate infecting tomato among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment “*” indicate same residues, “:” indicate similar residues and “.” Indicate dissimilar residues among the sequences

5'- terminus region of Rep protein



	10	20	30	40	50	60	70	80
AJE24789				Q.....	D.....			
AJE24782				N.....				
AJE24768								
AHA82196						T.....		
AHA61376				P.....		T.....		
CUU64520								
ABU95066		R.....	D.....		A.....			C.....
AAL60210		R.....			A.....	S.....		
CAF04471		S.....	R.....	V.....		K.....		
AJW80910		R.....			A.....			
AVH79736	MAPP	SRFR	INAK	NYFL	TPKCS	LTKEE	ALSQ	LQTL
AJE24761					A.....			
CBA13470		S.....	R.....	V.....		K.....		
AGV02043		S.....	R.....	V.....		D.....		K.....
AJE24803						A.....		
CUR44441		S.....	R.....	V.....		N.....		D.....
AAL60214		S.....	R.....	V.....		K.....		
ABB52040		S.....	R.....	V.....		R.....		
AJE24748		R.....		D.....		A.....		

	90	100	110	120	130	140	150	160
AJE24789						Q.....		
AJE24782						Q.....	D.....	
AJE24768						Q.....		
AHA82196		S.....		ER.....	VL.....	Q.....	D.....	
AHA61376				VL.....		Q.....	D.....	
CUU64520				VL.....		Q.....	D.....	
ABU95066				K.....	VL.....	Q.....	D.....	
AAL60210				VL.....		Q.....	D.....	
CAF04471				K.....	VL.....	Q.....	D.....	
AJW80910				K.....	VL.....	Q.....	D.....	
AVH79736	PSRS	AHFH	PNIG	QAKS	ASDV	KAYID	KDGD	DIVENG
AJE24761				VL.....		Q.....	D.....	
CBA13470				N.....	A.....	VL.....		
AGV02043				K.....	VL.....	Q.....	D.....	
AJE24803				A.....	VL.....	Q.....	D.....	
CUR44441				K.....	N.....	VL.....		
AAL60214				K.....	VL.....	Q.....	D.....	
ABB52040				K.....	VL.....	Q.....	D.....	
AJE24748				K.....	VL.....	Q.....	D.....	
AKL72077				K.....	VL.....	Q.....	D.....	
CAD98746				VL.....		Q.....	D.....	
BAF69033				VL.....		Q.....	D.....	

anti

	170	180	190	200	210	220	230	240
AJE24789								
AJE24782		Q.....	R.....		V.....	S.....		S.....
AJE24768		Q.....	R.....		V.....			
AHA82196		Q.....	R.....		L.....	V.....		
AHA61376		Q.....	R.....		V.....	V.....		
CUU64520		S.....		Q.....	R.....	A.....	VL.....	
ABU95066		V.....		Q.....	R.....		A.....	VS.....
AAL60210		NS.....		Q.....		L.....	VS.....	V.....
CAF04471		N.....		Q.....		AD.....	VS.....	V.....
AJW80910		N.....		Q.....		AD.....	VS.....	V.....
AVH79736	QFHN	LITN	LDRI	FKPP	SEVY	VSPF	SISS	IFDR
AJE24761		VS.....		Q.....		V.....	VS.....	V.....
CBA13470		N.....		Q.....		AD.....	VS.....	V.....
AGV02043		N.....		Q.....		AD.....	VS.....	V.....
AJE24803		S.....		Q.....		A.....	Y.....	VS.....
CUR44441		N.....		Q.....		L.....	VS.....	V.....
AAL60214		N.....		Q.....		L.....	VS.....	V.....
ABB52040		N.....		Q.....		L.....	VS.....	V.....
AJE24748		N.....		Q.....		L.....	VS.....	V.....
AKL72077		S.....		Q.....		L.....	I.....	VS.....
CAD98746		S.....		Q.....		L.....	I.....	VS.....
BAF69033		S.....		Q.....		L.....	I.....	VS.....

	250	260	270	280	290	300	310	320
AJE24789	I	I						
AJE24782	I	I						
AJE24768	I	I						
AHA82196	I	I						
AHA61376	I	I	V					
CUU64520	I	I					L	
ABU95066	I	I						
AAL60210	I	I						
CAF04471	I	I		N				
AJW80910	I	I						
AVH79736	CGHLDLSFKVYSNDAMYNVVDVDPHYLKHPKEFMGAQRDWQSNTKYGKFVMIKGGIPTIFLCNKGPNSSYKEYLDEEKN							
AJE24761	I	I						
CBA13470	I	I		C				
AGV02043	ES	I						
AJE24803	I	I						
CUR44441	E	I						
AAL60214	I	I						
ABB52040	E	I						
AJE24748	I	I						
AKL72077	E	I					T	
CAQ98746	I	TT						

3'- terminus region of Rep protein



	330	340	350	360
AJE24789	P			
AJE24782	D..P			
AJE24768	P			
AHA82196	A..A			
AHA61376	D..PQ			
CUU64520	P			
ABU95066	T			
AAL60210	P			
CAF04471				
AJW80910	T.A			
AVH79736	AALKQWAIKNAVFITLLEPLYSGRENIAPSEEEEEHSQEAS			
AJE24761	T			
CBA13470	LP			
AGV02043	A			
AJE24803	T			
CUR44441	A..T			
AAL60214	T...P..E			
ABB52040	T...T			
AJE24748	T			
AKL72077	T			
CAQ98746	T			
BAF69033	T			
	*****_**_* : **** ** *			

Multiple amino acid alignment of replication associated protein (AC1) gene of ToLCNDV- Manipur isolate infecting tomato, which overlapped on AC4 and AC2 ORFs among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment '*' indicate same residues, ':' indicate similar residues and '.' indicate dissimilar residues among the sequences

Activate

30/03/2020

	Motif I (15-21aa)	Motif II (57-62 aa)	Motif III (103-106 aa)	P loop (221-228 aa)	WalkerB (260-263 aa)
ToLCNDV-[PK:Rak]	FLTYPKC	HIHVLI	YINK	GDSRTGKT	IDDV
ToLCNDV-[PK:Mul]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	VDDV
ToLCNDV-[PK:Isl]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[IN:Cha]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[IN:Guj]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK:Fai]	FLTYPKC	HIHVLI	YIAK	GDSRTGKT	IDDV
ToLCNDV-[IN:Mus]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK:Che]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[IN:Var]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	VDDV
ToLCNDV-[IN:Man]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Jam]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Jes]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Joy]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[IR:Zab]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK:Dar]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Joy]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Chi]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[PK:Lab]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[BD:Tha]	FLTYPKC	HIHVLI	YIDK	GDSRTGKT	IDDV
ToLCNDV-[THA]	****.*	*****	:***	*****	*:**

Functional Putative motifs in AC1 protein of ToLCNDV-Manipur isolate and other ToLCNDV isolates. Red boxes shows identities and conserve in all the isolates and value within the motifs compared with ToLCJV-Kalyani isolate and ToLCJV isolates. Within the alignment ' * ' indicate same residues, ':' indicate similar residues and '.' indicate dissimilar residues among the sequences

5'- terminus region of TrAP protein



	10	20	30	40	50	60	70	80
AJE24788								V
AJE24781								V
AJE24767								V
AHA82197		L					X	V D
AHA61377			H			V	Y	VS
CUU64519		L		I				V
ABU95065		L		I				V
AAN76347		Q		I				V
CAF04470				I		K		V
AJW80911	R		Q	I		V		V
AJE24760				T	I	V		V
AVH79735	MQSSSHSKNHSIPVAKTSLPQKKKSIARRRV	DLPCGCSYVVSINCHDHGFTHRG	INHC	GSSREWR	IYLGNSKSP	LPQNF		
CBA13469		S		I				V
AGV02042				I		V	Y	V
AJE24802				Q	T	I	V	V
CUR44440		R	N		L			V
AAN76351	R		R		I			V
ABB52043				Q	I	V	A	V G
AJE24747			N	Q	T	I	V	V
AKL72076	L			Q	T	I	V	T
CAO98745				T	IT	V		V
BAF69032				T	T	V		V

3'- terminus region of TrAP protein



	90	100	110	120	130
AJE24788	AL	N	S		
AJE24781	AL	KT	N	R	
AJE24767	AL	KT	N	L	H
AHA82197	R	AL	NT	N	S
AHA61377	R	PAL	NTS	H	V
CUU64519	A	Q	AL	KT	N
ABU95065	A		AL	NT	H
AAN76347	A		AL	NT	H
CAF04470	A	R		AL	NT
AJW80911	AR		AL	NTT	H
AJE24760	R		AL	NT	N
AVH79735	ETPQQTFFQRRHRYQDSNTIQPQPAES	PGG	QVFSN	FPNLDGLT	PSDWD
CBA13469	R		AL	NT	N
AGV02042	R		AL	NT	N
AJE24802	R		AL	NT	N
CUR44440	A	R		AL	NT
AAN76351	A	R		AL	NTP
ABB52043	R	P	I	HQS	N
AJE24747	R		AL	NT	N
AKL72076	Q		L	KT	H
CAO98745	R		AL	NT	H
BAF69032	R		L	NT	N

Multiple amino acid alignment of transcriptional activator protein (AC2) gene of ToLCNDV- Manipur isolate infecting tomato, which overlapped on AC3 and AC1 ORFs among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment '*' indicate same residues, ':' indicate similar residues and '.' indicate dissimilar residues among the sequences

5'- terminus region of REn protein



	10	20	30	40	50	60	70
AJE24787	S.....					E	
AJE24780	S.....K.....Q.....					L	
AJE24766	S.....K.....Q.....					I	
AHA82198	S.....I.....K.....D.....TQ.....V.....					L	
AHA61378	T.S.....I.....LK.....D.....TH.....					L	
CUU64518	S.....K.....Q.N.....Q.....					L	
ABU95064	S.....K.....Q.....TQ.....					L	
AAN76346	S.....K.....Q.....TQ.....					L	
CAP04469	K.....K.....Q.D.....TQ.....					L	
AJW80912	S.....K.....D.....TQ.....					L	
AJE24759	S.....K.....D.....TQ.....					L	
MG649330	MITDSRTGEYITADRAENGVTWEIRNPLYFRILKHHSRPFLREHDIKIQIQFNHNLRRKALGVHKKFLT						
CBA13468	S.....K.....D.....TQ.....					L	
AGV02041	T.S.....K.....D.....TQ.....					L	
AJE24801	S.....K.....D.....TQ.....					L	
CUR44439	S.....K.....Q.D.....TQ.....					L	
AAN76350	S.....V.....K.....Q.D.....TH.....					L	
ABB52042	M.....H.....S.....PT.....VP.....K.....D.....TN.....V.....					L	
AJE24746	S.....S.....K.....D.....TQ.....					L	
AKL72075	S.....S.....H.....K.....N.....Q.....N.....					L	
BAF69031	S.....S.....K.....D.....TQ.....					L	
** ***** ***,*::,**,* : ****::**,****** ***,***** :*****							

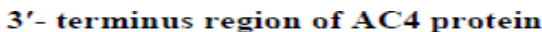
Continued

Activate W



	80	90	100	110	120	130
AJE24787	L.....V.....Q.I.....					
AJE24780	L.....V.....Q.I.....					
AJE24766	L.....G.....V.....Q.I.V.....M.....					
AHA82198	P.....L.....G.....V.....Q.I.V.T.....					
AHA61378	L.....L.....G.N.....V.....R.I.VQT.....					
CUU64518	L.....L.....G.....V.....M.Q.I.VCT.....					
ABU95064	K.....H.....L.....G.....V.....R.I.V.T.....					
AAN76346	K.....L.....G.....V.....R.I.V.AT.....					
CAP04469	K.....L.....G.....V.....M.R.I.V.T.....Y.....					
AJW80912	K.....K.....R.L.....G.....V.....K.I.V.T.....					
AJE24759	K.....P.....L.....R.....G.....V.....M.R.I.V.T.....					
MG649330	FQIWTGLRPQIGIFLRFVKTQVLKYPDMLGVISIYNVIRAAAYHVLNDVLEHTNDMWSSYDIKLDIY					
CBA13468	K.....L.....L.....G.....V.....Q.I.V.T.....					
AGV02041	K.....L.....L.....G.N.....V.....R.I.V.TT.....					
AJE24801	K.....P.....L.....R.....G.....V.....M.Q.I.V.T.....					
CUR44439	K.....L.....G.....V.....MVR.I.V.T.....					
AAN76350	K.....L.....L.....G.C.....L.....V.....R.I.V.T.....					
ABB52042	PT.....R.....R.LN.....VG.....V.....F.....K.I.V.T.....H.....					
AJE24746	K.....P.....L.....R.....G.....V.....M.Q.I.V.T.....					
AKL72075	K.....L.....R.....L.....R.....G.....V.....M.Q.I.V.T.....					
BAF69031	P.....L.....R.....G.....V.....Q.I.V.T.....					
*:** ***** ***,*::,**,* : ****::**,****** ***,***** :*****						

- Multiple amino acid alignment of replication enhancer protein (AC3) gene of ToLCNDV- Manipur isolate infecting tomato, which overlapped on AC2 ORFs among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment '*' indicate same residues, ':' indicate similar residues and '.' indicate dissimilar residues among the sequences.



Multiple amino acid alignment AC4 gene of ToLCNDV- Manipur isolate infecting tomato, which overlapped on AC1 ORFS among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment ‘*’ indicate same residues, ‘:’ indicate similar residues and ‘.’ Indicate dissimilar residues among the sequences

5'- terminus region of AC5 protein



	10	20	30	40	50	60	70
AJE24786	-----	-----	-----	-----	-----	-----	-----
AJE24779	-----	-----	-----	-----	-----	-----	-----
AJE24765	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
AHA82200	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
CUU64517	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
ABU95063	MHV	FHR	SC	AFI	VKHV	KNF	PKIL
AAN76345	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
AJW80914	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
AVH79733	-----	-----	-----	-----	-----	-----	-----
CBA13467	-----	-----	-----	-----	-----	-----	-----
AJE24800	-----	-----	-----	-----	-----	-----	-----
CUR44438	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
AAN76349	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC
ABB52045	-----	-----	-----	-----	-----	-----	-----
AJE24745	-----	-----	-----	-----	-----	-----	-----
AKL72074	MHVLHRC	CAFI	VKHV	KNF	PKIL	RG	SC

	80	90	100	110	120	130
140	AJE24786					
AJE24779	G.....G.....					
AJE24765	.Q.....A.....R.....R.....G.....I.F.....					
AHA82200	P.....K.....T.....A.....G.....					
CUU64517	T.....R.K.....T.....AS.....G.....					
ABU95063	S.....N.....R.K.....G.....G.....					
AAN76345	Y.....K.....T.....A.....G.....					
AJW80914	P.....T.....LTD.....Y.....R.KR.....T.....A.....G.....					
AVH79733	CESSSSGNITNAHDFANMRDIVPGFEGHLARAFSPWHVKTSIHVSFSGFSVHRPVCPLCFCDAGNGD					
CBA13467	T.....R.K.....FT.....A.....G.....G.....G.....C.....					
AJE24800	C.V.....T.....L.....Y.....KR.....T.....A.....G.....G.....					
CUR44438	G.....T.....K.....T.....A.....G.....G.....L.....					
AAN76349	Y.G.....T.....K.....T.....A.....G.....G.....					
ABB52045	P.....T.....R.K.....R.....P.....G.....G.....					
AJE24745	G.....C.....DT.....L.....V.....R.K.....T.....A.....G.....G.....					
AKL72074	R.....T.....N.....R.K.....T.....A.....G.....G.....					
	: * * * : : : * : : * * * * : * : * * * * * * * * * * : * * * *					

3'- terminus region of AC5 protein



	150	160
AJE24786		
AJE24779	...S.....	
AJE24765	...R..R.....S..L.Y.S	
AHA82200	...S.....S.....	
CUU64517	...S.....S..L....	
ABU95063	...S.....S.....	
AAN76345	...S.....S.....	
AJW80914	...S.....PS.....C	
MG649330	NCTCSIGAVEVETTAYFRCGR	
CBA13467	...S.....S..L....	
AJE24800	...S.....S..L....	
CUR44438	...S.....S.....	
AAN76349	...S..R.....S..L.G..	
ABB52045	...S.....S.....	
AJE24745	...S.....S..L....	
AKL72074	...S.K.....Y....	
	*** * ***** ;**.* *	

Multiple amino acid alignment AC5 gene of ToLCNDV- Manipur isolate infecting tomato, which overlapped on AV1 ORFs among the ToLCNDV infecting tomato, potato, okra or chilli respectively. Within the alignment ‘*’ indicate same residues, ‘:’ indicate similar residues and ‘.’ Indicate dissimilar residues among the sequences