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Comparison and molecular profiling of *Begomovirus* infecting chilli (*Capsicum annum*) in Gangetic alluvial zone of West Bengal

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Abstract
In our present investigation the leaf curl disease on chilli was observed up to an extent of 18-85% at different regions of Gangetic alluvial zone of West Bengal. The disease was successfully transmitted by whitefly (*Bemisia tabaci*), on experimental plants. The total DNA was extracted from infected leaf samples and PCR was carried out using a pair of begomovirus specific degenerate primers (SPG1/SPG2). The replication associated protein gene fragment (Rep; AC1) of 920 bp were amplified from naturally begomovirus infected and experimentally vector transmitted plants. NCBI blast analysis of partial nucleotide sequences of DNA-A revealed that Bongaon - North 24 Parganas - WB (Accession no. LT622253) and Dhatrigram - Burdwan - WB (Accession no: LT622254) begomovirus isolates shown 91% and 95% nucleotide sequence identity with Pepper leaf curl Bangladesh virus (Accession no: AF314531 & HM007111) respectively.
Moreover, these studies recount the effective method for detection of begomovirus, supply precise information about the frequency, distribution of distinct begomovirus isolates and specified the future possible reason of leaf curl outbreak in chilli in Gangetic alluvial West Bengal.

Keywords: Chilli, *begomovirus*, whitefly, NCBI, PCR, nucleotide sequence identity

1. Introduction
India is the largest producer, consumer and exporter of chilli (*Capsicum spp*.) and contributes 25% of total world production. Amongst the foliar diseases, virus diseases are very important and becoming the most limiting factors for better pepper production which affect the crop both qualitative and quantitatively [7, 9, 28]. Out of the 22 viruses infecting chilli, *Chilli leaf curl virus* (ChiLCV) is one of the major limiting constrain for chilli production in the Indian subcontinent and is consistently caused by begomoviruses [1, 24, 28]. Leaf curl disease of chilli shown typical symptoms of begomoviruses. The genus *Begomovirus* is the largest genus of family *Geminiviridae* & contains approximately 300 species [2], only infects dicotyledonous plants and is transmitted by the phloem feeding whitefly, *Bemisia tabaci* Genn. [10]. *Begomovirus* is characterized structurally by twinned (geminate) quasi-icosahedral capsids [15] of approximately 18*30 nm in size [18]. Most viruses belonging to the *Begomovirus* genus possess a bipartite genome, with two circular ssDNA molecules, DNA-A (2.6-2.8 kb) and DNA-B (2.5-2.8 kb) but single genome (DNA-A) monopartite viruses are being reported like recently a new monopartite *Begomovirus* species, *Chilli leaf curl Vellanad virus* and associated beta-satellites infecting chilli in the Vellanad region of Kerala, India reported [12]. Begomoviruses having two groups like new world (NW) and old world (OW) begomoviruses [19, 20]. Both worlds differ from each other in different aspects, where all NW begomoviruses are bipartite, while a large portion of OW begomoviruses are monopartite with association of satellite molecules and a few of them having bipartite genome. Chilli leaf curl etiology was established early in the 1960s in India [6, 17]. In 2004, Chilli leaf curl disease epidemics occurred in Jodhpur, the main chilli-growing area in Rajasthan, India. Later on *chilli leaf curl virus* have been reported on chilli crop from Jodhpur (Rajasthan) and shown closest sequence identity (96.1%) with an isolate of ChiLCV infecting potato in Pakistan and sequence diversity up to 12.3% among the ChiLCV isolates of India and Pakistan [24]. In India, *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) was recently shown to be associated with chilli leaf curl disease occurring in Lucknow [11]. *Tomato leaf curl Joydebpur virus* (reported earlier from Joydebpur, Bangladesh in tomato) has also been found to be associated with chilli leaf curl disease in Punjab [26].

An isolate from Kalyani (West Bengal) shared maximum nucleotide sequence identity with *Pepper leaf curl Bangladesh virus* (96.3%), while three isolates from Rajasthan shown nucleotide identity of 88.9 to 91.1% with *Chilli leaf curl virus*-Multan and 8 isolates (2 each from Delhi, Haryana, Punjab and Uttar Pradesh) shared nucleotide identity of 92.4 to 98.5% with *Tomato leaf curl New Delhi virus* [21]. Recently identification of *Tomato Leaf Curl Virus* (ToLCV) strain causing leaf curl disease in Tomato and Chilli in Maharashtra and reported to cause 90-100% yield loss [4].

The rapid evolution of virus variants, the massive increase of vector populations (with the appearance of whitefly 'B' biotype) and the introduction of modem human agricultural practices have contributed to a rapid and worldwide spread of the virus [30].

However, attempt was taken in the present investigation to study the incidence, transmission, detection, characterization and sequence variations of the ssDNA virus (*Begomovirus*) isolates from different locations of West Bengal (Gangetic alluvial zone) and phylogenetic relationship with other related begomoviruses for further analysis of the viral genome of the begomoviruses infecting chilli in that region.

2. Materials and Methods

2.1 Survey of disease incidence & sample collection

Samples showing begomovirus infection symptoms (upward leaf-curling, distortion, vein yellowing, stunted growth & sometimes with bushy appearance of plants) were collected from North 24-Parganas (Bongaon), Burdwan (Dhatrigram), Kalyani (Nadia) districts of West Bengal, similarly healthy leaf samples also collected from above mentioned regions. Both the infected and healthy leaf samples were brought in cool boxes, kept in -20 ºC for further experiments. In addition to that single severely infected/ stunted chilli plant was uprooted with soil from each of these locations and maintained in glasshouse at optimum growth condition in Directorate of Research farm, BCKV, Kalyani, West Bengal.

The survey was conducted during July- August, 2015 when the crop was at 2-3 month during. The disease diagnosis in the field was based on typical chilli leaf curl symptoms. The percent disease incidence was recorded at randomly in different locations in the field by counting total no. of plants and no. of plants showing chilli leaf curl symptoms using the formula [23] given below-

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total Number of plants observed}} \text{X100}$$

2.2 Artificial transmission by whitefly

Transfer of begomovirus from the pre-collected infected chilli plants was carried out during September, 2015 using whitefly vector (*Bemisia tabaci*). Newly emerged whitefly adults were collected carefully from chilli fields of Gayeshpur, BCKV, Nadia using a whitefly aspirator made with tube (Bib size) and released in some cotton plants inside a wooden-net rearing cage (45 x 45 x 30 cm) for rearing and maintenance of the whiteflies.

Whiteflies were released on infected chilli plants of different region, maintained at glass house, for 24 hours virus acquisition, then the viruliferous whiteflies were removed and allowed to feed on 18-20 days old healthy chilli seedlings (var: Bullet), at the rate of 10 whiteflies per seedling for 24 hours inoculation feeding. The inoculated seedlings of chilli were sprayed with insecticides to kill the white flies and kept in the insect proof condition for symptoms development up to 40 days [8]. Inoculated chilli seedlings showing chilli leaf curl symptoms were subjected for PCR for confirmation of begomovirus infection.

2.3 DNA isolation and PCR detection of virus

Total DNA were extracted from 100 mg of infected and healthy plants using Cetyl trimethyl ammonium bromide (CTAB) method [5, 25]. Presence of begomovirus was confirmed by PCR amplification with a begomovirus specific degenerate primer pairs SPG1/SPG2 (5'-CCCCKGTGCGWRAATCCAT-3'/5'–ATCCVAAYWTYCAGGGAGCTAA-3' [16], which amplified a partial segment of replication associated protein (AC1) gene. The PCR amplification was carried out in a thermal cycler (Mastercycler, Eppendorf, AG 22331, Germany). The PCR reaction mixture contained 2 µl DNA sample, 1 µl of each primers (Conc. 10 mM), 1 µl dNTPs (10mM), 1.5 µl $Mgcl_2$ (25 mM), 2.5 µl 10x reaction buffer, 0.5 µl Taq polymerase and 15.5 µl sterile HPLC-H2O, that will give rise to a volume of 25 µl PCR reaction. The conditions for PCR reaction were, an initial denaturation at 94 ^{0}C for 5.00 minute followed by 34 cycles of denaturation at 94 ^{0}C for 30 sec., annealing at 59^{0}C for 30 sec and extension for 1 minute at 72^{0}C. Then final extension at 72^{0}C for 7 minute was included. Amplified PCR product were separated by electrophoresis on 1.5% agarose gel and DNA fragments were visualized using ethidium bromide strain, here 1 kb DNA ladder (Fermentas, Life Science) is used & documented by Gel documentation system (Vilber- Lormout, France). The expected amplicon of 920 bp were obtained from the DNA of all diseased leaf samples chosen. PCR product is further amplified up to 200 µL and run on 1% agarose gel and finally the desired band (920 bp) were purified using gel extraction kit (Gene JET Gel Extraction kit, Thermo Scientific, Lithuania).

The amplified products were stored in -4 ^{0}C for further use.

2.4 Sequence and Phylogenetic analysis

The complete nucleotide sequences were subjected for similarity search using BLASTn search program (http://www.ncbi.nlm.nih.gov/BLAST/). The sequence was submitted to GenBank with legal annotations. The accessions of GenBank were also received (Accession numbers - LT622253 & LT622254). The sequences were compared with equivalent sequences from a range of other geminiviruses present in GenBank. Multiple sequence alignment was carried out using the software ClustalW and ClustalX version 2.0 [14]. The pair-wise alignments and Percent Identity Matrix of nucleotide and amino acid sequences were performed using ClustalW2 programme from EMBL Sequence Database online. Phylogenetic trees were constructed on matrices of aligned sequences with 1000 bootstrap replicates by using MEGA version 7.0 at its default settings using best fit model.

Table 1: List of begomoviruses compared with the Bongaon-North 24Parganas-West Bengal, Dhatrigram-Burdwan-West Bengal begomovirus isolates in the present study, their origin and NCBI gene accession numbers

Virus strains	Acronyms	Origin	Gene Accession No.
Pepper leaf curl Bangladesh virus segment A, complete sequence	PepLCBV-BB	Bangladesh	AF314531
Pepper leaf curl Bangladesh virus India [India/Mograhat/2007] clone pChMogK4 segment DNA-A, complete sequence	PepLCBV-IM	India	HM007111
Tomato leaf curl Liwa virus isolate Tom43, complete genome	ToLCLV-OB	Oman	KF111685
Tomato leaf curl Liwa virus isolate Tom44, complete genome	ToLCLV-OB	Oman	KF111686
Tomato leaf curl Liwa virus isolate Tom42, complete genome	ToLCLV-OB	Oman	KF111684
Pepper leaf curl Lahore virus isolate Nagpur segment DNA-A, complete sequence	PepLCLV-IN	India	JN663864
Tomato leaf curl Liwa virus isolate LW1, complete genome	ToLCLV-O	Oman	HF912280
Tomato leaf curl Joydebpur virus clone Moh9, complete genome	ToLCJV-IM	India	KJ028211
Pepper leaf curl Bangladesh virus-India isolate Chhapra segment DNA-A, complete sequence	PepLCBV-IC	India	JN663853
Chilli leaf curl virus isolate Sh1, complete genome	ChiLCV-O	Oman	JN604491
Papaya leaf curl virus av2 gene, av1 gene, ac5 gene, ac3- truncated gene, ac2 gene, ac1 gene and ac4- truncated gene, clone MI68	PapLCV-Pk	Pakistan	FM955602
Chilli leaf curl virus-[Pakistan] segment DNA A, complete sequence	ChiLCV -Pk	Pakistan	DQ114477
Chili leaf curl Pakistan virus isolate Khanewal 2 clone PC5 segment A, complete sequence	ChiLCPV-Pk	Pakistan	DQ116879
Tomato leaf curl virus [India:Kalyani-B.C.K.V.:Ridgegourd:2013] partial AC1 gene for replication initiation protein, isolate kly-bck-ridg-9	ToLCV-IK	India	HF679119
Pepper leaf curl virus isolate ANGC_CREP replication initiation protein gene, partial cds	PepLCV-I	India	JN887127
Pepper leaf curl virus isolate CAK226 replication initiation protein gene, partial cds	PepLCV-I	India	JN887125
Pepper leaf curl virus isolate CESC_CREP replication initiation protein gene, partial cds	PepLCV-I	India	JN887128

3. Results and Discussion

3.1 Disease symptoms and incidence

All the locations surveyed had a varying degree of leaf curl incidence with mild to severe upward leaf curl symptoms with interveinal & marginal chlorosis (Fig. 1a-e) resemble the begomovirus infection on chilli occurring in India and outside of India. The severely infected plants produced no fruit; however, plants with mild infection produced fruits with reduced sizes. The disease incidence ranged from 18-85%. In-some places chilli plants with mixed infection of mites along with begomovirus was noticed.

Fig 1a-e: Variable symptoms of *Begomovirus* infection chilli in West Bengal

3.2 PCR detection of the virus

One set of universal degenerate primers (SPG1/SPG2) were used for amplification and confirmation of the partial replication associated protein gene (AC1) with expected DNA fragment of 920 bp (Fig. 2) from the virus infected samples of Bongaon - North 24 - Parganas and Dhatrigram - Burdwan chilli leaf curl & Nadia samples.

The samples were considered to contain begomovirus when the molecular weight of the amplified products matched with standard reported molecular weights. The Bongaon - North 24 Parganas - WB and Dhatrigram - Burdwan- WB samples containing viral DNA were then further purified and subjected for sequencing.

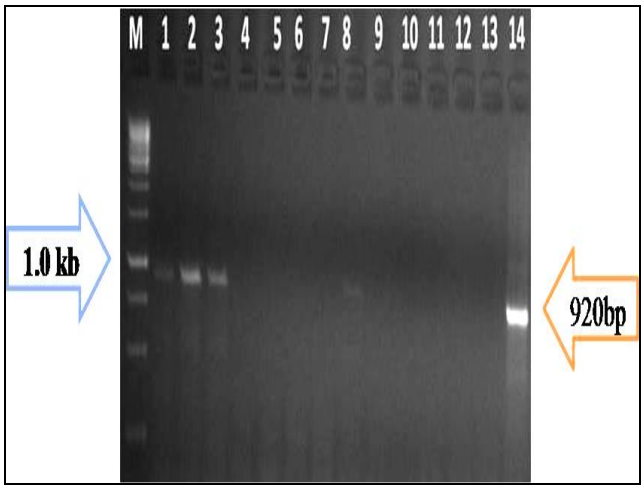

Fig 2: PCR amplification of collected begomovirus samples using degenerate primer pair SPG1/SPG2 sowing the size of amplicon 920 bp: Lane M - 1kb DNA ladder, 1-3: N 24p, 8: Nadia, 14: Burdwan

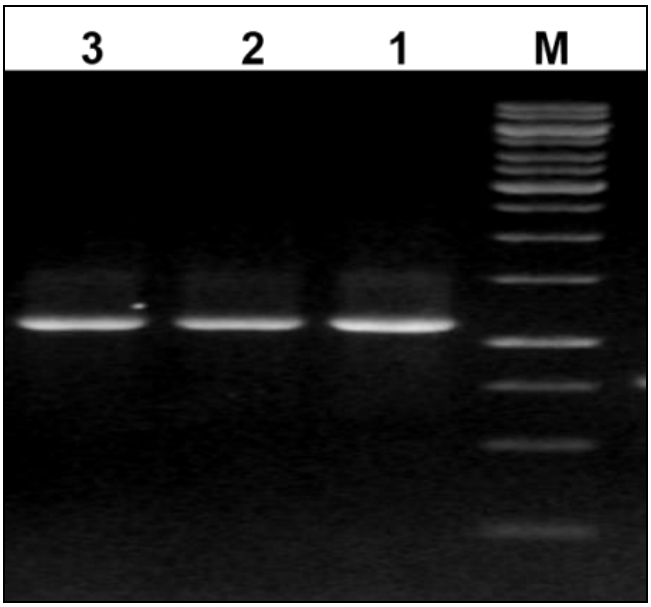

Fig 3: PCR amplification of begomoviruses of whitefly inoculated chilli plants: Lane M - 1kb DNA ladder; lane 1, 2, 3 - N24p, Nadia, Burdwan

3.3 Transmission efficiency

The disease was successfully transmitted to healthy chilli plant (Var. Bullet) using whitefly as vector. Begomoviruses of all the samples were transmitted to healthy chilli seedlings with transmission efficiency of 15-75% depending on the samples. Typical begomovirus infection symptoms were produced in the inoculated plants within 17-30 days after whitefly inoculation. Seedlings showing leaf curl symptoms gave typical 920 bp band on PCR amplification (Fig. 3). The present data indicated that the ChiLCD in chilli was caused by whitefly-transmitted Begomovirus species.

3.4 Sequencing and phylogenetic analysis

The partial nucleotide sequence of begomovirus infecting chilli was deposited in the GenBank and assigned with accession numbers LT622253 (554 bp) and LT622254 (638 bp).The *Begomovirus* associated with Bongaon - North 24 Parganas (LT622253) and dhatrigram - Burdwan, West Bengal isolate (LT622254) DNA-A containing replication associated protein (AC1) gene partial and shown highest nucleotide sequence identity (nsi) with *Pepper leaf curl Bangladesh virus* segment A, complete sequence (91%) [Acc. No.- AF314531] and Pepper leaf curl Bangladesh virus India [India/Mograhat/2007] clone pChMogK4 segment DNA-A, complete sequence (95%) [Acc. No.- HM007111] respectively. The nucleotide sequence of Bongaon - North 24 Parganas - West Bengal and Dhatrigram - Burdwan- West Bengal begomovirus isolate was compared with 17 previously reported begomovirus isolates infecting chilli, peeper, tomato, cucurbit, legume & black-nightshade (Table – 1) and respective begomovirus accessions available from the GenBank (EMBL). For LT622253 (North 24 Parganas) & LT622254 (Burdwan), out of 17 accessions, 7 begomovirus belongs to PepLCV isolates, 6 to ToLCLV, 3 to ChiLCV & 1 to PapLCV.

Multiple sequence alignment was carried out using ClustalW and ClustalX version 2.0 [14]. Bongaon - N24P - WB begomovirus isolate gave maximum nucleotide sequence identity with one isolate from Bogra, Bangladesh [Acc. No.- AF314531] 91%, whereas Dhatrigram - Burdwan - WB begomovirus isolate gave maximum nucleotide sequence identity with two GenBank accession within the range of 91% to 95, reported from mograhat, India [HM007111] 95% and bogra, Bangladesh [AF314531] 91%. The pairwise sequence comparison with the help of PASC programme (BLAST based Alignment) for Bongaon-N24P-WB begomovirus isolate and Dhatrigram - Burdwan – WB begomovirus isolate registered a maximum of 89.69% pairwise alignment with AF314531 (Table - 2) and 91.19% with HM007111 begomovirus isolate respectively (Table- 2). A phylogenetic tree was drawn using the MEGA version 7.0 to analyze the phylogenetic relationship of the sequenced genome of begomoviruses under study with a number of selected begomovirus DNA sequence retrieved from GenBank. Constructed phylogenetic tree shown LT622253 (North24-Parganas) & LT622254 (Burdwan) isolate has many main and sub-clusters. The LT622253 (North24-Parganas) & LT622254 (Burdwan) chilli begomoviorus isolate, India was segregated from other chilli infecting begomoviruses (Fig. 4). Phylogenetic tree clearly indicates that Bongaon- North 24 pargana-WB (LT622253) chilli begomovirus isolate clustered with Pepper leaf curl Bangladesh virus segment A, complete sequence [Acc. No.-AF314531] (Fig. 4) and dhatrigram-Burdwan-WB (LT622254) chilli begomovirus isolate is clustered and most closely related to Pepper leaf curl Bangladesh virus India [India/Mograhat/2007] clone pChMogK4 segment DNA-A, complete sequence [HM007111] (Fig. 4). Both the isolates (LT622253 & LT622254) also have shown evolutionary relationship with Pakistan, Oman, and Bangladesh begomovirus isolates.

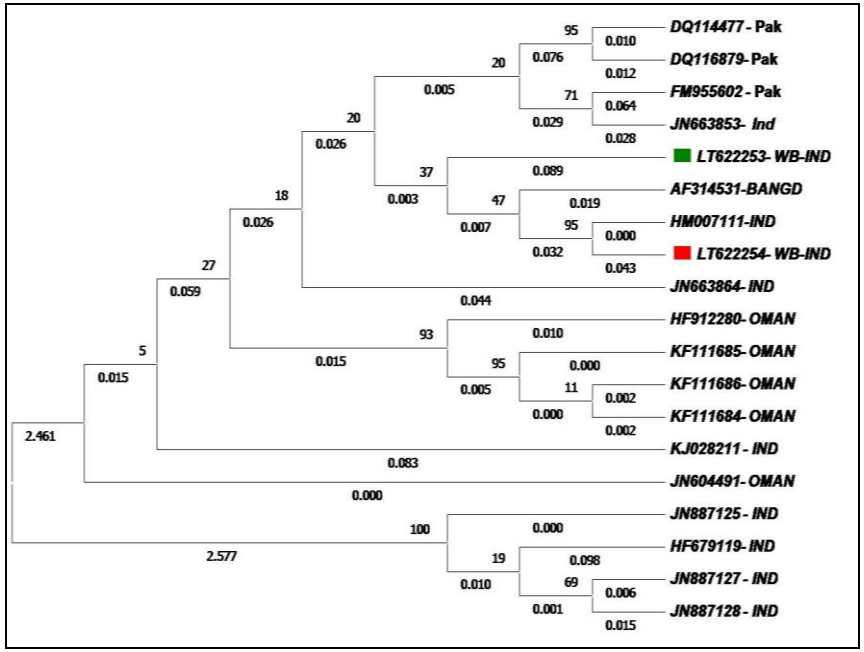

Fig 4: Phylogenetic relationship of partial nucleotide sequences of DNA-A between begomoviruses infecting chilli of Bongaon-24Parganas-WB, Dhatrigram - Burdwan - WB and other related begomoviruses. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura M., 1980). The tree with the highest log likelihood (-3504.78) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5562)]. Evolutionary analyses were conducted in MEGA7 [13].

Table 2: Pair wise matrix based on percent nucleotide sequence identity of Bongaon- North 24 parganas and Dhatrigram-Bardwan-WB begomovirus isolates infecting chilli and other begomovirus infecting chilli, peeper, tomato, black nightshade, cucurbit, legume were created by ClustalW2 programme from EMBL database.

Accession No.	HF679119	JN887127	JN887128	JN887125	FM955602	HF912280	KF111686	KF111684	KF111685	DQ114477	DQ116879	JN663864	LT622253	JN663853	LT622254	AF314531	HM007111	KJ028211	JN604491
HF679119	100.00																		
JN887127	87.20	100.00																	
JN887128	87.10	94.87	100.00																
JN887125	86.47	94.72	96.58	100.00															
FM955602	42.65	42.66	41.46	43.78	100.00														
HF912280	43.17	44.19	43.55	44.92	85.36	100.00													
KF111686	42.27	43.42	42.76	44.16	85.16	96.81	100.00												
KF111684	42.53	43.68	43.02	44.42	86.00	97.61	98.84	100.00											
KF111685	42.53	43.68	43.02	44.42	86.00	97.64	98.88	99.67	100.00										
DQ114477	42.27	42.78	41.72	43.78	87.42	83.14	82.87	83.64	83.64	100.00									
DQ116879	42.01	42.53	41.46	43.53	87.42	83.18	82.91	83.67	83.67	98.91	100.00								
JN663864	41.37	42.66	41.59	43.65	84.95	85.17	84.39	85.27	85.27	86.88	86.77	100.00							
LT622253	45.89	45.47	44.63	46.32	85.90	86.80	87.34	87.34	87.52	85.53	84.99	86.26	100.00						
JN663853	42.91	43.42	42.37	44.16	87.53	85.17	84.76	85.38	85.45	87.61	87.75	87.83	86.26	100.00					
LT622254	43.95	43.24	42.53	43.59	83.65	84.28	84.75	84.91	85.06	83.96	83.65	85.06	87.09	85.85	100.00				
AF314531	42.65	42.78	41.85	43.91	87.12	84.83	84.42	85.00	85.07	88.47	88.44	89.68	89.69	92.73	87.74	100.00			
HM007111	42.53	42.66	41.85	43.91	86.51	84.55	84.18	84.83	84.94	87.79	87.65	89.36	88.61	92.81	91.19	96.08	100.00		
KJ028211	42.40	43.04	41.59	44.04	81.16	78.87	78.28	79.08	79.16	80.58	80.62	80.90	85.17	85.59	82.08	86.09	85.84	100.00	
JN604491	41.62	43.17	41.59	43.53	79.65	83.84	82.96	83.73	83.84	79.10	79.07	83.02	84.63	80.07	79.25	80.17	79.85	86.01	100.00

4. Discussion

The PCR detection of begomovirus using degenerate primers confirmed the association of begomoviruses with chilli grown in West Bengal. Farmers' chilli field shown mild to severe upward leaf curl symptoms along with interveinal & marginal chlorosis, stunting and less or no fruit on infected plant. The variation of symptoms between different chilli growing areas indicated mixed infections with different begomoviruses and mites.

In the last 10-20 years, the commercial management practice employed against chilli leaf curl virus disease was intensive use of insecticides targeting the insect vector and this could be of no use in upcoming days due to increasing insecticide tolerance. The severity and incidence of the chilli leaf curl disease on chilli in West Bengal gives an alarming signal against economic production. And the PCR technique using degenerate primer pair (SPG1 & SPG2) allowed rapid, sensitive, and accurate detection of begomoviruses in chilli with minimal sample preparation, thus it could be very effective method for depicting the current situation of begomovirus in a region. Phylogenetic tree analysis of Bongaon - North 24 Parganas (LT622253) and Dhatrigram-Burdwan - West Bengal (LT622254) begomovirus isolate shown closer relationship with *Pepper leaf curl Bangladesh virus* isolates. As the identities of two different sequences are ≥ 91%, they are considered to be the variant of same virus [3, 31]. Thus LT622253 & LT622254 can be considered as the variant of *Pepper leaf curl Bangladesh virus* isolates (AF314531 and HM007111).

5. Conclusion

The study provides evidence for the possible risk of trans-boundary movement of the virus through plant materials and insect vectors and increase in begomovirus share under chilli. Due to continuous resurgence of whitefly vectors & indiscriminate use of chemical insecticides, there is a strong need to develop resistant cultivar against the virus dominating in major chilli growing areas of West Bengal & carrying out local level publicity campaign to create awareness amongst farmers' community towards possible risks.

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7. References

1. Briddon RW, Bull SE, Amin I. Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. Virology. 2003; 312:106-121
2. Brown JK, Fauquet CM, Briddon RW, Zerbini M, Moriones E, Navas-Castillo J. Family *Geminiviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (Eds.), Vitus taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, Elsevier-Academic Press, Amsterdam, The Netherlands, 2011, 351-373
3. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC *et al*. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Archives Virology. 2015; 160:1593-1619
4. Chavan AK, Choudhary RS, Narwade RB, Autade RH. Identification of Tomato Leaf Curl Virus (ToLCV) Strain Causing ToLCV in Tomato and Chilli. International Journal of Science and Research (IJSR). ISSN (Online). 2013, 2319-7064
5. Dellaporta SL, Wood J, Hicks JB. A plant DNA mini preparation: Version III. Plant Molecular Biology Reporter. 1983; 1:19-21
6. Dhanraj KS, Seth ML. Enation in *Capsicum annum* L. (chilli) caused by a new strain of leaf curl virus. Indian Journal of Horticulture. 1968; 25:70-71
7. George TE, Anand N, Singh SJ. Influence of potato virus Y (PVY) and cucumber mosaic virus (CMV) on growth and yield of *bell* pepper (*Capsicum annum* L.). Indian Cocoa, Arecanut and Spices Journal. 1995; 19:1-4
8. Govindappa MR, Shankergoud I, Shankarappa KS, Wickramaarachchi WART, Reddy RB, Rangaswamy KT. Molecular Detection and Partial Characterization of Begomovirus Associated with Leaf Curl Disease of Sunflower (*Helianthus annuus*) in Southern India. Plant Pathology Journal. 2011; 10(1):29-35
9. Hernandez HJ. Yield performance of Jalapeno pepper cultivar (*Capsicum annum* L.). Capsicum and Eggplant-Newsletter. 1995; 14:50-53
10. Kalaria R, Mahatma MK, Mahatma L. Molecular Characterization of Begomovirus Infecting Abutilon Glaucum in South Gujarat Region. The Bioscan. 2013; 8(1):105-107.
11. Khan MS, Raj SK, Singh R. First report of Tomato leaf curl New Delhi virus infecting chilli in India. Plant Pathology. 2006; 55:289
12. Kumar RV, Singh AK, Chakraborty S. A new monopartite begomovirus species, Chilli leaf curl Vellanad virus, and associated betasatellites infecting chilli in the Vellanad region of Kerala, India. New Disease Reports. 2012; 25:20
13. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 2016; 33:1870-1874
14. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H *et al*. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23:2947-2948
15. Lazarowitz SG. Geminiviruses: Genome and structure and gene function. Critical Reviews in Plant Sciences. 1992; 11:327-349
16. Lotrakul P, Valverde RA, Clark CA, Sim J, De La Torre R. Detection of a geminivirus infecting sweet potato in the United States. Plant Disease. 1998; 82:1253-7
17. Mishra MD, Roy-choudhuri SP, Ashrafi J. Virus causing leaf curl of chilli (*Capsicum annum* L.). Indian Journal of Microbiology. 1963; 3:73-76
18. Padidam M, Beachy RN, Fauquet CM. Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. Journal of General Virology. 1995; 76:25-35
19. Padidam M, Sawyer S, Fauquet CM. Possible emergence of new geminiviruses by frequent recombination. *Virology*. 1999; 265:218-225
20. Paximadis M, Idis AM, Torres-Jerez I, Villarreal A, Rey ME, Brown JK. Characterization of tobacco geminiviruses in the Old and New World. Archives of *Virology*. 1999; 144:703-717
21. Reddy KM, Reddy MK. Breeding for virus resistance, In: Kumar R, Rai AB, Rai M, Singh HP (Eds.), Advances in Chilli Research, Studium Press Pvt. Ltd., New Delhi, India, 2010, 119-132

22. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987; 4:406-425
23. Sankara RK, Acharyya P. Incidence of yellow vein mosaic virus disease of okra [*Abelmoschus esculentus* (L.) Moench] under summer and rainy environments. International Journal of Current Research. 2012; 4(5):18-21.
24. Senanayake DMJB, Mandal B, Lodha S, Varma A. First report of Chilli leaf curl virus affecting chilli in India. Plant Pathology. 2006; 56:343
25. Sharma R, Mahila HR, Mohapatra T, Bhargava SC, Sharma MM. Isolating plant genomic DNA without liquid nitrogen. Plant Molecular Biology Reporter. 2003; 21:43-50
26. Shih SL, Tsai WS, Green SK, Singh D. First report of Tomato leaf curl Joydebpur virus infecting chilli in India. New Disease Reports. 2006; 14:17
27. Shih SL, Tsai WS, Green SK. Molecular characterization of tomato and chilli leaf curl begomoviruses from Pakistan. Plant Disease. 2003; 87:200
28. Singh SS, Singh HB, Patel KK. Effect of chilli mosaic virus infection on total yield of chilli var. Jwala in Faizabad. Journal of Living World. 1996; 3:5-8
29. Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution. 1992; 9:678-687.
30. Varma, A, Malathi VG. Emerging geminivirus problems: A serious threat to crop production. Annals of Applied Biology. 2003; 142:145-164.
31. Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J *et al*. ICTV Virus Taxonomy Profile: *Geminiviridae*. Journal of General Virology. 2017; 98:131-133.