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Molecular identification and *in silico* characterization of coat protein in chilli leaf curl virus associated in chilli from south Gujarat region of India

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Abstracts
Chilli leaf curl disease (CLCVD) incited in different species of crops and one of the most significant biotic disease in limiting production and productivity in India and worldwide. In this study, Natural incidence of CLCVD disease on chilli was observed in March, 2019 at Gujarat, India. The infected plants revealed systemic viral symptoms as leaf curling and thickening and swelling of veins, stem twisted and stunted. Molecular methods were followed to detection and characterized coat protein (CP) gene of Chilli leaf curl virus using specified design primers. The purified PCR product (~900 bp) was sequenced and further analysed showed that the virus had 99.00% sequence identities and closest phylogenetic relationships with isolates of Chilli leaf curl virus-India isolate Ahmadabad segment DNA-A, complete sequence (JN663846.1),Therefore name as Chilli leaf curl virus isolate NAU Gujarat coat protein (AV1) gene and submitted to NCBI (MK955890.1). ChiLCV coat-protein sequence was computationally analysed for presence of conserved domain as Gemini super family and further modelled to predict structure using swiss model that further validated using Ramachandran plot showing 89.10% with 172 amino acid in most favourable region. Thus, the present study will be helpful to understand evolutionary classification and the molecular host pathogen interaction to develop robust resistant strategies against *Gemini viruses*, particularly ChiLCV in the nearby future.

Keywords: Chilli leaf curl virus diseases (ChiL CVD), *Begomovirus*, AV1 gene, PCR, whiteflies and Chilli leaf curl virus (ChiLCV)

Introduction

Chilli (*Capsicum annuum* L., family *Solanaceae*) is an important vegetable and spices crop grown in worldwide. Commercial production of chilli is mainly limit in tropical regions of the world. India one of the largest producer of chilli crop in world with average 25 per cent contribution with area of 7.44 lakh hectares with an annual production of 14.53 lakh tonnes with the productivity of 1953 kg/ha (Akhter *et al*. 2009 [1]; Anon.,2015 [2]; Kareem and Byadgi, 2017 [11]; Ansar *et al.* 2018 [3]). In last few decades, the productivity of chilli decreases due to its susceptibility to various others pests and diseases result in significant yield losses. The crops had been significantly reported by many pathogen mainly viral diseases worldwide. Chilli cultivars are susceptible to broad range of host with different varients in genomic viruses, including *Begomovirus* es as Chilli leaf curl virus disease (ChiLCVD) cause severe damages to crops at all the stages in field. ChiLCD is one of the devastating pathogens and poses a severe threat to chilli production in tropics and sub-tropics region of world and also threatening to several other economically significant crops and weeds. In India, it is more over spreaded across many regions of India (Subban and Sudaram, 2012 [26]; Kalaria, *et al.* 2013 [10]; Matylda *et al.* 2015 [15]; Zehra *et al.* 2017 [31]; Thakur *et al.* 2018 [27]).

Chilli leaf curl viruses (ChiLCV) are the plant viruses belongs to bipartite *Begomovirus* species (genus *Begomovirus*, family *Geminiviridae*). ChiLCV under the genera *Begomovirus* may be present in bipartite type (both DNA-A and DNA-B of 2.7kb bp with a mutual ~200 bp region) or monopartite type circular ssDNA (very much similar to DNA-A) (Khuswaha *et al.* 2015 [14]). In India, the first natural occurrence of chilli leaf curl virus with typical leaf curl symptoms was reported to be caused by ToLCV (Mishra *et al.* 1963 [16]; Pruthi and Samuel, 1942 [21]). There are several reports of species or strains of chilli leaf curl of *Geminiviruses* causing Chilli leaf curl virus diseases (ChiLCVD) in India and abroad (Husain, 1932 [8];

Thakur *et al.* 2018 [27]). The affected plants are severely stunted, leaf become reduced in size, wrinkle, curl upwards, become distorted and produced no fruit. The virus is transmitted by vector as whiteflies (*Bemisia tabaci*) that are attracted to young leaves and growing tips. The vector live in a circulative and persistent manner which attack mainly dicotyledonous crop plants species (Thakur *et al.* 2018 [27]).

In Indian subcontinent, Most of the ChiLCV are monopartite and only contain DNA-A (Briddon, 2008 [4]). The function of DNA-A component is to encode signals for six most important viral factors such as- the coat protein (CP) - AV1; pre-coat protein-AV2; the viral replication associated protein (Rep) -AC1; the transcription activator protein (TrAP) -AC2; the replication enhancer protein- AC3; and protein for putative symptom expression-AC4. DNA-B encodes two important movement proteins, BV1 and BC1, responsible for nuclear shuttling and cell-to-cell long distance movement (Kumar *et al.* 2015 [12]; Thakur *et al.* 2018 [27]). Eradicating viruses as pathogens in chilli one of the major challenges due to limitation of Insecticides. Therefore either molecular breeding along with transgenic approaches required for virus resistance varieties by targeting viruses genome component (Olorunju and Ntare, 2001 [18]; Waliyar *et al.* 2007 [29]). Detection of coat protein (CP) gene infected leaves DNA can assure the presence of ChiLCV and thus help in diagnosing leaf curl diseases in field. This CP is responsible for the attachment of the virus and accumulation of ssDNA into it. Many literature reported use of plant virus CP as a potential sources for development of virus resistant varieties (Kumal *et al.* 2015 [12]; Kushwaha *et al.* 2015 [14]; Kareem and Byadgi, 2017 [11]; Ansar *et al.* 2018 [3]). It is important that viruses occurring in a specific geographical area wise need to be identified and characterized prior to developing sustainable, environment-friendly disease management strategies (Green and Kim, 1991 [7]). Hence, tremendous consideration should be given to study the phenotypic and molecular properties of this severe causal agent. Spotting of ChiLCVD in Gujarat and its devastating effect in production emerged the importance of studying this disease at molecular and computational approaches. A leaf curl disease in chilli characterized by curling and chlorosis in leaves and overall stunted plant growth is observed in the experimental field of Navsari Agricultural University, Navsari. In the present study, we characterized coat protein (AV1) gene of ChiLCV for the evolutionary. Further, molecular modelling of ChiLCV coat protein provided a topology for understanding protein folding and functional structure that help to understanding the virus infection and provide way to combating against infection.

Materials and Methods

Molecular Characterization

Sampling and Genomic DNA extraction

The primary detection was done by observing the whitefly association with chilli plants near ASPEE Horticulture and Forestry Farm, Navsari Agricultural University, Navsari. The infected plants were dwarfed due to stunted growth, reduced leaf size with curling and puckering of leaflets. Modified CTAB method (Kalaria *et al.* 2013 [10]; George *et al.* 2014 [6]) was used to isolate the total DNA from infected young leaves.

AV1 coat protein gene amplification

A specific primers pair of *Begomovirus* coat protein (CP) gene were designed in silico using tool FAST PCR(*https://primerdigital.com/fastpcr.html*) and synthesised as CLCVF (GTATAGAAGCCCAGATGTGCCT) and reverse primer as CLCVR (AGGGCCT GCTCC TTCGAT) are designed from conserved part of DNA-A genome of ChiLCV (IDT integrated DNA Technologies Coralville, IA). PCR was performed with some slight modification in parameter for the amplification of coat protein (AV1) gene. Further amplified fragments were analysed by 1.5% agarose gel electrophoresis and visualized in trans illuminator (Snehi *et al.* 2016 [25]; Saeed *et al.* 2017 [23]).

Purification and sequencing

Amplified fragment was purified using Big Dye® Terminator v3.1 Cycle Sequencing clean up method with some modification used further for sequencing. Retrieved sequence was further analysed for similarity index using NCBI-BLASTN for the nomenclature of sequence and submitted to NCBI database using Bank It.

Phylogenetic trees were constructed using full optimal alignment in the Clustal X version 2.0 Software and neighbor-joining method with 1000 bootstrap replications available in the MEGA version 6.0 (Kalaria *et al.* 2013 [10]; George *et al.* 2014 [6]).

Insilico characterization of CLCV protein sequence

Coat protein Sequence analysis

Different ORFs from the sequence were obtained using ORF finder tool (*https:// www.ncbi. nlm.nih.gov/orffinder/*) of the NCBI that were further checked in NCBI-BLASTN for ChiLCV protein sequence identities. The ChiLCV protein sequence were also check for the presence of conserved domain using NCBI-CDD tool
(https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml)
(Prajapat *et al.* 2010 [20]; Kumar *et al.* 2012 [13]; Khuwaha, *et al.* 2015 [14]). MEENA *et al*: IN SILICO ANALYSIS OF COAT-PROTEIN OF GALIC COMMON LATENT VIRUS 483 Secondary Structure Prediction

Structure Modelling of Coat Protein

BLASTP based homolog search with RCSB-PDB (PDB; http://www.rcsb.org/ pdb/home/home.do) was also carried out with Complete ChiLCV Coat-protein sequence to find out the similar homologs in database. After performing BLASTP, template sequences with fasta format more than 30% sequence identity were retrieved from RCSB-PDB. As these procedures yielded one close homologs.

So, we choose to go for homology modelling using online swiss model web server of ChiLCV coat protein (https://swissmodel.expasy.org/)
(Kumar *et al.* 2012 [13]; Patel and Kalaria, 2018 [19]).

Structure Validation of Models and Energy Minimization

Template sequence in PDB extention was retrieved from swiss model web server for visualizing and validation of the model. Evaluation of built model quality using swiss model was examined for amino acid region in Ramachandran plot. (https://servicesn. *mbi.ucla.edu/PROCHECK/*) (Prajapat *et al.* 2010 [20]; Patel and Kalaria, 2018 [19]). Based on the percentage of favourness amino acid and frequency of outliers, the models were selected and could be used for further experiment.

Result and discussion

Primary confirmation of disease incidence

Fig 1: ChiLCV infected Chilli plants exhibiting severe leaf curl, blistering reduction of leaf size and stunting of whole plant (a) and its close view of chilli leaves (b).

In March, 2019 Chilli plants with severe ChiLCV were observed near ASPEE Horticulture and Forestry Farm, Navsari Agricultural University, Navsari (20° 92' 49.342" N) and 72° 90' 79.144" E) of south Gujarat region in India. Symptomatic leaves with severe infections are documented by many investigators (Mishra *et al*. 1963 [16]; Kalaria *et al*, 2013 [10]; Kumar *et al*. 2015 [12]; Ansar *et al.* 2018 [3]; Thakur *et al.* 2018 [27]) were collected and further carried forward for molecular approaches (Fig.1).

Molecular identification of coat protein gene

Begomovirus genera infected chilli leaf were further subjected to genomic DNA isolation to diagnose the presence of the suspected causal agent (Fig. 2). In *Begomovirus*, AV1 gene corresponds to coat protein, one most conserved part in viral genome. (Kumar *et al.* 2015 [12]). The Genomic DNA was further taken for molecular conformation using amplification of AV1 coat protein gene using specific design primer as CLCV-CPF and CLCV-CPR. Amplification of AV1 gene for *Begomovirus* detection had been earlier used by different scientists (Chakraborty *et al*., 2003 [5]; Hussain *et al*., 2004 [9]).The presence of *Begomovirus* in infected DNA can be implied by amplification of ~900bp fragment (Fig.3). On sequencing, a 790bp long sequence of AV-1 of DNA-A of the *Begomovirus* corresponding as ChiLCV coat protein gene. Similarly Saeed *et al.* (2017) [23] obtained amplicon of ~771 bp from ChiLCV infected mint.

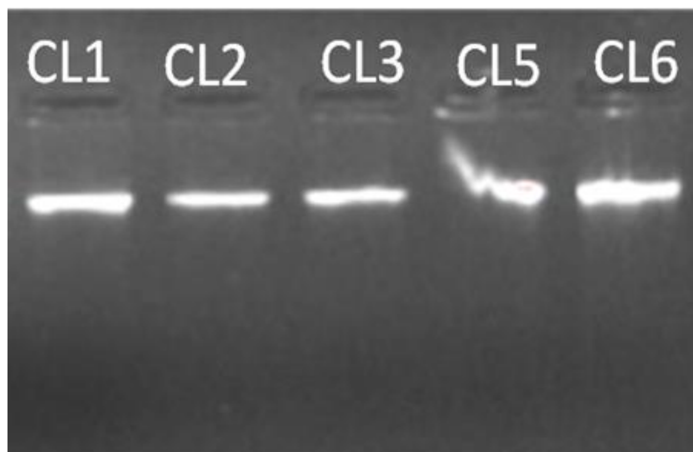

Fig 2: Total Genomic DNA isolation from ChiLCV infected Chilli

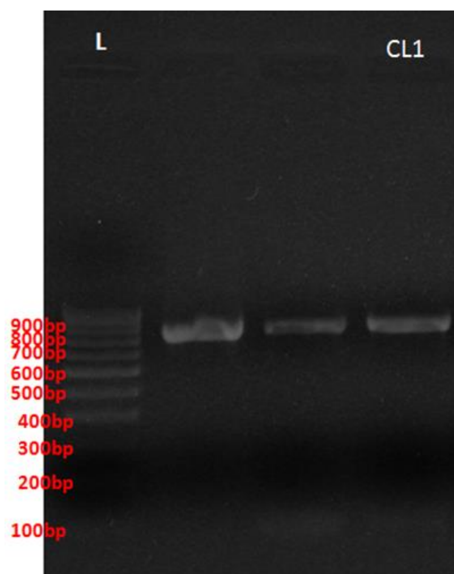

Fig 3: Amplification of ChiLCV coat protein AV1 gene from Chilli

Molecular relationship of ChiLCV with other *Begomovirus* es.

Obtained results from the BLAST database showed that all the sequence have more than 99.00% nucleotide (NT) identities mainly with Chilli leaf curl virus-India isolate from India and nearby Indian subcontinent (Table.1). In BLAST pairwise sequence comparison analysis, obtain sequence has maximum 99.75 per cent sequence identities with Chilli leaf curl virus-India isolate Ahmadabad segment DNA-A, complete sequence (JN663846.1), while average 99.37% with different Chilli leaf curl virus complete genome, clones.

Table 1: Percent identities (nucleotide) between parts of Chilli leaf curl virus isolates NAU Gujarat coat protein (AV1) gene (MK955890) with reported *Begomovirus es* worldwide.

S. No.	Accession	Name of sequence	Query cover	E-value	Percentage identity
1	JN663846.1	Chilli leaf curl virus-India isolate Ahmedabad segment DNA-A, complete sequence	100%	0.0	99.75%
2	LN886660.1	Chilli leaf curl virus complete genome, clone RM283	100%	0.0	99.37%
3	LN886659.1	Chilli leaf curl virus complete genome, clone RM282	100%	0.0	99.37%
4	LN886658.1	Chilli leaf curl virus complete genome, clone RM281	100%	0.0	99.37%
5	LN886657.1	Chilli leaf curl virus complete genome, clone RM280	100%	0.0	99.37%
6	LN886656.1	Chilli leaf curl virus complete genome, clone RM279	100%	0.0	99.37%
7	LN886655.1	Chilli leaf curl virus complete genome, clone RM277	100%	0.0	99.37%
8	LN886654.1	Chilli leaf curl virus complete genome, clone RM276	100%	0.0	99.37%
9	LN886653.1	Chilli leaf curl virus complete genome, clone RM273	100%	0.0	99.37%
10	LN886652.1	Chilli leaf curl virus complete genome, clone RM270	100%	0.0	99.37%
11	LN886651.1	Chilli leaf curl virus complete genome, clone RM269	100%	0.0	99.37%
12	LN886650.1	Chilli leaf curl virus complete genome, clone RM267	100%	0.0	99.37%
13	LN886649.1	Chilli leaf curl virus complete genome, clone RM262	100%	0.0	99.37%
14	LN886648.1	Chilli leaf curl virus complete genome, clone RM259	100%	0.0	99.37%
15	KT835648.1	Chilli leaf curl virus-India coat protein (AV1) gene, complete cds	99%	0.0	99.37%
16	KF515609.1	Chilli leaf curlvirus [Anand:Tomato:2012] isolate, complete sequence	100%	0.0	99.92%
17	HM007114.1	Chilli leaf curlvirus [Noida/2007] clone pChNoiB7 segment DNA-A, complete sequence.	100%	0.0	99.92%
18	KJ700656.1	Chilli leaf curl virus isolate RK02, complete genome	100%	0.0	98.99%
19	KJ700653.1	Chilli leaf curl virus isolate RK01, complete genome	100%	0.0	98.99%
20	HM007104.1	Chilli leaf curl virus-India [India/Jodhpur/2009] clone pChJodB26 segment DNA-A, complete sequence	100%	0.0	98.99%
21	EU939533.1	Chilli leaf curl virus isolate Narwan segment DNA A, complete sequence	100%	0.0	98.99%
22	KX533940.1	Chilli leaf curl virus isolate New Delhi, complete genome	100%	0.0	98.86%
23	KX499526.1	Chilli leaf curl virus isolate New Delhi, complete genome	100%	0.0	98.86%
24	KJ649706.1	Chilli leaf curl India virus isolate Sonipat:TC290:2010 segment DNA-A, complete sequence	100%	0.0	98.86%

These result clearly indicated that the suspected pathogen was *Begomovirus* infection as Chilli leaf curl virus. Rybicki 1998) [22] has been reported that more than 90% nucleotide sequence identity has been suggested as a guideline for predicting as viral strain for *Begomovirus* nomenculture. Accordingly, the above result, strain was named as Chilli leaf curl virus isolate NAU Gujarat coat protein (AV1) gene and sequence submitted to NCBI database as MK955890. Multiple sequence alignments of all the sequence were carried out to find the conserved sequences among all. During phylogenetic analysis of chilli isolate under study (MK955890.1) showed closest relationships with all isolates as shared a common ancestor falls in the same cluster except KT835648 and EU939533 which belong to another cluster due to variation in nucleotide sequences (Fig. 4 A,B). Ansar *et al.* (2018) [3] also carried the phylogenic analysis of AV1 fragments of isolate (KY010624) had nucleotide identity ranging between 94 and 98 per cent with isolates of tomato leaf curl Joydebpur virus. Saeed *et al.* (2017) reported BLASTn analysis of amplified AV1 fragments revealed highest similarity of 98% with Chilli leaf curl India virus (FM877858) from Mint. Similar work reported by Vanthana *et al.* (2017) [28] and Kalaria *et al.* (2013) [10] for the characterization of coat protein (CP) gene of *Begomovirus* es.

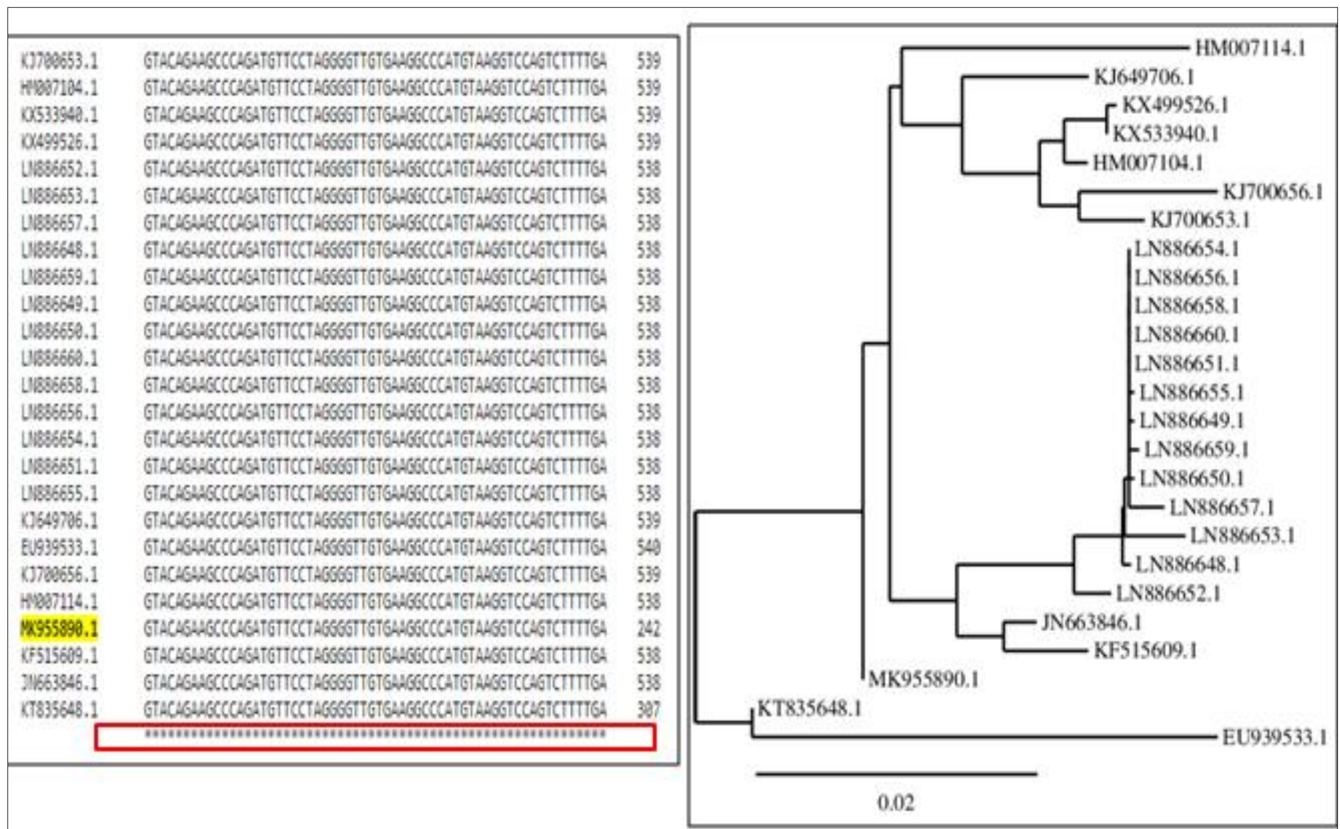

Fig 4: (A,B) Phylogenetic analysis of complete coat protein (Av1) gene of ChiLCV under study infecting Chilli (MK955890) compared with various *Begomovirus* coat protein

Insilico characterization of ChiLCV protein sequence
One ORF obtain from ChiLCV protein sequence showed the presence of conserved domain as Geminivirus coat protein/nuclear export factor BR1 family from Pfam database acc. no. Pfam00844 with E-value 2.07ee-99 (Fig.5). Similar work also carried out in ToLCV coat protein sequence by Kumar *et al.* (2012) [13] and Papaya Leaf curl virus coat protein by Patel and Kalaria (2018) [19].

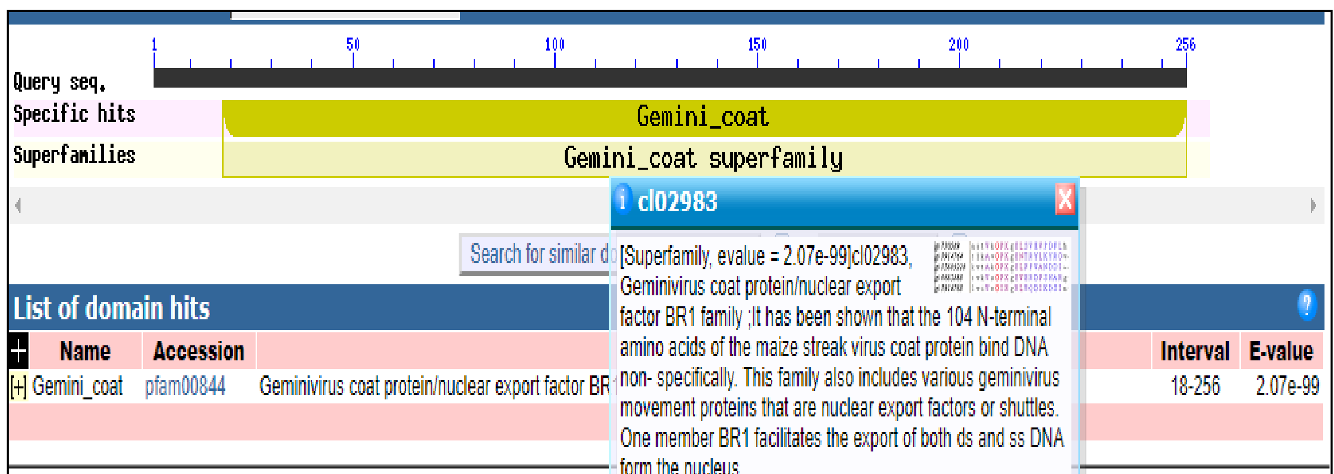

Fig 5: Presence of Gemini coat protein as Conserved domain in ChiLCV coat protein

Homology Modelling of ChiLCV Coat Protein:
BLASTP of ChiLCV coat protein sequence with PDB database result showed 82.79% similarity with *Aqueratum yellow vein virus* (6f2s.1 J). ChiLCV coat protein sequence was further carried forwarded for homology modelling using swiss model web server for structure prediction (Fig 6). QMEAN score found to be -2.52 with positive sign (Table 2), but the stability of the structure will depend more on ramachandran plot of residues. Similar work had also been carried out by Patel and kalaria (2018) for homology modelling of Papaya Leaf curl virus coat protein using *Aqueratum yellow vein virus* (6f2s.1 J) having similarity more than 30 per cent. Yadav *et al.* (2011) [30] also performed the homology modelling of coat-protein of Mungbean Yellow Mosaic India Virus.

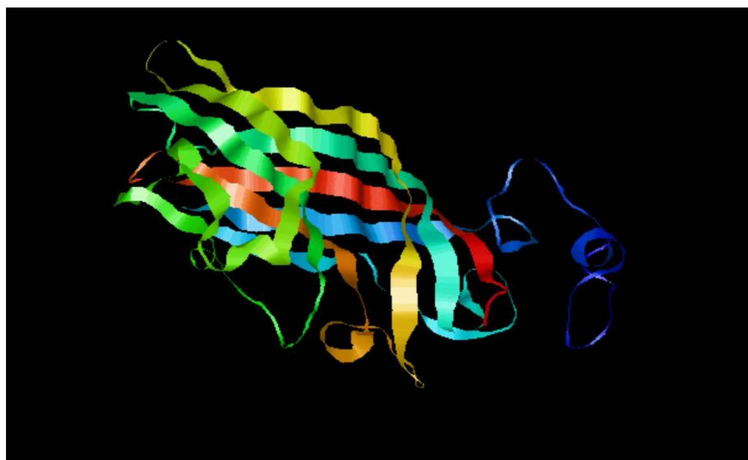

Fig 6: Rasmol visualization of predicted ChiLCV coat protein structure using Swiss Model

Table 2: Homology modelling of ChiLCV coat protein using Swiss Model

S. No.	Parameters	ChiLCV coat protein
1	QMEAN	-2.52
2	GMQE	0.75
3	Template	6f2s.1 J
4	Sequence identity	82.79%

Validation of Homology Modelling

In homology modelling, evaluation of model quality one of the important step. Once the model built, the final model must be validated in order to confirm whether the model's stereochemistry is stable and reliability with typical values originate in crystal structures. Ramachandran plot calculation in PROCHECK tool used to measured the quality of the modelled structure by analyzing residue region (Morris, *et al.* 2012) [17]. The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. The darkest areas represented to the "core" regions representing the most favorable combinations of phi-psi values. The percentage of residues in the "core" regions is one of the best guides to stereo-chemical quality (Patel and kalaria, 2018) [19]. Predicted ChiLCV coat protein model subjected to ramachandran plot with 89.1% amino acid in most favourable region (>90%) with 172 amino acid (Fig 7). Prajapat *et al.* (2010) [20] also studied ramachandran plot for AC4 build protein of *Abutilon mosaic virus* having 89.40% residues in most favoured region. Similar work also carried out predicted for homology model of antioxidant proteins in Spinach (Sahay and Shakya, 2010) [24] and Papaya Leaf curl virus coat protein by Patel and Kalaria (2018) [19].

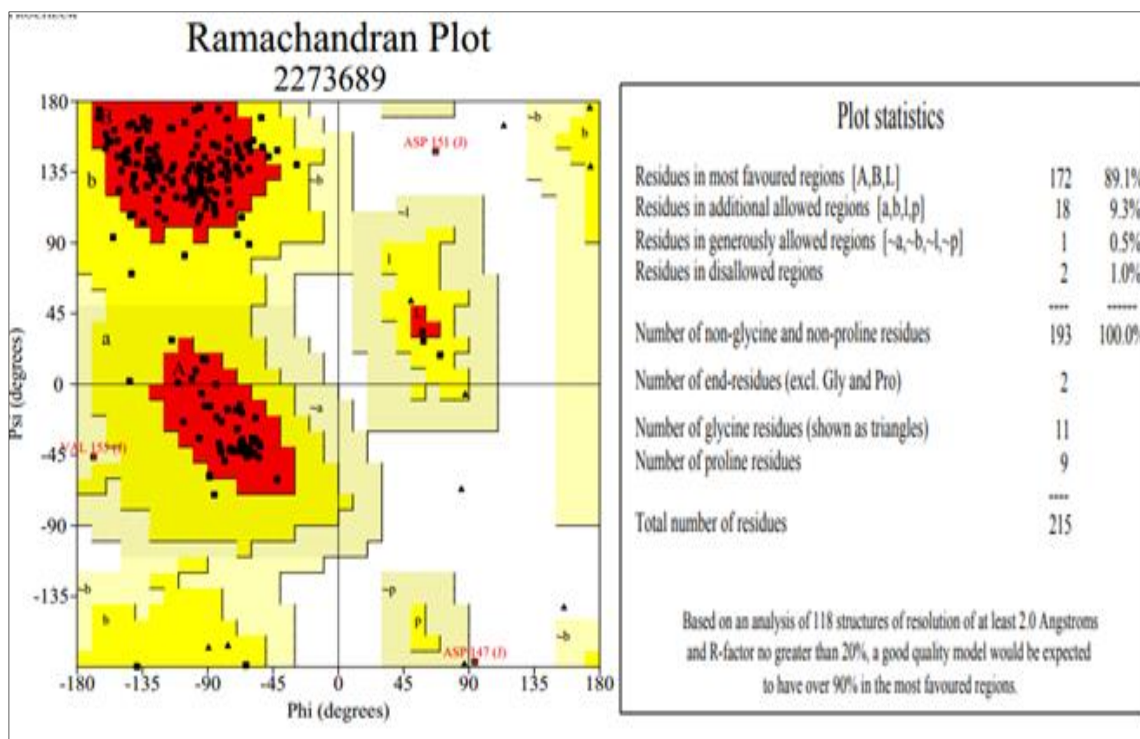

Fig 7: Ramachandran Plot of ChiLCV coat protein model using Swiss model

Conclusion

Despite the severe losses due to ChiLCD, a systematic study of the AV1 gene associated with ChiLCV carried out to analyse the genetic diversity and the phylogeographical distribution of ChiLCD-associated *Begomovirus* es (Patel and Kalaria, 2018). Therefore, more studies required to focus on developments in the plant–virus interactions that promise more avenues of *Begomovirus* management. The present

study was undertaken for molecular and in silico characterization of ChiLCV coat proteins of *Begomovirus* in Chill from south Gujarat region of India. Amplification of AV1 gene of ~900bp conformed the *Begomovirus* infection in chill and further validated on sequencing as Chilli leaf curl virus isolate NAU Gujarat coat protein (AV1) gene [MK955890.1]. Coat protein sequence was also conformed presence of conserved domain as Geminivirus coat superfamily with acc. no. Pfam00844. Based on BLASTP result, The ChiLCV coat proteins was further taken for homology modelling based structure prediction using swiss model and revealed a very good structure with QMEAN score was -2.52 further validated with Ramachandran plot showed 89.1% amino acid in most favourable region with 172 amino acid of template result in great reliability of structure. As chilli associated *Begomovirus* es and betasatellites are enlarging geographical region including host (George *et al.* 2014) [6]. These information help in functional analysis and protein folding of ChiLCV that helps in developing of diseases resistance strategy for various crops against *Begomovirus* es to minimize the yield losses.

Reference

1. Akhter A, Qazi J, Saeed M, Mansoor SA. Severe Leaf Curl Disease on Chilies in Pakistan is Associated with Multiple *Begomovirus* Components. Plant Diseases. 2009; 93(9):962.
2. Anonymous. Pocket Book of Agriculture Statistics, Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Govt. of India, 2015, 53.
3. Ansar M, Akram M, Agnihotri AK, Srinivasaraghavan A, Saha T. Naimuddin Characterization of leaf curl virus in chili and overwintering role of nightshade in linkage between chilli and tomato, Journal of Plant Pathology, 2018, 1-8.
4. Briddon RW, Brown JK, Moriones E. Recommendations for the classification and nomenclature of the DNA-βsatellites of *Begomovirus* es, Archives of Virology. 2008; 153(4):763-781.
5. Chakraborty S, Pandey PK, Banerjee MK, Kalloo, G, Fauquet CM Tomato leaf curl Gujarat virus, a new *Begomovirus* species causing a severe leaf curl disease of tomato in Varanasi, India Phytopathology. 2003; 93:1485-1495.
6. George B, Vinoth, RK, Chakraborty S. Molecular characterization of Chilli leaf curl virus and satellite molecules associated with leaf curl disease of *Amaranthus* spp Virus Genes. 2014; 48:397-401.
7. Green SK, Kim JS. Characterisation and control of viruses infecting peppers: A literature review. AVDRC, 1991, 60-66.
8. Husain MA. Leaf curl in cotton and other plants. Nature. 1932; 130, 312.
9. Hussain M, Mansoor S, Iram S, Zafar Y, Briddon RW. First report of Tomato leaf curl New Delhi virus affecting chilli pepper in Pakistan. Plant Pathology Journal. 2004; 53, 794.
10. Kalaria RK, Mahatma MK, Mahatma L. Molecular Characterization of *Begomovirus* infecting *Abutilon glaucum* in south Gujarat region, The Bioscan. 2013; 8(1):105-107.
11. Kareem MA, Byadgi AS. Molecular Identification and Characterization of Virus Associated with Murda Complex Disease in Chilli (Cv. *Byadgi Dabbi*). International Journal of Current Microbiology and Applied Sciences. 2017; 6(11):2837-2844.
12. Kumar RV, Singh, AK, Singh AK, Yadav T, Basu S, Kushwaha N, Chattopadhyay B, Chakraborty S. Complexity of *Begomovirus* and betasatellite populations associated with chilli leaf curl disease in India, Journal of General Virology, 2015; 96: 3143-3158.
13. Kumar SP, Patel SK, Kapopara RG, Jasrai YT, Pandya HA. Evolutionary and Molecular Aspects of Indian Tomato Leaf Curl Virus Coat Protein, International Journal Plant Genomics, 2012, 1-12.
14. Kushwaha N, Sahu PP, Prasad M, Chakraborty S. Chilli leaf curl virus infection highlights the differential expression of genes involved in protein homeostasis and defense in resistant chilli plants, Applied journal of Microbiology and Biotechnology. 2015; 99:4757-4770.
15. Matylda JS, Kalaria R, Kauranne T. A dynamic model for epidermic outbursts by *Begomovirus* population clusters. Ecology Modelling. 2015; 297:60-68.
16. Mishra MD, Raychaudhuri SP, Jha A. Virus causing leaf curl of chilli (*Capsicum annuum L.*) Indian Journal of Microbiology. 1963; 3:73-76.
17. Morris AL, MacArthur MW, Hutchinson EG, Thornton JM. Stereo chemical quality of protein structure coordinates, Proteins. 1992; 12:345-364.
18. Olorunju PE, Ntare BR. Combating viruses and virus disease of groundnut through the use of resistant varieties: A case study of Nigeria. Plant virology in Sub-Saharan Africa, 2001, 189-202.
19. Patel J, Kalaria RK. In-silico Analysis & Homology of Papaya Ring Spot Virus & Papaya Leaf curl virus coat protein, Bulletin journal of Environment, Pharmacology and Life Science. 2018; 7(8):19-28.
20. Prajapat R, Guar RK, Raizada R, Gupta VK. Insilico analysis of genetic diversity of *Begomovirus* using Homology modelling, Journal of Biolgical science. 2010; 10(3):217-223.
21. Pruthi HS, Samuel CK. Entomological investigations on the leaf curl disease of tobacco in northern India. V. Biology and population of the white-fly vector (*Bemisia tabaci* Gen.) in relation to the incidence of disease. Indian Journal of Agricultural Science. 1942: 12:35.
22. Rybicki EP. "A proposal for naming geminiviruses; a reply by the geminiviridae study group chair. Achieves of Virology. 1998; 143:421-424.
23. Saeed ST, Kumar B, Shasany AK, Samad A. Molecular identification of Chilli leaf curl India virus along with betasatellite molecule causing leaf curl disease of menthol mint (*Mentha arvensis* var. Kosi) in India, Journal of General Plant Pathology and Plant Diseases. 2017; 98(1):164.
24. Sahay A, Shakya M. In silico Analysis and Homology Modelling of Antioxidant Proteins of Spinach, Journal of Proteomics and Bioinformatics. 2010; 3(5):148-154.
25. Snehi SK, Parihar SS, Gupta G, Singh V, Purvia AS. Molecular Detection and Identification of *Begomovirus* Isolate on Tomato from Central Region of India Journal of Plant Pathology and Microbiology. 2016; 7:389.
26. Subban R, Sundaram K. Effect of Antiviral Formulations on Chilli Leaf Curl Virus (CLCV) Disease of Chilli Plant (*Capsicum annuum* L), Journal of Pharmacy Research. 2012; 5(12):5363-5366.
27. Thakur H, Jindal SK, Sharma A, Dhaliwal MS. Chilli leaf curl virus disease: a serious threat for chilli cultivation,

Journal of Plant Diseases and Protection. 2018; 125 (3): 239-249.

28. Vanthana M, Mahatma L, Ghevariya, TV, Saranya R. Molecular Characterization of Tomato Leaf Curl Virus (ToLCV) In South Gujarat. International Journal of Current Microbiology and Applied Sciences. 2017; 6(3): 473-481.
29. Waliyar F, Kumar PL, Monyo E, Nigam SN, Reddy AS, Osiru M, Diallo ATA. Century of Research on Groundnut Rosette Disease and its Management. Technical Report. International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. 2007; 75:1-44.
30. Yadav NK, Sarika-Iquebal MA, Akram M. In-silico analysis and homology modelling of coat-protein of Mungbean Yellow Mosaic India Virus. Journal Food Legumes. 2011; 24(2):138-141.
31. Zehra SB, Ahmad A, Sharma A, Sofi S, Lateef A, Bashir Z, Husain M, Rathore JP. Chilli Leaf Curl Virus an Emerging Threat to Chilli in India, International Journal of Pure and Applied Bioscience. 2017; 5(5):404-414.