MOLECULAR CHARACTERIZATION OF *CHILLI LEAF CURL VIRUS* INFECTING CHILLI AND PREDICTION OF ITS COAT PROTEIN STRUCTURE

PREETHI, C.
PALB 6303

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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in

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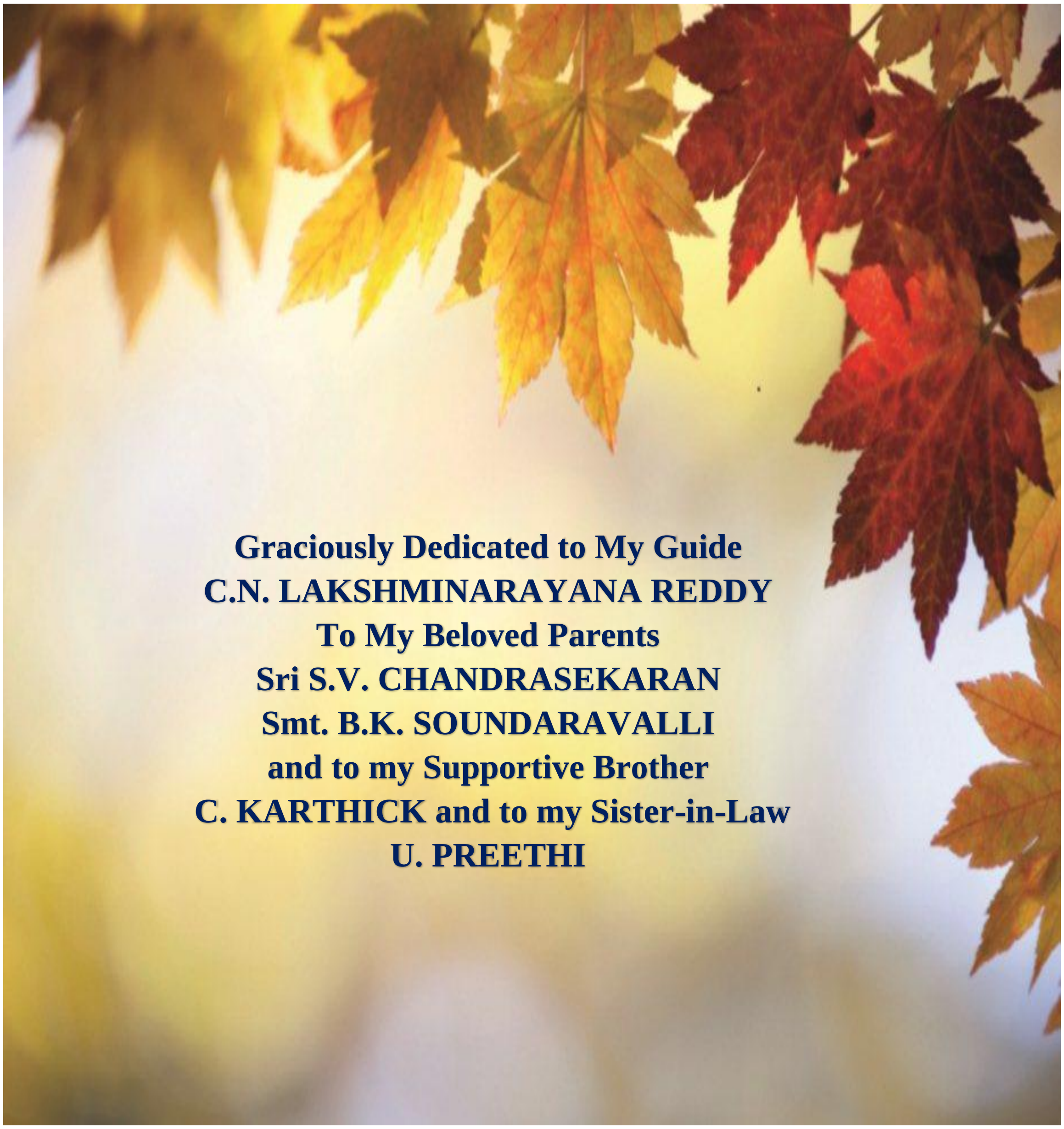

Graciously Dedicated to My Guide
C.N. LAKSHMINARAYANA REDDY
To My Beloved Parents
Sri S.V. CHANDRASEKARAN
Smt. B.K. SOUNDARAVALLI
and to my Supportive Brother
C. KARTHICK and to my Sister-in-Law
U. PREETHI

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR CHARACTERIZATION OF *CHILLI LEAF CURL VIRUS* INFECTING CHILLI AND PREDICTION OF ITS COAT PROTEIN STRUCTURE"** submitted by **Ms. Preethi, C., ID. No. PALB 6303** for the award of degree of **MASTER OF SCIENCE (Agriculture) in PLANT PATHOLOGY** to the University of Agricultural Sciences, Bangalore is a record of *bona-fide* research work done by her during the period of her study in this university under my guidance and supervision. This thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
November, 2021

(C. N. LAKSHMINARAYANA REDDY)
(Major advisor)

Approved by

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Last and most importantly, it is with pleasure that I record here my deep sense of gratitude to ***University of Agricultural Sciences, Bangalore*** *for all the facility and help. It makes me immensely proud and honored to be a student of this legendary and prestigious University. Last but not the least I extend my gratitude to all the people who have helped, supported and encouraged me in many ways for the completion of my postgraduate degree programme*

………any omission in this small manuscript doesn't mean lack of gratitude.

Bengaluru

November, 2021 *PREETHI, C.*

MOLECULAR CHARACTERIZATION OF *CHILLI LEAF CURL VIRUS* INFECTING CHILLI AND PREDICTION OF ITS COAT PROTEIN STRUCTURE

PREETHI, C.

THESIS ABSTRACT

Chilli (*Capsicum annum* L.) suffers from a plethora of biotic stresses including many viral diseases. Among viral diseases, *chilli leaf curl virus* (ChiLCV) is one of the most widespread and destructive disease. An attempt was made to study the biological transmission of ChiLCV to chilli and *Nicotiana* spp, genome sequencing of ChiLCV and *in-silico* analysis of ChiLCV coat protein (CP) in the current study. ChiLCV was transmitted to chilli (Arka Lohith), *Nicotiana glutinosa*, *N. benthamiana* and *N. tabacum* using whiteflies and they produced characteristic leaf curl symptoms. Molecular detection of ChiLCV was done using begomovirus specific primers, which produced an expected amplicon of 1400 bp. The sample gave negative amplification to DNA B, indicating that, the virus isolate is monopartite. Complete genome sequencing of DNA A homologue and its analysis revealed that it is closely related to ChiLCV and *Pepper leaf curl virus* with the sequence similarity of 97.5 to 99.2 per cent and 85.4 – 92.2 per cent, respectively. This was further supported by phylogenetic analysis. The recombination events detected using RDP4 revealed that the virus under the current study is a recombinant. Computation of physico-chemical properties revealed that ChiLCV CP was negatively charged with 29.66 kDa molecular weight, instability index of 49.20 and it was also found to be thermostable and hydrophilic. The secondary structure of ChiLCV CP revealed its good quality with no disordered residue and binding sites which was also well supported by the PSIPRED cartoon. The tertiary structure of the protein was created using homology-based modelling and this can be further utilized in studies such as protein- protein interaction and protein ligand interactions.

November, 2021

Department of Plant Pathology **(C. N. LAKSHMINARAYANA REDDY)**

UAS, GKVK, Bengaluru- 560065 Major Advisor

ಮೆಣಸಿನಕಾಯಿಗೆ ಸೋಂಕು ತಗುಲಿಸುವ ಮೆಣಸಿನಕಾಯಿ ಎಲೆ ಸುರುಳಿಯ ನಂಜಾಣುವಿನ ಅಣು ಗುಣಲಕ್ಷಣಗಳು ಮತ್ತು ಅದರ ಕೋಟ್ ಪ್ರೋಟಿನ್ ರಚನೆಯ ಮುನ್ಸೂಚನೆ

ಪ್ರೀತಿ, ಸಿ

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಮೆಣಸಿನಕಾಯಿ (*ಕ್ಯಾಪ್ಸಿಕಂ ಆನಮ್* ಎಲ್.) ಅನೇಕ ನಂಜಾಣು ರೋಗಗಳನ್ನು ಒಳಗೊಂಡಂತೆ ಜೈವಿಕ ಒತ್ತಡಗಳಿಂದ ಬಳಲುತ್ತಿದೆ. ನಂಜಾಣು ಕಾಯಿಲೆಗಳಲ್ಲಿ, ಮೆಣಸಿನಕಾಯಿ ಎಲೆ ಸುರುಳಿಯ ನಂಜಾಣು ರೋಗವು ಅತ್ಯಂತ ವ್ಯಾಪಕ ಮತ್ತು ವಿನಾಶಕಾರಿ ಕಾಯಿಲೆಯಾಗಿದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಮೆಣಸಿನಕಾಯಿ ಮತ್ತು ನಿಕೋಷಿಯಾನಾ ಸಸ್ಯಗಳಲ್ಲಿ ಮೆಣಸಿನ ಕಾಯಿ ಎಲೆ ಸುರುಳಿ ನಂಜಾಣು (ಚಿಎಲ್‌ಸಿವಿ) ಜೈವಿಕ ಪ್ರಸರಣ, ಅಣ್ವಿಕ ಗುಣಲಕ್ಷಣ ಮತ್ತು ಕೋಟ್ ಪ್ರೋಟಿನ್‌ನ ಇನ್ ಸಿಲಿಕೊ ವಿಶ್ಲೇಷಣೆಯ ಅಧ್ಯಯನ ಮಾಡಲು ಪ್ರಯತ್ನಿಸಲಾಯಿತು. ಚಿಎಲ್‌ಸಿವಿಯನ್ನು ಮೆಣಸಿನಕಾಯಿ (ಅರ್ಕಾ ಲೋಹಿತ್), *ನಿಕೋಷಿಯಾನಾ ಗ್ಲುಟಿನೋಸಾ*, *ಎನ್. ಬೆಂಥಾಮಿಯಾನಾ* ಮತ್ತು *ಎನ್. ಟಬ್ಯಾಕಮ್* ಸಸ್ಯಗಳಿಗೆ ಬಿಳಿನೊಣಗಳನ್ನು ಬಳಸಿ ಸೋಂಕಿಸಲಾಯಿತು, ಮತ್ತು ಅವು ವಿಶಿಷ್ಟವಾದ ಎಲೆ ಸುರುಳಿಯ ಲಕ್ಷಣಗಳನ್ನು ತೋರಿಸಿದವು. ಚಿಎಲ್‌ಸಿವಿಯ ಅಣ್ವಿಕ ಪತ್ತೆಯನ್ನು ಬೆಗೊಮೊ ನಂಜಾಣುವಿನ ನಿರ್ದಿಷ್ಟ ಪ್ರೈಮರ್‌ಗಳನ್ನು ಬಳಸಿ ಮಾಡಲಾಯಿತು, ಇದು ೧೪೦೦ ಬಿಪಿಗಳ ನಿರೀಕ್ಷಿತ ಉತ್ಪನ್ನವನ್ನು ತೋರಿಸಿತು. ವೈರಾಣುವಿನ ಅನುವಂಶಿಕ ರಚನೆಯಲ್ಲಿ ಡಿಎನ್‌ಎ ಬಿ ಅನುಪಸ್ಥಿತಿ ಇರುವುದರಿಂದ ಇದನ್ನು ಮೊನೊಪಾರ್ಟೈಟ್ ಬೆಗೊಮೊ ನಂಜಾಣು ಎಂದು ಖಚಿತಪಡಿಸಲಾಯಿತು. ಈ ನಂಜಾಣುವಿನ ಡಿಎನ್‌ಎ ಎ ಹೊಮೊಲಾಗ್‌ನ ಸಂಪೂರ್ಣ ಅನುವಂಶಿಕ ರಚನೆಯ ಅನುಕ್ರಮದ ವಿಶ್ಲೇಷಣೆಯು ಪ್ರಸ್ತುತ ನಂಜಾಣುವು ಚಿಎಲ್‌ಸಿವಿ ಮತ್ತು ಮೆಣಸಿನ ಎಲೆ ಸುರುಳಿ ನಂಜಾಣುವಿಗೆ ನಿಕಟ ಸಂಬಂಧ ಹೊಂದಿದೆ ಎಂದು ತಿಳಿದು ಬಂದಿದೆ, ಇದು ಅನುಕ್ರಮವಾಗಿ ಶೇಕಡಾ ೯೭.೫ ರಿಂದ ೯೯.೨ ಮತ್ತು ೮೫.೪–೯೨.೨ ರಷ್ಟಿದೆ. ಇದು ಫೈಲೋಜೆನೆಟಿಕ್ ವಿಶ್ಲೇಷಣೆಯಿಂದ ಮತ್ತಷ್ಟು ಬೆಂಬಲಿತವಾಗಿದೆ. ಆರ್‌ಡಿಪಿ ೪ ರ ಸಂಯೋಜನೆ ಬ್ರೇಕ್‌ಪಾಯಿಂಟ್ ವಿಶ್ಲೇಷಣೆಯು ನಂಜಾಣುವಿನ ಆಂತರಿಕ–ಪ್ರಭೇದಗಳ ಮರುಸಂಯೋಜನೆಯನ್ನು ಸೂಚಿಸುತ್ತದೆ. ಭೌತ–ರಾಸಾಯನಿಕ ಗುಣಲಕ್ಷಣಗಳ ಲೆಕ್ಕಾಚಾರವು ಚಿಎಲ್‌ಸಿವಿ ಸಿಪಿ ೨೯.೬೬ ಕೆಡಿಎ ಅಣ್ವಿಕ ತೂಕ, ೪೯.೨೦ ರ ಅಸ್ಥಿರತೆಯ ಸೂಚ್ಯಂಕದೊಮದಿಗೆ ಋಣಾತ್ಮಕವಾಗಿ ಚಾರ್ಜ್‌ನ್ನು ಹೊಂದಿದೆ ಎಂದು ತಿಳಿದುಪಟ್ಟಿದೆ ಮತ್ತು ಇದು ಥರ್ಮೋಸ್ಟೆಬಲ್ ಮತ್ತು ಹೈಡ್ರೋಫಿಲಿಕ್ ಎಂದು ತಿಳಿದು ಬಂದಿದೆ. ಚಿಎಲ್‌ಸಿವಿ ಕೋಟ್ ಪ್ರೋಟಿನ್‌ನ ದ್ವಿತೀಯಕ ರಚನೆಯು ಯಾವುದೇ ಅಸ್ತವ್ಯಸ್ತವಾಗಿರುವ ಶೇಷ ಮತ್ತು ಬೈಂಡಿಂಗ್ ಸೈಟ್‌ಗಳಿಲ್ಲದೆ ಅದರ ಉತ್ತಮ ಗುಣಮಟ್ಟವನ್ನು ಬಹಿರಂಗಪಡಿಸಿತು, ಇದು ಪಿಎಸ್‌ಐಪಿಆರ್‌ಎಡಿ ಕಾರ್ಟೂನ್‌ನಿಂದ ಉತ್ತಮವಾಗಿ ಬೆಂಬಲಿತವಾಗಿದೆ. ಪ್ರೋಟೀನಿನ ತೃತೀಯ ರಚನೆಯನ್ನು ಹೋಮಾಲಜಿ–ಆಧಾರಿತ ಮಾಡೆಲಿಂಗ್ ಬಳಸಿ ರಚಿಸಲಾಗಿದೆ ಮತ್ತು ಇದನ್ನು ಪ್ರೋಟೀನ್–ಪ್ರೋಟೀನ್ ಪರಸ್ಪರ ಕ್ರಿಯೆ ಮತ್ತು ಪ್ರೋಟೀನ್–ಲಿಗ್ಯಾಂಡ್ ಸಂವಹನ ಅಧ್ಯಯನಗಳಲ್ಲಿ ಮತ್ತಷ್ಟು ಬಳಸಿಕೊಳ್ಳಬಹುದು.

ನವೆಂಬರ್, ೨೦೨೧ **(ಪಿ. ಎನ್. ಲಕ್ಷ್ಮೀನಾರಾಯಣ ರೆಡ್ಡಿ)**
ಸಸ್ಯರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃ. ವಿ. ವಿ., ಬೆಂಗಳೂರು ಮುಖ್ಯ ಸಲಹೆಗಾರರು

Biological and Molecular Characterization of *Chilli Leaf Curl Virus* Infecting Chilli

C. PREETHI, PALB6303

Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru-560 065

INTRODUCTION

- ✓ Chilli *(Capsicum annuum* L.) belongs to family *Solanaceae* (2n=24) and is an important vegetable and spice crop grown throughout the tropical and subtropical world. Major production of chilli comes from India.
- ✓ Chilli is affected by many diseases, among them viral disease are the major constraints.
- ✓ Leaf curl disease caused by *Chilli leaf curl virus* (ChiLCV) emerged as major constraint for the production of chilli in India
- ✓ ChiLCV belongs to the family *Geminiviridae*, genus *Begomovirus* and has ssDNA (Harrison *et al.*, 1977). It is transmitted by whitefly (*Bemisia tabaci)* (Varma, 1962) in persistent mode.
- ✓ Recombination and pseudo-recombination and re-assortment of genomic components are resulting in emergence of new species and strains of begomoviruses at a very faster rate.
- ✓ In the present study, attempt has been made to characterize ChiLCV isolate infecting chilli

OBJECTIVES

1.Collection, maintainance and confirmation of virus isolate infecting chilli

2.Biological and molecular characterization of ChiLCV isolate

MATERIAL AND METHODS

- ✓ The ChiLCV infected chilli plants were collected from farmers field, Guntur district of Andhra Pradesh . The virus isolate was maintained on chilli variety Arka Lohith, by inoculating the virus through viruliferous whiteflies (Senanayake *et al.,* 2007).
- ✓ Non viruliferous whiteflies were used for acquisition of virus particles from chilli leaf curl infected leaf sample for a period of 24 hr, further these viruliferous whiteflies were released on healthy chilli and *Nicotiana* spp.
- ✓ The extraction of total genomic DNA from the healthy and ChiLCV infected plants was done by CTAB method. The PCR detection and molecular characterization of ChiLCV was done by using below mentioned primers

Sl.no	Primers	Amplicon length(bp)
1	MKBEGF4 MKBEGR5	1400
2	OY2395F OY680R	1200
3	GEMF1223 GEMR2542	1300

- ✓ PCR product obtained from ChiLCV sample was separated by electrophoresis in 0.8 % agarose gel. Fragment with sizes corresponding to the expected amplified sequence was excised from the gel and eluted using the gel purification kit.
- ✓ The sequence of virus isolate under this study was compared with the other sequences of selected *Begomoviruses* obtained from the GenBank database by using Clustal W algorithm, MEGA-7 version and Species Demarcation Tool (SDT).

RESULTS

- ➢ Transmission of ChiLCV isolate from chilli leaf samples showing symptoms similar to begomovirus infection to Arka Lohith chilli seedlings using viruliferous whiteflies resulted in the development of characteristic leaf curl symptoms after 25 days of post inoculation (plate. 1-A).
- ➢ Further Transmission of ChiLCV isolate by viruliferous whiteflies to *Nicotiana* spp. seedlings resulted in the development of characteristic leaf curl symptoms after 20 days of post inoculation (plate.1-B, C, and D).
- ➢ The PCR detection and full length genome amplification of ChiLCV isolate done by using DNA-A/DNA-A homologue specific primers (Forward and reverse) as mentioned in material and methods such as MK , OY , GEM resulted in amplified products of 1400 , 1200 and 1300bp, respectively (plate .2).
- ➢ The sample gave positive amplification for primers specific to β satellite and alpha satellite
- ➢ Sequence analysis of DNA-A homologue using SDT revealed , the current isolate is having 97.5 to 99.2%, 85.4 – 92.2% and 87.9 – 90.8 % identity with ChiLCV, Pepper leaf curl virus and Tomato leaf curl Joydebpur virus, respectively. This results indicated that the current isolate is ChiLCV (plate.4).
- ➢ The phylogenetic analysis also revealed close clustering of the present isolate with ChiLCV isolates infecting chilli (Plate .5)
- ➢ The virus isolate in the current study is *Chilli leaf curl virus* transmitted by *B. tabaci* and gave positive amplification to sub-genomic components β- satellite and alpha satellite of begomoviruses.

DISCUSSION

- ➢ The begomoviruses emerged as a single major constraint in the production of many crops across the world (Varma & Malathi., 2003).
- ➢ From the past few years chilli was devastated in India due to the begomovirus infection resulting in loss up to 100 per cent (Kumar *et al*., 2012).
- ➢ In the current study, begomovirus isolate infection chilli from Guntur was characterized. The tools used for the characterization of the virus was similar with the previous works (Senanayake *et al.,* 2013). Biological characterization using whitefly transmission and host range, genome amplification and sequencing, sequence analysis using SDT and phylogenetic analysis together with currently existing criteria (Kumar *et al*, 2015) for the species demarcation of begomoviruses has shown, that the isolate in the present study is a *Chilli leaf curl virus.*

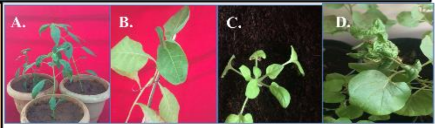

Plate 1: Symptoms of ChiLCV isolate on
A.Chilli B.*Nicotiana tabacum* C.*N.glutinosa*D.*N.benthamiana*

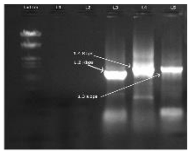

Plate 2: Ladder-1kb marker , L1- water control , L2- healthy control , L3-L5 – ChiLCV infected chilli samples

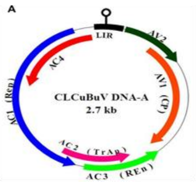

Plate 3: Genome organization of begomovirus

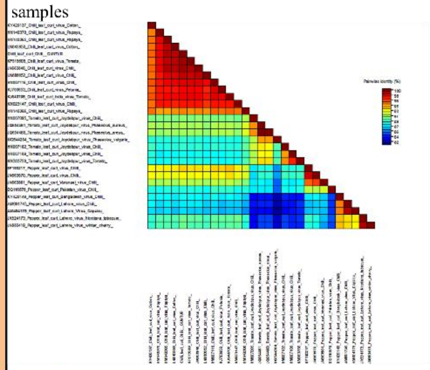

Plate 4:- Identity matrix generated for ChiLCV isolate

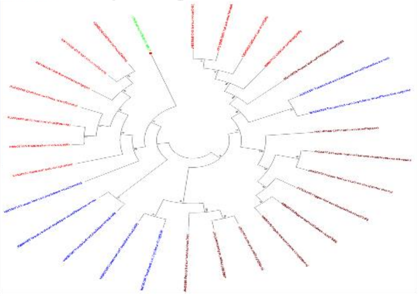

Plate 5: Phylogenetic tree of ChiLCV

SUMMARY

- ➢ ChiLCV isolate was transmitted to chilli, *N. tabacum, N. benthamiana and N. glutinosa* using whiteflies and they produced characteristic leaf curl symptoms
- ➢ Molecular characterization of virus isolate in the current study using the species demarcation tool and phylogenetic analysis revealed it as *Chilli leaf curl virus.*
- ➢ The virus is associated with sub-genomic components β- satellite and alpha satellite

Advisory committee

Chairperson	:	Dr. C. N. Lakshminarayana Reddy
Co – Chairperson	:	Dr. M. Saifulla
Members	:	Dr. N. Nataraja Karaba
	:	Dr. T. L. Mohan Kumar

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I INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important spice crop grown for its fruits, which are used in green as well as ripe dried form for its pungency. Chilli belongs to the genus *Capsicum* and family *Solanaceae*. There are mainly four cultivated *Capsicum* species which are originated from South America. Commercial cultivation of chilli is mostly confined to the tropical regions of the world, since it requires long and warm season for its growth and development. Chilli is known from prehistoric period in Peru and was widely cultivated in Central and South America in early times. It was first introduced to India by Portuguese towards the end of 15th century.

The chilli fruits are rich in capsaicinoids and carotenoids. Pungency is a common feature of chilli and contains unique group of alkaloids called as Capsaicinoids (Hoffman *et al.*, 1983). Capsaicin and dihydrocapsaicin are responsible for 90 per cent of pungency of spices (Kawada *et al.*, 1984). Capsaicinoids are unique to the Capsicum genus, which are produced in glands on the placenta of the fruit and they are odourless, colourless, flavourless, non-nutrient compounds. Capsaicin is a major alkaloid among capsaicinoids (Andrews, 1995), which has wide applications in the food, medicine and pharmaceutical industries (Min *et al.*, 2004). The carotenoids act as dietary precursors for vitamin A, which plays an important role in the regulation of vision, growth and reproduction (Ong and Choo, 1997). In many industries carotenoids from pepper are used as natural colorants. Chilli is a rich source of vitamin A, C and E. Recently, Russian scientists have identified vitamin “P” in green chillies which is considered to be important as it protects from secondary radiation injury (Verghese, 1999). It contains small quantities of protein, fats, carbohydrates and minerals like phosphorus, iron and calcium. Whereas, the ripe and dry chilli contains per 100 g: protein 15.9 g, fat 6.2 g, carbohydrates 31.6 g, fibre 30.6 g, mineral matter 6.1 g, calcium 0.16 g, phosphorus 0.37 g, iron 0.0023 mg, Vitamin C 50 mg and vitamin A 576 mg.

Chilli is cultivated in different parts of the world including India, China, Pakistan Nigeria, Mexico, Indonesia and the Korean Republic. India is the world’s largest producer and exporter of chilli and it contributes 25 per cent of world’s total chilli production. In

India, green chilli grown in an area of 2.87 lakh hectare with a production of 34.06 lakh tones and dry chilli is grown in an area of 8.31 lakh hectares with a production of 18.72 lakh tones (Anon, 2017). Major green chilli producing states in India are Karnataka, Bihar, Chhattisgarh, Madhya Pradesh and Maharashtra, while dry chilli grown in Andhra Pradesh, Telangana, Karnataka, Madhya Pradesh, West Bengal and Orissa. Karnataka is the second largest producer contributing 12 per cent to the total production. Though India has substantial share in the world with respect to area under chilli, productivity was very low as compared to the other chilli growing countries like Korea and Indonesia (2-3 t/ha) (Arun Kumar, 2006); China (6.82 t/ha), Pakistan (2.31t/ha) and Thailand (2.23 t/ha) during 2013 (Geetha and Selvarani, 2017).

In Karnataka, Dharwad, Belgaum, Shimoga, Belgaum, Haveri, Raichur, Gadag, Ballary Kolar, Hassan and Mysore are the major chilli producing Districts. Though the area under chilli cultivation in Karnataka is more, its production was low as compared to the other states because, many biotic and abiotic stresses like viruses, fungi, drought etc. are causing huge yield losses depending on the stage of crop they are affecting.

Chilli leaf curl disease (ChiLCD) causes severe crop loss in the tropical and subtropical regions of the Indian subcontinent and emerged as a major factor limiting chilli cultivation (Chattopadhyay *et al*., 2008; Mishra *et al*., 1963; Senanayake *et al*., 2007, 2013; Varma and Malathi, 2003). The symptoms of ChiLCD-infected plants are leaf curling, leaf rolling, puckering and reduced leaf size; in severe cases, the infected plants remain stunted and fail to bear any fruit, resulting in complete crop loss. Although the first incidence of ChiLCD was reported in the 1960s, its association with begomoviruses was confirmed only recently (Chattopadhyay *et al*., 2008; Khan *et al*., 2006; Kumar *et al*., 2012; Senanayake *et al*., 2007). During the past few years, monopartite begomoviruses and betasatellites associated with ChiLCD have spread to major chilli-growing regions of the Indian subcontinent. Nearly sixty-five (65) plant viruses are so far reported to infect chili worldwide (Devi and Devi, 2020). To date, in India more than 16 different mono and bipartite begomviruses are reported to be associated with ChiLCD, which includes *Chilli leaf curl virus* (ChiLCV), *Chilli leaf curl India virus* (ChiLCINV), *Chilli leaf curl Vellanad virus* (ChiLCVV), *Cotton leaf curl virus* (CLCuV), *Tomato leaf curl Karnataka virus*

(ToLCKV), *Chilli leaf curl Gonda virus* (ChiLCGV), *Chilli leaf curl Kanpur virus* (ChiLCKaV), *Chilli leaf curl Nagpur virus* (ChiLCNV), *Chilli leaf curl Lahore virus* (ChiLCLV), *Pepper leaf curl Bangladesh virus* (PepLCBdV), *Pepper leaf curl Lahore virus* (PepLCLaV), *Chilli leaf curl Ahmedabad virus* (ChLCuAV), *Chilli leaf curl Bhavanisagar virus* (ChiLCBV), *Chilli leaf curl Bijnour virus* (ChLCBiV), *Tomato leaf curl Joydebpur virus* and *Tomato leaf curl New Delhi virus* (ToLCNDV) transmitted by morphologically indistinguishable cryptic species of whitefly (*Bemisia tabaci*) in a persistent circulative manner under natural condition (Khan *et al*., 2006; Senanayake *et al*., 2007; Shih *et al*., 2007, Chattopadhyay *et al*., 2008; Kumar *et al*., 2011; Kumar *et al*., 2012; Kumar *et al*., 2015). In India, diverse agro-climatic conditions and mixed cropping pattern supporting the year-round survival of the whitefly vector responsible for widening and overlapping the host range of begomoviruses reported earlier (Malathi *et al*., 2017).

Viruses belongs to the genus begomovirus (family *Geminiviridae*) causes economically important diseases on many crops throughout tropical and subtropical regions. Begomoviruses are circular single-stranded (ss) DNA viruses comprising either one or two genomic components (DNA-A and DNA-B) of approximately 2.7 kb each and are encapsidated within twined icosahedral virions of 18 x 30 nm in size. Based on genome organization, host range and vector specificity, they are divided into 14 genera, *Begomovirus, Capulavirus, Citlodavirus, Curtovirus, Eragrovirus, Grablovirus, Maldovirus, Mastrevirus, Mulcrilevirus, Opunvirus, Topilevirus, Topocuvirus* and *Turncurtovirus,* and includes more than 525 species (https://talk.ictvonline.org/ictvreports/ictv_online_report/ssdnaviruses/w/geminiviridae). Of these genus begomovirus is the most economically significant and threatening cultivation many crops in world. Based on the presence of one or two genomic components, it has been further divided into either bipartite (DNA-A and DNA-B) or monopartite (genome is homologue of the DNA-A molecule of bipartite begomoviruses). The DNA-A virion-sense strand encodes two open reading frames (ORFs) (AV1 and AV2) and complementary-sense strand encodes four ORFs (AC1, AC2, AC3 and AC4). The DNA-B encodes two ORFs, one at virion-sense strand (BV1) and another BC1 at complementary-sense strand. Both the genomic components (DNA-A and DNA-B) share

~200 nt in a common region (CR), which is present within the intergenic region that includes the replication origin.

Additionally, the monopartite begomoviruses are associated with sub genomic ssDNA molecules known as alpha (DNA 1), beta (Mansoor *et al*., 1999, Nawaz-ul-Rehman and Fauquet, 2009) and deltasatellites (Fiallo-Olivé *et al*., 2010). Betasatellites implicated in symptoms modulation are true satellites, which have approximately half the size (approximately 1.3 kb) of their helper virus and depend on their helper virus for encapsidation and transmission (Briddon *et al*., 2002). The structure of betasatellites is highly conserved comprising of a sequence rich in adenine (A-rich), a sequence conserved between all betasatellites, known as the satellite conserved region (SCR), that contains a predicted hairpin structure with the Nano nucleotide sequence TAATATTAC forming a part of the loop, and a single conserved (between all betasatellites) gene with a capacity to encode an ≥118 amino acid product known as s βC1 (Briddon *et al*., 2003). A class of autonomously replicating satellite-like molecules identified as alpha satellites (approximately 1.3 kb) with circular ssDNA molecules. These are not true satellite they are depending on the virus for transmission, movement, and encapsulation and play role in symptoms attenuation and reduction in accumulation of betasatellite in the affected host plants (Briddon and Stanley, 2006; Nawaz-ul-Rehman and Fauquet, 2009).

Delta satellites are true satellites, approximately half the size of betasatellites (~700 nt) completely dependent on the helper viruses for its replication and movement (Fiallo-Olive *et al*., 2010). However, recently it has been recorded the association of satellite molecules with the bipartite viruses infecting different crops in Indian subcontinents (Venkataravanappa *et al*., 2019).

In silico prediction of physico-chemical properties, structure and function of viral proteins has gained its importance in the recent years (Esfandiari and Sefidbakht, 2018). The predicted three-dimensional structure of viral protein has been utilized for interaction studies which provide an insight in to the pathways involved in the process of pathogenesis.

The ChiLCV affects all the growth stages of chilli plants and causes up to 100 per cent yield loss in marketable fruits and sometimes resulting in abandoning the fields prior to harvest. The affected leaves exhibit varied kind of symptoms such as curling, puckering and distortion with blistering of inter-veinal areas and shortening of internodes (Arun Kumar, 2006). The leaf curl complex is one of the most important diseases of chilli in India and causes substantial losses every year from several decades. The disease also threatens the commercial cultivation of chilli particularly during *kharif*. Because of intensive cultivation, use of same varieties and occurrence of different insects, the management of the disease has become a major challenge not only for the farmers, but also for the scientific community.

With this backdrop information, the current study was designed.

Objectives:

1. Biological transmission of *Chilli leaf curl virus* isolate using whitefly, *Bemisia tabaci* to select host plants.
2. Complete genome amplification, sequencing and phylogenetic analysis of *Chilli leaf curl virus* infecting chilli.
3. Detection of recombination events in *Chilli leaf curl virus.*
4. *In silico* analysis of coat protein of *Chilli leaf curl virus.*

II REVIEW OF LITERATURE

2.1 Importance of Chilli leaf curl disease and its causal agent ChiLCV:

Chilli leaf curl disease is important component of chilli murda, complex viral diseases and insect pests on chilli in India. Early reports suggest that mites (*Polyphagotarsonemus latus* (Banks) and thrips (*Scirtothrips dorsalis* Hood) are responsible for causing curling in chilli plants. Again, Johnpulle (1939) considered it to be caused by both mites and thrips. Further, Amin (1979) concluded that leaf curl in chilli was due to feeding of thrips and mites and not by an infectious agent. Puttarudraiah (1959) reported the involvement of thrips and mites in murda complex in Karnataka. The natural occurrence of tobacco leaf curl was observed on chilli in India by Pal and Tandon (1937) and Seth and Dhanraj (1972).

Chilli is known to be affected by 42 viruses. Twenty-two of them reported to occur naturally and twenty viruses can infect on artificial inoculation. Among the twenty-four viruses reported to occur naturally on chilli, eleven viruses have been reported from India, *viz.*, *Cucumber mosaic virus* (CMV) (Anjaneyulu and Appa Rao, 1967), *Tobacco leaf curl virus* (TLCV) (Jagdale and Ghosh, 2019), *Indian chilli mosaic virus* (Jha and Rayachaudhuri, 1956), *Potato virus Y* (PVY) (Joshi and Bhargava, 1962) *Potato virus X* (PVX), *Tobacco ring spot virus* (TRSV), *Pepper veinal mottle virus* (PVMV), *Pepper vein banding virus* (PVBV) and *Chilli leaf curl virus* (ChiLCV)(Senanayake *et al*., 2007) and *Tomato leaf curl New Delhi virus* (ToLCNDV) (Khan *et al*., 2006 and Ilyas and Khan,1996).

Leaf curl disease of chilli has emerged as a serious problem in the Jodhpur District, the major chilli growing area of Rajasthan state. During December 2004, very high disease incidence (up to 100% of plants) was observed in farmers' fields in Narwa and Tinwari villages. The characteristic field symptoms were upward curling, puckering and reduced size of leaves. Severely affected plants were stunted and produced no fruit. The virus from field samples from Narwa village was transmitted by whitefly (*Bemisia tabaci*) to 50–100 per cent of chilli test plants, which produced vein clearing, curling and stunting symptoms. Electron microscopic examination of field samples revealed few, typical geminate particles. The presence of a begomovirus was confirmed by PCR using the degenerate primers AVF28 5′-GCCCACATYGTC TTYCCNGT-3′and AV29R 5′-GGCTTYCTRTACATRGG-3′, which gave 1.0 kb product. Cloning and sequencing of the PCR product yielded a 995 bp sequence (Acc. No. DQ445255). A BLAST search of GenBank revealed close similarity of the sequence with the intergenic region and part of the replication initiator protein, AV1 and AV2 genes of ChiLCuV-[Pk:Mul]; AF336806) reported from Pakistan (Shih *et al*., 2007). In India, ToLCNDV was recently shown to be associated with chilli leaf curl disease occurring in Lucknow (Khan *et al*., 2006). Two more Begomovirus species, *Cotton leaf curl Multan virus* (CLCuMV) (Akhtar *et al*., 2004) and *Pepper yellow leaf curl Indonesia virus* (PepYLCIDV), have been reported to be associated with chilli leaf curl in Pakistan and Indonesia, respectively.

Sequence analysis showed that the virus isolated from Jodhpur was distantly related (59.1-67.9 % identity) to CLCuMV, PepYLCIDV and ToLCNDV. However, it shared 96.5 per cent identity with ChiLCuV-[Pak:Mul]. Given the close sequence identity with ChLCuV-[Pk: Mul], the virus isolated from Jodhpur is considered to be ChiLCuV. This was the first report of ChiLCuV affecting chilli in India and the recent report of Khan *et al*. (2006) show that chilli leaf curl in India is caused by more than one begomovirus. Chilli leaf curl locally known as murda is a most destructive disease of chilli in India.

2.2 Symptomatology of the Chilli leaf curl disease:

Leaf crinkle or leaf curl complex was observed on chillies by Hussan (1932). The vein clearing and leaf malformation was reported. Curling of leaf margin, reduction in leaf size, vein clearing was observed in India, Sri Lanka and USA (Puttarudraih, 1959). Abaxial curling of the leaves accompanied by puckering, thickening and swelling of the veins were observed by Mishra and Lamba (1929) and Muniyappa and Veeresh (1984). Appearance of most prominent symptoms such as vein clearing followed by veinal distortion, swelling of veins and veinlets on dorsal side were reported.

Appearance of most prominent symptoms such as vein clearing followed by veinal distortion, swelling of veins and veinlets on dorsal side were reported.

2.3 Whitefly, *Bemisia tabaci* (Gennadius):

Whitefly, *B. tabaci* (Hemiptera; *Aleyrodidae*) have been recorded from all the tropical, subtropical and temperate regions of the world (Gonsebatt *et al*., 2012). Amongst the known whitefly species, *B. tabaci* is considered as economically most important insect attacking over 600 plant species. *B. tabaci* is multivoltine and polyphagous and a vector of economically important plant viruses. In the year 1989, it was first collected and described as *Aleyrodes tabaci* (Gennadius) from tobacco (*Nicotiana* spp.) at Greece. It was subsequently renamed as *B. tabaci* (Rusell, 1975).

In India, *B. tabaci* was first recorded in 1905 (Mishra and Lamba, 1929) and its centre of origin was suggested to be the Indian subcontinent because of the presence of a large number of natural enemies against it were from the subcontinent (Fishpool and Burban, 1994).

Firdaus *et al*. (2013) suggests that sequencing a 657 bp portion of mtCOI helped to identify new species of *B. tabaci* and established the new term “cryptic species complex”. On the basis of 3.5 per cent pairwise divergence in mtCOI sequences within *B. tabaci* species, 42 distinct species have been reported; Africa, Asia I, Asia I-India, Asia II 1–12, Asia III, Asia IV, Asia V, Australia, Australia/Indonesia, China 1–5, Indian Ocean, Middle East Asia Minor I-II (MEAM), Mediterranean (MED), MEAM K, New World 1–2, Japan 1–2, Uganda, Italy 1, and Sub Saharan Africa 1–5.

In India recently, phylogenetic analysis using mtCOI gene subunit and Bayesian methods revealed the existence of three cryptic species namely Asia I, Asia II 1, and Asia II 7. Out of these three cryptic species, Asia II 1 was found to be predominant with wide spread distribution across the surveyed regions from cool temperate zones to hot and humid tropical plains. On the contrary, cryptic species Asia II 7 showed localized distribution. There was a significantly high genetic differentiation among these three cryptic species (Prasanna *et al*., 2015). The cryptic species identified from India so far are Asia I, Asia I-India, Asia-1, Asia - 5, Asia- 7, Asia-11, Asia -13, MEAM K, China 3, MEAM1 (Ellango *et al*., 2015).

Asian subcontinent is known for the diversity of *B. tabaci*. The similar kind of diversity analysis of *B. tabaci* from China, which shares major border with India (Lee *et al.*, 2013). This revealed the taxonomic status and the number of species comprising the *B. tabaci* complex based on 1059 nts length mtCOI sequences of *B. tabaci*. The genetic divergence within *B. tabaci* was conspicuously higher (on average, 11.1%), and the results indicates that *B. tabaci* is composed of multiple species that may belong to different genera or subfamilies. A phylogenetic tree reveals that the *B. tabaci* complex is composed of a total of 31 putative species, including a new species. However, genetic divergence within six species (Asia II 1, Asia II 7, Australia, Mediterranean, New World, *and* Sub Saharan Africa 1) was higher than 3.5 per cent (having less than 96.50 per cent similarity), which has been used as a threshold for species demarcation within the *B. tabaci* complex (Dinsdale *et al*., 2010, De Barro *et al*., 2011).

Pakistan, which also shares major border with India, begomoviruses transmitted by *B. tabaci* are major problem in several crops (Varma and Malathi, 2003) and often witnessed spread of whitefly vectored viral diseases across the border. The diversity and geographic distribution of *B. tabaci* cryptic species in Pakistan using mtCOI determined for 285 adult whiteflies found to represent six cryptic species. The most numerous being Asia II-1 and Middle East Asia Minor 1 (MEAM-1), the latter also referred to as the B-biotype, which was confined to Sindh and Punjab province. The endemic Asia I was restricted to Sindh province, while an individual in the Asia II-8 was identified in Pakistan for the first time (Masood *et al*., 2017).

2.4 Transmission of *Chilli leaf curl virus* (ChiLCuV) by *Bemisia tabaci*

The largest and most economically significant groups of plant viruses transmitted by *B. tabaci* are the begomoviruses. The ability of biotype of *B. tabaci* to transmit Geminiviruses has impact on agricultural production (Jyothsna *et al*., 2013).

Hussan (1932) reported that the leaf curl or leaf crinkle occurring on chilli was caused by *B. tabaci*. Mishra *et al*. (1963), Muniyappa and Veeresh (1984) reported the transmission of chilli leaf curl by means of whitefly (*Bemisia tabaci*). Inoculated chilli plants showed typical leaf curl symptoms after two-six weeks.

2.5 Crop loss / Importance of chilli leaf curl disease:

Chilli is one of the most valuable cash crops of India. It is a common and widely cultivated spices crop almost all over the world. Chilli is a richest source of vitamin C and A (Howard *et al*., 2000). Andhra Pradesh is the largest producing state of chilly. Indian chillies are mostly exported to Sri Lanka, USA, Nepal, Mexico, Malaysia and Bangladesh. Chilli suffers from a large number of viral, fungal, bacterial, nematode and phytoplasma diseases. Viruses is known to cause different symptoms like mosaic, ring spot, curling, yellowing etc. on chilly and these symptoms result heavy economic losses of about 15billion US Dollar per annum worldwide.

Begomoviruses (family *Geminiviridae*) are the most destructive plant viruses that cause diseases like leaf curl, mosaic, yellow mosaic and yellow vein mosaic in numerous crop plants in the tropical and subtropical world. Leaf curl disease in chili is known to cause by several begomoviruses of which Chili leaf curl virus (ChiLCV) is the most predominant in India (Mishra *et al.,* 1963). ChiLCV is efficiently spread by whitefly (*B. tabaci*) which is abundant year-round in tropical and subtropical climates where wide variety of hosts serves as reservoir (Senanayake *et al.*, 2007). Leaf curl in chili (ChiLCD) is a common disease but recent frequent epidemic outbreaks witnessed repeatedly in the chili growing areas of Central and Southern India is now a growing concern. ChiLCD is the most damaging in terms of yield loss across the cropping regions (Nigam *et al*., 2015). In severe cases, even up to 100 per cent losses of marketable fruit have been reported (Thakur *et al.*, 2018). In absence of epidemiological information so far no definite management strategy has been evolved.

Sastry and Singh (1973) reported that (TLCV) infected plants produced very few fruits when infected within 20 days after planting and resulted in up to 92.3 per cent yield loss. Plants infected 35 and 50 days after transplanting resulted in 74 and 22.9 per cent yield loss, respectively.

2.6 Detection and identification of ChiLCV using molecular techniques – PCR method

Polymerase chain reaction (PCR), cloning and sequencing are considered to be the three basic tools of modern molecular biology. Among these, PCR is versatile in practice and the oldest in theory which was proposed by H. Ghobind Khorana and his colleagues in 1970s (Kleppe *et al*., 1971). At that time genes were neither sequenced nor thermostable polymerases were identified and synthesis of oligonucleotide primers was not contemplated. Hence, the ideas of Khorana could not become practicable. After a lapse of 15 years the idea was conceived by Kary Mullis and his colleagues of Cetus corporation, Ushyho described *in vitro* amplification of single – copy beta – globin mammalian gene using the Klenow fragment of *Escherichia coli* Polymerase 1 (Saikia and Muniyappa, 1989). However, the use of the thermostable polymerase from *Thermus aquaticus* (Saikia and Muniyappa, 1989) increased the efficiency of PCR and lead to the automation of the method. Now cloning is not the only way to isolate genes and DNA sequencing has become a routine after the arrival of automated sequencers for which asymmetric PCR is the pre-requisite. PCR has become a cornerstone in molecular analysis including the detection of plant pathogens/viruses.

Degenerate oligonucleotide primers for the detection of whitefly transmitted Geminiviruses were designed by Deng *et al*. (1994). These were designed for amplification of an approximately 500 bp fragment of DNA-A of five well characterized whitefly transmitted Geminiviruses in polymerase chain reaction. Thirteen Geminiviruses from the leaf samples and six viruses in single viruliferous whiteflies were detected. Viruses were also distinguished by the pattern of DNA fragments obtained with restriction endonucleases on the PCR products.

Capsicum frutescens (chilli) plants exhibiting leaf curl symptoms, collected from Palampur, Himachal Pradesh region of India, were found associated with a begomovirus and a betasatellite-like molecule. Viral DNA was amplified, cloned and sequenced. The begomoviral genome and the betasatellite consisted of 2775 and 1376 nucleotides respectively. The virus appeared to be monopartite. The genome sequence had <87.9 per cent identity with all other begomovirus sequences, below the threshold for species demarcation, suggesting that the isolate represents a distinct species for which the name Chilli leaf curl Palampur virus (ChiLCPaV) was proposed. Infectious clones consisting of partial tandem repeats of the viral genome (1.9-mer) and the betasatellite (1.7-mer) were constructed in the binary vector pCAMBIA-1300 and agroinoculated to chilli and *Nicotiana benthamiana*. The viral clone did not produce leaf curl symptoms when inoculated alone but the plants were stunted compared to controls. Typical leaf curling and stunting symptoms were observed when the viral clone was inoculated with the betasatellite. In a phylogenetic analysis with other closely related begomoviruses, the new sequence grouped with an isolate of *Papaya leaf curl virus*-[Pakistan:2008] from *Rhynchosia capitata* and *Tomato leaf curl New Delhi virus*-[India:2006] from tomato. The betasatellite showed most identity (94%) with an isolate of *Chilli leaf curl betasatellite* from Pakistan. Using the RDP3 recombination detection program, a recombination event involving *Croton yellow vein mosaic virus*-[India:2007] as a major parent and *Tomato leaf curl Karnataka virus*-[India:2007] as a minor parent was detected in the ChiLCPaV sequence. No significant recombination event was detected in the betasatellite sequence (Kumar *et al.,* 2011).

Among the 14 ToLCV infected samples, comparisons of partial DNA-A sequences amplified with degenerate primers confirmed the existence of several types of begomoviruses. The complete DNA-A sequences of four isolates (Y25, Y41, Y72, Y161) corresponding to the four groups, were determined. Sequence comparisons and phylogenetic analysis revealed that they corresponded to four previously identified begomoviruses. Groups I, II and IV are most closely related to *Tomato yellow leaf curl China virus* (TYLCCNV), *Tobacco curly shoot virus* (TbCSV) and *Tobacco leaf curl Yunnan virus* (TbLCYNV), respectively, while Group III showed close relationships with *Tomato yellow leaf curl Thailand virus* (TYLCTHV). Sequence analysis showed that Y72β, Y77βand Y79β seemed to be different from other characterized DNA betasatellite, sharing the maximum nucleotide sequence identity with DNA betasatellite of TbCSV.

Srivastava *et al*. (1995) isolated the double stranded total DNA from infected tomato plants and amplified using virus specific primer. The amplified product was cloned and sequenced and they reported that either DNA-A (2.75 kb) or DNA-B (2.55 kb) are present. Sequences of cloned DNA-A and DNA-B when used as probes could detect DNA-A/DNA-B in the nucleic acid obtained from fresh diseased tissue. Both DNA-A and DNA-B are needed for infection and they have a common region of 166 bases with about 94 per cent nucleotide sequence homology, a characteristic of all bipartite Geminiviruses. Comparison of the amino acid sequence of the putative coat protein product of (TLCV) with some other mono- and bipartite begomoviruses revealed a maximum of 86 per cent homology with *Indian cassava mosaic virus* (ICMV).

DNA extracts of tomato plants infected with TYLCV in field conditions and whitefly DNA lysates were amplified with specific primers using PCR. Amplification was highly reproducible and confirmed the presence of TYLCV.

Ramos *et al*. (1996) carried out the PCR amplification using DNA extracts of tomato plants infected with TYLCV. Full nucleotide sequence of the virus was determined. Comparison of sequence data with other isolates revealed that sequence similarity ranged between 85.7 and 97.3 per cent. The ToLCV-Pan has a bipartite genome (DNAs A and B) and computer analysis showed that genome resembles that of other bipartite whitefly-transmitted begomoviruses. DNA-A (2584 nt) and B (2542 nt) have little sequence homology other than within the common region. Homology analysis of DNA-A and B showed that ToLCV-Pan is closely related to *Potato yellow mosaic virus* (PYMV) from Venezuela.

Chakraborty *et al.* (2003) reported that in November 2001, leaf curl disease of tomato manifested by yellowing of leaf lamina, upward leaf curling, leaf distortion, shrinking of leaf surface, and stunted plant growth was observed in tomato- growing areas in the Varanasi and Mirzapur districts of Eastern Uttar Pradesh, India. Based on the viral DNA sequences, the new begomoviruses isolate was identified as *Tomato leaf curl Gujarat virus*.

Ribeiro *et al*. (2003) reported a large-scale genetic diversity of tomato-infecting begomoviruses. Viruses were detected by polymerase chain reaction (PCR) using universal virus specific primers for the genus, Begomovirus. PCR-amplified fragments were cloned and sequenced. Based on sequence comparisons and phylogenetic analyses, at least seven previously undescribed species of begomoviruses were found. Four of the new viruses were found exclusively in the Southern states, two exclusively in the Northern states, and one was found in both regions. Sequence comparisons reveal strong evidence of recombination among the Brazilian begomoviruses.

(Ong and choo, 1997) amplified, cloned and sequenced replicase protein gene of three isolates of whitefly transmitted begomovirus causing leaf curl disease of tomato in India. Nucleotide sequence and the derived amino acid sequence for the replicase gene of these isolates was determined and analyzed with the published sequences. Phylogenetic relationship clearly indicates two subsets, one belonging to *Tomato leaf curl virus* (ToLCV) having bipartite genome and the other having the monopartite genome.

The complete nucleotide sequence of the DNA-A and DNA-B components for the first time for *Tomato leaf curl Sinaloa virus* (ToLCSinV) was done (Rojas *et al*., 2005). In addition, the complete nucleotide sequence was determined for the DNA-A component of two isolates of *Tomato severe leaf curl virus* (ToSLCV). The genome organization of ToLCSinV and ToSLCV was identical to the bipartite genomes of other begomoviruses described from the America. Phylogenetic analysis of the DNA-A and DNA-B components and their open reading frames indicated that ToLCSinV and ToSLCV belong to different clades: ToLCSinV to the *Abutilon mosaic virus* clade and ToSLCV to the *Squash leaf curl virus* clade. The two Nicaraguan isolates of ToSLCV showed a close relationship with ToSLCV from Guatemala (ToSLCV-[GT96-1] and *Tomato chino La Paz virus* (ToChLPV) but different significantly in the AV1 and AC1 regions. Computer based predictions indicated that recombination with another begomovirus had taken place within AV1 of ToSLCV dividing this species into two strains. A high probability was also found that ToChLPV might have involved in the evolution of ToSLCV.

2.7 Phylogenetic and sequence analysis of *Chilli leaf curl virus:*

All characterized whitefly-transmitted begomoviruses with origins in the New World (NW) have bipartite genomes composed of a DNA-A and DNA-B component.

Recently, an NW begomovirus lacking a DNA-B component associated with tomato leaf curl disease (ToLCD) in Peru, *Tomato leaf deformation virus* (ToLDeV) was reported (Melgarejo *et al*., 2013). Isolates of ToLDeV associated with ToLCD in Ecuador and Peru have a single, genetically diverse genomic DNA that is most closely related to DNA-A components of NW bipartite begomoviruses. Agroinoculation of multimeric clones of the genomic DNA of three ToLDeV genotypes (two variants and a strain) resulted in the development of tomato leaf curl symptoms indistinguishable from those of ToLCD in Ecuador and Peru. Biological properties of these ToLDeV genotypes were similar to those of Old World (OW) monopartite tomato-infecting begomoviruses, including lack of sap transmissibility, phloem limitation, a resistance phenotype in tomato germplasm with the Ty-1 gene, and functional properties of the V1 (capsid protein) and C4 genes.

Together, these results establish that ToLDeV is an emergent NW monopartite begomovirus that is causing ToLCD in Ecuador and Peru (Melgarejo *et al*., 2013). Tomato is widely grown vegetable crop in India and viral diseases are major constraint for its production in the country. The *Chilli leaf curl virus* (ChiLCV) infecting chilli reported from the Indian subcontinent is found to be associated with tomato enation leaf curl disease in tomato growing areas of India. The leaf samples showing enation leaf curl symptoms were collected from tomato fields located in Sonipet (ten infected and one healthy sample) of Haryana State and Varanasi (five infected and one healthy sample) of Uttar Pradesh State, India. Full length genome of begomovirus and associated betasatellite were amplified, cloned and sequenced. The Viral sequences represented in the begomovirus clones showed 89–100 per cent nucleotide sequence identity, suggesting that they represent a single species. Comparisons to sequences available in the databases showed nucleotide sequence identities of 87.3–91.8 per cent for TC-Vns and 87.8–98.4 per cent for TC287 and TC290, with Indian isolates of ChiLCV. The betasatellite sequences obtained had 83.1–94.1 per cent identity with tomato leaf curl Bangladesh betasatellite (ToLCBDB). An analysis for recombinant origin of genome and betasatellite showed major part of their genome was likely to be originated by recombination of begomoviruses infecting different host species resulting in evolution of new recombinant virus. The ChiLCV-tom reported in the current study is another distinct strain of ChiLCV identified in tomato causing enation leaf curl disease in India (Venkataravanappa *et al*., 2017).

Begomoviruses are dicot-infecting, whitefly-transmitted viruses with a genome comprised of one or two molecules of circular, single-stranded DNA. In Brazil, tomato-infecting begomoviruses have emerged as serious pathogens since the introduction of a new biotype of the insect vector in the mid-1990's. *Tomato rugose mosaic virus* (ToRMV) and *Tomato severe rugose virus* (ToSRV) are often found in tomato fields. The complete sequence of the DNA-B components of ToSRV and ToRMV shown an identity of 98.2 per cent. Additionally, the high nucleotide identity (96.2%) between their common regions indicates that these two viruses may share the same DNA-B (Silva *et al*., 2014).

2.8 *In silico* analysis of *chilli leaf curl virus:*

The word *in silico* is derived from Latin referring to silicon in computer chips. The *in-silico* experiments comprise of computer simulations that create model for natural or laboratory processes. Efforts have been made by many scientists to establish computer models of cellular behaviour. Various *in silico* analysis have been carried with respect to plant viruses in the recent past (Kumar *et al*., 2013; Esfandiari and Sefidbakht, 2018).

Sarwar *et al*. (2019) calculated the physico-chemical properties of βC1 protein encoded by *Cotton leaf curl Multan betasatellite* (CLCMulB). The features based on the properties of amino acid is essential and reasonable to compare proteins and study their function.

Protein structure prediction is the inference of the three-dimensional structure of a protein from its amino acid sequence, that is, the prediction of its secondary and tertiary structure from primary structure. The secondary and tertiary structure of *Potato virus X* (PVX) was predicted using I-TASSER and further it was used for molecular dynamic simulation (Esfandiari and Sefidbakht, 2018). Sarwar *et al*. (2019) built the three dimensional structure of βC1 protein encoded by CLCMulB using homology modelling with the aid of SWISS-MODEL web server.

Knowledge of protein three-dimensional structure or tertiary structure (3D) is a basic prerequisite for understanding the function of a protein. The predicted proteins can be further utilized for protein-ligand docking, protein-protein interaction and molecular dynamic studies.

III MATERIAL AND METHODS

Understanding the host – virus-vector interactions are very essential to contain the viral diseases. This study was conducted to understand the biological transmission of the virus and its molecular characteristics. Sequence and phylogenetic analysis was also determined. All the experiments were conducted at the Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru (13.0777^{o} North, 77.5805^{o} East).

3.1 Collection and maintenance of vector culture and virus isolate

3.1.1 Raising and maintenance of Host plants:

Chilli (variety: Arka Lohith) and tobacco species (*Nicotiana tabacum*, *N. glutinosa* and *N. benthamiana*) plantlets were raised in pro-trays filled with sterilized coir pith. After the seedlings attained two-three leaf stage, they were transplanted in to plastic pots containing soil and compost mixture in the ratio of two:one and maintained under greenhouse conditions.

3.1.2 Maintenance of *Bemisia tabaci* whitefly culture:

The culture of non-viruliferous whitefly *B. tabaci* maintained on the cotton (*Gossypium hirsutum* L. cv. Laxmi) plants in the glasshouse at Department of Plant Pathology, UAS, GKVK, Bengaluru was used for all studies. The old host plants were replaced periodically with healthy young plants for the proper maintenance of the whitefly culture periodically (Plate 1).

3.1.3 Collection, maintenance and biological transmission of *Chilli leaf curl virus*

Leaf sample from chilli plants showing symptoms typical to begomovirus infection such as vein clearing, yellowing, leaf curling and leaf puckering was collected from the farmer field at Guntur, Andhra Pradesh. The transmission of virus by *B. tabaci* was carried out as described by (Venkataravanappa *et al.,* 2017). Whiteflies were given acquisition on the infected sample of chilli collected from the field (Acquisition Access Period: 24 h) and were allowed on to feed (Inoculation Access Period: 24 h) on healthy chilli (variety Arka Lohith) and tobacco plants raised and maintained in the glasshouse.

Initially, inoculation cages were prepared from transparent plastic cylindrical tubes with 8 cm in height and 7 cm in diameter by closing with muslin cloth to provide aeration for vectors and avoid the accumulation of excess moisture inside during transmission of virus by *B. tabaci*. A small hole of about 0.5 cm was made at the middle portion of the tubes to aid in release of whiteflies. The non-viruliferous whiteflies maintained in the glasshouse were given 24 h acquisition on *Chilli leaf curl virus* (ChiLCV) infected sample collected from glasshouse-maintained virus culture to make them viruliferous. The prepared inoculation cage was placed over the test seedlings at two-three leaf stage and 10 viruliferous whiteflies were released on to each test plants covered with inoculation cage through the small hole made on the cages. After 24 h of inoculation access period, inoculation cages were removed, the plants were sprayed with the systemic insecticide (Movento (Spirotetramat 11.01% + Imidacloprid 11.01%) @ 0.3 mL per L) and kept for symptom development and maintenance of virus culture for further experiments.

Similarly, the ChiLCV isolate collected from the Guntur was transmitted to the *N. glutinosa*, *N. benthamiana, and N. tabacum* plants raised in the glasshouse and maintained for conducting the further experiments in the study. The observations on days taken for symptom expression, type of symptoms were recorded. Further, their infection the virus was confirmed by PCR detection using begomovirus specific primers.

3.2 PCR detection of *Chilli leaf curl virus*

3.2.1 DNA extraction from *Chilli leaf curl virus* infected plant leaf sample

The total DNA was extracted from both symptomatic and asymptomatic leaf samples of chilli, *N. glutinosa*, *N. benthamiana, and N. tabacum* plants using cetyl trimethyl ammonium bromide (CTAB) method described by Lodhi *et al.* (1994). The detailed procedure followed was,

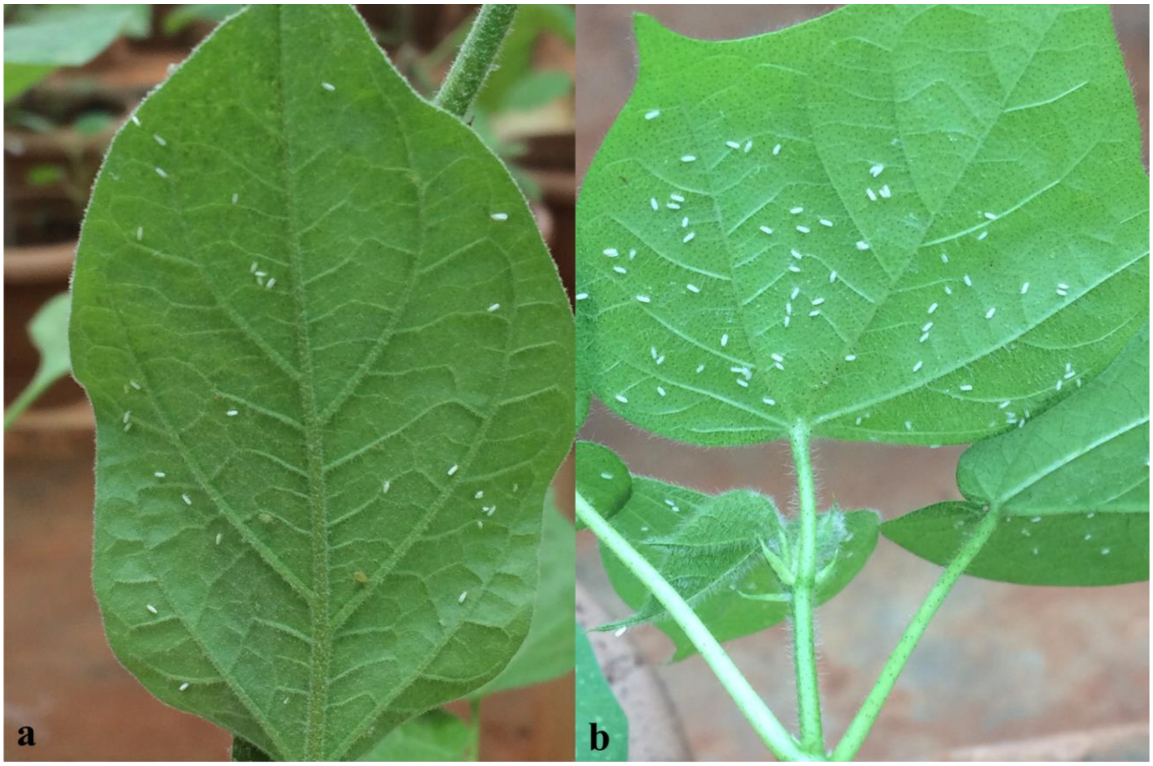


Plate 1: Rearing and maintenance of *Bemisia tabaci* whitefly culture on a) Brinjal (*Solanum melongena* L.) b) Cotton (*Gossypium hirsutum* L. cv. Laxmi).

Plate 2: Leaf curl symptoms on chilli plants (variety Arka Lohith). The chilli plants were inoculated with the *Chilli leaf curl virus* Guntur isolate through whitefly (*Bemisia tabaci*) transmission.

3.2.1.1 Reagents used

a. Preparation of reagent stock solutions

Reagents	For 100 mL volume of distilled water
5M Sodium chloride (NaCl)	29.22 g
0.5M EDTA- Ethylene diamino tetra acetic acid (pH 8.0)	18.61 g
1M Tris HCl	15.78 g
70% (v/v) Ethanol	70 mL ethanol in 30 ml sterile distilled water

b. CTAB buffer preparation

Stock solution	For 100ml of buffer preparation
Distilled Water	57.8 mL
1M Tris HCl (pH-8.0)	10 mL stock solution in 100 mL preparation yields required 100 mM Tris HCl concentration
0.5M EDTA (Ethylene diamino tetra acetic acid, pH-8.0)	4 mL stock solution in 100 mL preparation yields required 20 mM concentration
5M NaCl	28 mL stock solution in 100 mL preparation yields required 1.4 M concentration
CTAB	2g
β- Mercaptaethanol	200µL

3.2.1.2 Protocol for DNA Extraction

1. Approximately 100 mg of virus infected leaf tissue was ground with 1 mL of pre-warmed (65 ºC) DNA extraction buffer (2% CTAB (w/v); 1.4 M NaCl; 20 mM EDTA of pH 8.0; 100 mM Tris-HCl of pH 8.0; 2% polyvinyl pyrrolidone (PVP) and 0.2% β-mercaptaethanol) added *in situ* just before DNA extraction using sterilized mortar and pestle. The whole crude sap was transferred into a fresh 1.5-mL Eppendorf tube and then placed in a water bath for 30 min with occasional stirring.

2. The supernatant (750 µL) was transferred into a new 1.5 mL Eppendorf tube and add equal volume (750 µL) of chloroform: isoamyl alcohol (24:1), vortexed and then centrifuged at 13000 rpm for 10 min using micro centrifuge.
3. The upper aqueous layer was transferred in to a fresh 1.5 mL Eppendorf tube.
4. The DNA was precipitated by mixing with 300 µL of chilled isopropanol and incubated at -20 ºC for at least 1 h.
5. The samples were centrifuged at 13000 rpm for 10 min and the supernatant was discarded.
6. The resulted pellet was washed with 70 per cent (v/v) ethanol (500 µL) by vortexing and dried in a vacuum drier for 10 min.
7. The dried pellet was suspended in 100 µL of TE buffer (10 mM Tris-HCl of pH 8.0 and 1 mM EDTA) of pH 8.0 and stored at -20 ºC.

3.2.1.3 Quantification and quality assessment

Extracted DNA samples were qualitatively monitored by subjecting to one per cent agarose gel electrophoresis. The concentration was spectrophotometrically determined based on optical density value at 260 nm and 280 nm. A value of OD 260 = one corresponded to 50 µg/µL DNA solution. The OD260/OD280 ratio for good quality of DNA extracted should be ranging from 1.8-2.0.

3.3 PCR amplification of complete genome of *Chilli leaf curl virus* (ChiLCV)

To confirm the status of begomovirus associated leaf curl disease of chilli and different tobacco plants, PCR amplifications were carried out using total genomic DNA isolated from chilli and different tobacco as template and primers specific to begomovirus (Venkataravanappa *et al*., 2012**)** and DNA satellites (Briddon *et al*., 2002; Kumar *et al*., 2010). The details of the primers used for this purpose are given below:

Sl. No.	**Genome component**	**Primer pairs used**	**Expected amplicon size**
1	DNA-A	MKBEGF4/ MKBEGR5	1.2 kb
		OY2395F/ OY680R	1.2kb
		GEMF1223/ GEMR2542	1.3kb
2	Alpha satellite	ALPHAF/ ALPHAR	1.4kb
3	Beta satellite	BETA01F/BETA01R	1.4kb

3.3.1 Protocol

1. 0.2 mL PCR tubes were selected, labelled and kept on ice.
2. Reactions included template DNA extracted from healthy and ChiLCV infected plants and water control
3. The 25 µL PCR mixture was prepared by adding the following ingredients into the Eppendorf tube.

Reagents used are listed below,

Reagents	**Volume (µL)**
Template DNA	2.0
Primer-F (20pmol/µL)	0.5
Primer-R (20pmol/µL)	0.5
10 x PCR buffer	2.5
25 mM $MgCl_2$	1.5
2.5 mM dNTP mixture	1.5
Taq polymerase	0.3
Sterile distilled water	16.2
Total	**25 µL**

The DNA template was added at last and tubes were spun briefly. The PCR was performed in a thermocycler (Eppendorf) with the following amplification conditions.

Thermocyclic conditions for amplification of DNA-A and betasatellite of ChiLCV

Steps	Temperature
Step1: Initial denaturation	94 ºC for 5 min
Step 2: Denaturation	94 ºC for 1 min
Step 3: Annealing	55 ºC for 1min
Step 4: Extension	72 ºC for 1.30 min
Step 5: Final extension	72 ºC for 10 min
Step 6: Cooling	4 ºC for ∞
Number of cycles	35

After the completion of the reaction, the products were kept at 4 ºC prior until gel analysis.

3.3.2 Analysis of PCR products by agarose gel electrophoresis

Analysis was performed according to the procedure outlined by Sambrook and Russel (2001).

Procedure

The gel casting tray was cleaned by washing and subsequent wiping with 70 per cent ethanol. Required volume of 0.8 per cent agarose was prepared by melting agarose in 0.5X Tris borate EDTA (TBE) buffer (0.8 g agarose in 100 mL 0.5X TBE buffer) in a microwave oven. Once agarose solution was cooled up to 45 ºC, Ethidium bromide was added at the rate of 0.5 µg/mL of agarose. UV-transparent plastic (UVTP) Gel tray with the desired number of wells was placed in casting tray for closing the ends. After comb was placed in the boat, molten agarose was poured in to the boat without forming air bubbles and allowed 25-30 min for proper solidification (polymerization). The 25 µL of each PCR product was separately mixed with 4.2 μL of loading dye on a clean polythene strip. After removing the gel tray from the casting tray, gel tray was placed in the electrophoresis tank filled with 0.5X TBE buffer maintaining the buffer level 2 mm above the gel slab. The comb was removed carefully. Samples of PCR products were loaded into wells of the gel along with 1 kb DNA ladder and run at 85 V for 1 h until the dye front reached the other end of the gel. Gel was removed from the casting tray and visualized under UV and photographed in a gel documentation unit.

Following electrophoresis, the DNA bands of desired length was excised by using sterile blade under UV-trans illuminators and collected in 1.5 mL tubes and further purified the samples by using gel purification kit (Qiagen gel elution kit; *Cat No*./ID: *28706)* and sent for sequencing.

3.3.4 Sequence comparison and phylogenetic analysis

Sequence similarity searches were done by comparing sequences obtained in the present study to all sequences available in GenBank using Basic Local Alignment Search Tool (BLASTn). Sequences showing the highest identity scores with the chilli isolate were retrieved. Sequence identity matrices for begomovirus isolates were generated using the Bioedit Sequence Alignment Editor (Version 5.0.9; Hall, 1999). Full length genome of chilli begomovirus isolates and other selected genome of begomovirus species were aligned using Clustal W.

DNA Plotter can be used to generate a circular and linear DNA map which displays the regions and features of interest. It is a Java based application in Artemis library and provides information regarding the distribution of guanine (G) and cytosine (C) in the genome. The DNA-A nucleotide sequence in the FASTA format was uploaded to Artemis DNA plotter analysis tool v18.1.0. (http://www. sange r.ac.uk/Softw are/Artemis) (Carver *et al*., 2009). The circular genome with GC-plot was generated with the following parameters; window size- 100, step size-1, graph height-0.7 and track size-0.2.

In order to know the taxonomic position of the current isolate the Sequence Demarcation Tool (SDT) analysis was carried with other selected begomoviruses. A phylogenetic tree was constructed using the neighbour-joining method with 1,000 bootstrap replicates by MEGA X tool (Kumar *et al*., 2018). In order to know the possible recombination events in DNA-A components of begomovirus isolate, split - decomposition tree was constructed with 1000 bootstrap replicates in Split Tree version 4.11.3 with default settings. Further, recombination breakpoints analysis was carried out using Recombination Detection Program (RDP), GENECOV, Max Chi, Chimara, Si Scan, 3Seq integrated in RDP 4. Default RDP settings with 0.05 P-value cut off with standard Bonferroni correction for multiple testing was used throughout the study (Martin, 1987).

3.4 *In silico* analysis of ChiLCV coat protein

3.4.1 Physico-chemical characterization of coat protein (CP) of ChiLCV

The DNA-A nucleotide sequence of ChiLCV was subjected to NCBI ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) and amino acid sequences CP of ChiLCV was obtained and uploaded to the Expasy-ProtParam tool (https://web.expasy.org/protparam/) (Gasteiger *et al*., 2003). In ProtParam tool, physico-chemical properties of a protein such as number of amino acid, molecular weight, theoretical isoelectric point, total number of positively charged residues, the instability index, aliphatic index (AI) and grand average of hydropathicity (GRAVY) were calculated.

3.4.2 Prediction of secondary, tertiary structure and active amino acid residues of ChiLCV-CP

The secondary structure prediction is the base to infer structural properties of an unknown protein, it also helps in predicting the 3D structure. The secondary structure of CP of ChiLCV Guntur isolate was predicted using PSIPRED (http://bioinf.cs.ucl.ac.uk/index.php?id=779) online tool. It incorporates two feed-forward neural networks which perform an analysis on output obtained from PSI-BLAST (Position Specific Iterated - BLAST). Hence, the predicted secondary structure is accurate and reliable. Further, the amino acid sequence of CP was subjected to BLAST analysis against protein data bank (PDB) to find the availability of templates for homology modelling. After confirming the availability of template, the amino acid sequence of ChiLCV Guntur isolate

CP was uploaded to SWISS-MODEL workspace (https://swissmodel.expasy.org/) (Waterhouse *et al*., 2018).

SWISS-MODEL is an automated protein structure homology modelling online tool, accessible via ExPASy web server. The models with the best Z- score value, Global Model Quality Estimation (GMQE) and QMEANDisCo Global statistical parameter were selected. The missing atoms and side chain refinement was carried using ModRefiner online tool (https://zhanglab. dcmb.med.umich.edu/ModRefiner/) (Adiyaman and McGuffin, 2019). To calculate the root mean square deviation between the 3D model obtained from SWISS-MODEL and refined model obtained from ModRefiner the 3D structures were over superimposed in Chimera software (Pettersen *et al*., 2004) using matchmaker tool. Further, to validate the stereo chemical quality of the 3D models in each step of refinement, they were uploaded to SAVES online tool (https://servicesn.mbi.ucla.edu/SAVES/) (Daróczi, 2013) to obtain the Ramachandran plot in PROCHECK. The models with the good quality were visualized in Biovia Discovery Studio 2020 (BIOVIA, 2020) and PyMOL (DeLano, 2009).

IV RESULTS AND DISCUSSION

4.1 Virus transmission assay

The virus from the infected leaf sample from farmer's filed at Guntur, Andhra Pradesh was successfully transmitted to seedlings of chilli (cv Arka Lohith) and tobacco (*Nicotiana tabacum, N. glutinosa and N, benthamiana*) using whitefly, *Bemisia tabaci*. The percentage of transmission by whitefly was recorded to be100 per cent in chilli. The inoculated chilli plants showed typical leaf curl symptoms same as natural infection after 8 - 10 days of inoculation. The initial symptoms appeared as curling of leaves followed by reduction in leaf size (Plate :2 and 3).

4.2 PCR amplification of begomovirus associated with leaf curl disease of chilli

The total DNA was extracted from five chilli samples showing the leaf curl disease symptoms using CTAB method. The DNA concentration was measured using spectrometer before amplification. The average yield of DNA obtained was 1500ng/µL in the final suspension used for PCR confirmation. Initially to know the status of begomovirus associated with leaf curl disease of chilli, the total DNA isolated from of five chilli samples was subjected to PCR amplification using three sets of overlapping degenerative primers OY2395F/OY680R, MKBEGF4/ MKBEGR5, GEMA1215F/ GEMA2542R which resulted in PCR amplicons of 1.2, 1.2 and 1.5 kb in size, respectively in all five samples (Plate 3).

There was no such amplification observed in healthy chilli samples. The amplified products were purified and cloned in to cloning vector (pTZ57R/T) (Thermo Fisher Scientific Inc., PA). The insert in the clones were confirmed by restriction digestion. The recombinant clones were sequenced in both orientations was used for analysis.

4.3.1 Genome organization of *chilli leaf curl virus* (ChiLCV) Guntur isolate

The nucleotide sequences of DNA-A of chilli Guntur isolate were analyzed for prediction of translation products and to know the data on coding regions, predicted protein products, probable function attributed to each product in analogy with other begomoviruses. The complete genome of begomovirus amplified from chilli Guntur isolate was determined to be 2761 nt in length and showed genome typical to the Old World (OW) monopartite begomovirus, with five ORFs such as AV2 (147-503/118/12.98), AV1 (307-1077/256/28.16) in sense and AC3 (1074-1478/134/14.74), AC2 (1219-1623/134/14.74), AC1 (1526-2611/361/39.71) and AC4 (C4 protein, 2161-2460/99/10.89) in antisense sense strand of DNA-A component.

Using the ORF information, circular DNA map of ChiLCV was generated with the aid of Artemis DNA Plotter (Fig. 1). Guanine-cytosine (GC) content refers to the proportion of guanine (G) and cytosine (C) in a given stretch fragment of the genome. The GC plot graph represents the distribution of GC content throughput the genome. Varied GC content was observed in the whole genome. However, the region encoding AC2 protein showed high GC content.

4.3.2 DNA-A genome homologies to begomoviruses

The complete genome (DNA-A component) of chilli Guntur isolate collected from farmer field was compared with sequences of other whitefly transmitted begomoviruses infecting chilli and other crops using Sequence Demarcation Tool (SDT) to calculate the pair wise identity of chilli isolate and other begomoviruses available in the NCBI database.

The SDT analysis showed that Guntur isolate isolated from chilli, shared maximum nucleotide identity of 99.1 to 99.3 % with *Chilli leaf curl virus* (MK161454, KF515609, JN663846). However, the isolate also showed less than 98 per cent nucleotide similarity with other chilli infecting viruses from the different parts of the Indian subcontinents. This result was also supported by a two-dimensional color-coded matrix of pairwise identity scores (Fig.2) generated by Sequence Demarcation Tool (SDT). These identities are above the threshold for defining distinct begomovirus species as per the presently applicable species demarcation criteria for begomoviruses 91 per cent, and within the range for strains (<94%) and variants (>94%) of a species. Thus chilli Guntur isolate showed nucleotide (nt) identity above 94 per cent nt similarity with *Chilli leaf curl virus*. Therefore, Guntur isolate in the current study was considered as a variant of *Chilli leaf curl virus*.

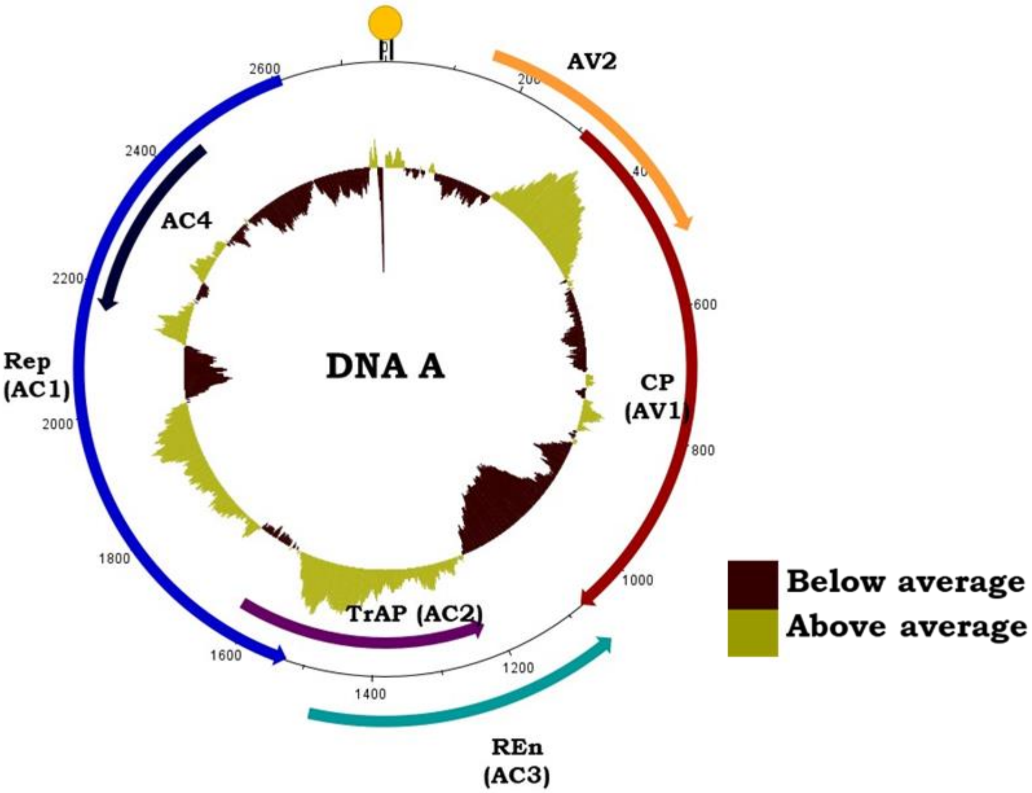


Figure 1: The GC content analysis of the genome of *Chilli leaf curl virus*. The outermost circle represents the nucleotide position in the circular genome. The arrows with different colour code represent the ORFs encoded by the genome, which was deduced with the help of NCBI ORF Finder. The innermost circle represents the GC content above and below average with different colour codes.

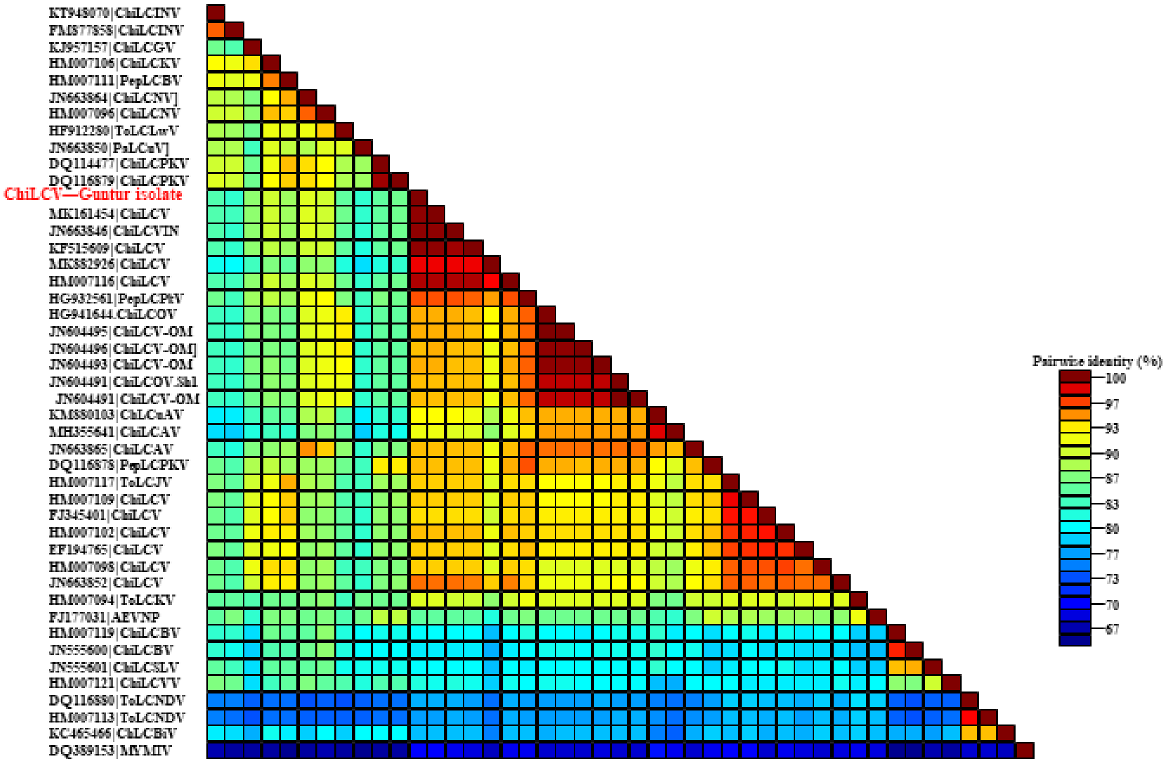


Figure 2: Graphical representation of percentage pairwise genome scores and nucleotide identity plot of full genomes of chilli infecting begomvirus prepared using SDTv2.0 (Species Demarcation Tool) (http://web.cbio.uct.ac.za/SDT)

Plate 3: Leaf curl symptoms on *Nicotiana spp*. a) *N. tabacum* b) *N. glutinosa* c) *N. banthamiana*. The tobacco plants were inoculated with the *Chilli leaf curl virus* Guntur isolate through whitefly (*Bemisia tabaci*) transmission.

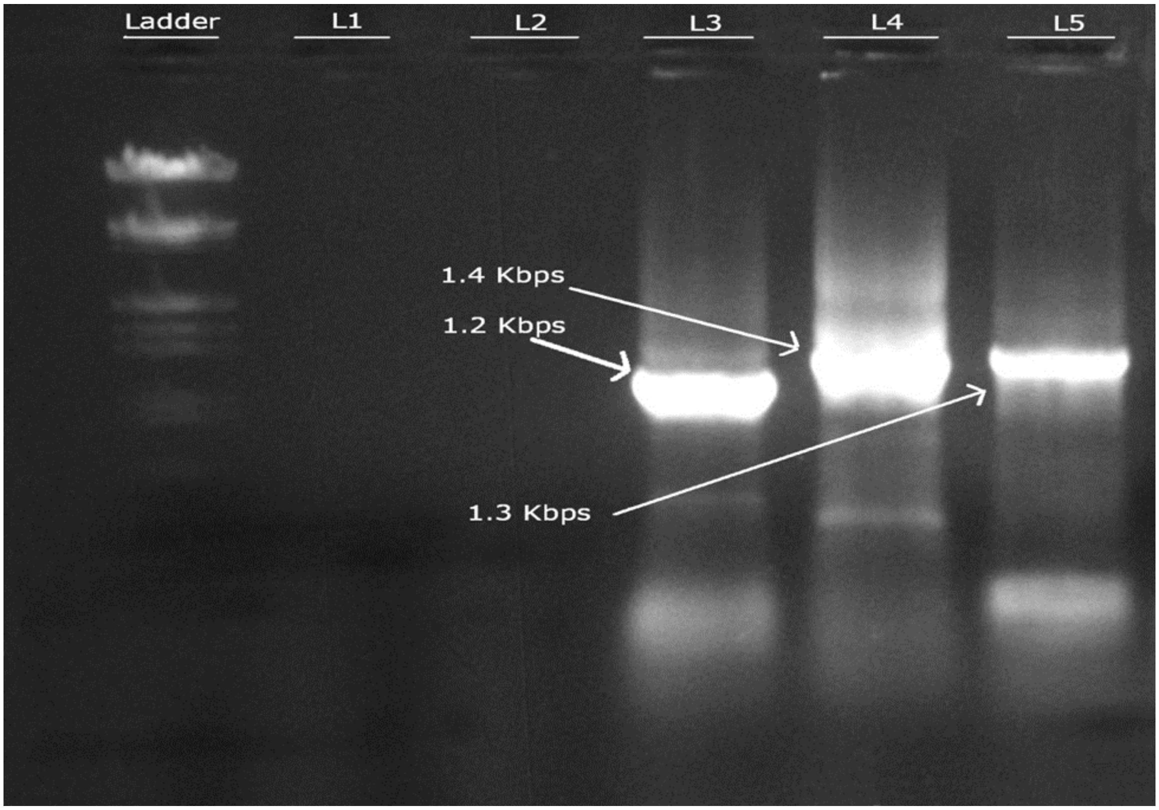


Plate 4: **The DNA isolated from the chilli sample subjected to the PCR using begomovirus specific primers. Agarose gel showing PCR amplified begomovirus specific genome segments.**

4.3.3 Phylogenetic analysis of DNA-A component

The phylogenetic tree was constructed based on nucleotide sequence data of begomovirus isolated from chilli Guntur isolate and other selected begamovirus retrieved from NCBI database. A phylogenetic tree was established through Neighbor-Joining method by taking the 45 representative viral genome sequences such as *chilli leaf curl India virus* (ChiLCV-IN) (11 sequences), *ageratum enation virus* (AEV) (1), *chilli leaf curl Vellanad virus* (ChiLCVV) (1), *tomato leaf curl Karnataka virus* (ToLCKV) (1), *chilli leaf curl Gonda virus* (ChiLCGV) (1), *chilli leaf curl Kanpur virus* (ChiLCKaV) (1), *chilli leaf curl Nagpur virus* (ChiLCNV) (2), *chilli leaf curl Lahore virus* (ChiLCLV), *pepper leaf curl Bangladesh virus* (PepLCBDV) (2), *chilli leaf curl Ahmedabad virus* (ChLCAV) (3), *chilli leaf curl Bhavanisagar virus* (ChiLCBV) (2), *chilli leaf curl Bijnour virus* (ChLCBiV), *tomato leaf curl Joydebpur virus*, *Chilli leaf curl Kanpur virus* (ChiLCKaV), *tomato leaf curl Liwa virus* (ToLCLwV), *pepper leaf curl Pakistan virus* (PepLCPKV) (2), *chilli leaf curl Sri Lanka virus* (ChiLCSLV), *chilli leaf curl Oman virus* (ChiLCOMV), *chilli leaf curl virus from Oman* (ChiLCV-OM) (3), *tomato leaf curl New Delhi virus* (ToLCNDV) (2) by using MEGA X program (Kumar *et al*., 2018) with 1000 bootstrap replicates using *mungbean yellow mosaic India virus* (MYMV)(Acc.No. DQ389153) as out group. The phylogenetic analysis showed the chilli Guntur isolate was closely clustered with already reported ChiLCV isolates (KF515609, MK161454, MK882926, JN663846) in India infecting tomato and chilli with high bootstrap values (Fig 3) and designated it as ChiLCV Guntur isolate.

When individually encoded protein of ChiLCV Guntur isolate was compared with different begomoviruses infecting chilli was retrieved from NCBI database, the pre-coat (AV2), coat protein (CP) and rep (C1) gene, TrAP (C2), REn (C3) and C4 regions showed highest amino acid sequence similarities with ChiLCV-IN (MK882926, MK161454, KF515609, HM007116, JN663852, JN663846) (Table 1). Similarly, the phylogenetic tree was generated by MEGA X programme based on amino acid sequence of begomovirus isolated from chilli and other selected begomovirus retrieved from the NCBI database. The analysis showed that the pre-coat (AV2), coat protein (CP) and rep (C1) gene, TrAP (C2), REn (C3) and C4 region of CHL1 isolate was closely cluster with already reported ChiLCV

Table 1. Pairwise percent nucleotide sequence identities comparisons between the complete genome sequence (DNA-A) and intergenic regions (IR) of the *Chilli leaf curl virus* Guntur isolate from chilli in the current study with other selected begomoviruses available in the databases

Begomovirus species$^{\#}$	Accession No	Host	Location	Genome	IR	Gene (percentage amino acid sequence identity)					
						V2	CP (AV1)	Rep (AC1)	TrAP (C2)	REn (C3)	C4
ChiLCV-IN	MK882926	Chilli	India	96.1	97.9	100	99.2	87.2	83.5	91.7	85.8
ChiLCV-IN	MK161454	Chilli	India	99.3	99.3	100	99.6	98.0	99.2	100	97.9
ChiLCV-IN	KF515609	Chilli	India	99.1	98.9	100	99.6	98.0	98.5	99.2	93.9
ChiLCV-IN	HM007116	Chilli	India	98.1	99.3	100	100	98.0	94.7	98.5	96.9
ChiLCV-IN	JN663852	Chilli	India	91.2	85.9	89.8	96.4	95.5	96.2	93.2	91.9
ChiLCV-IN	JN663846	Chilli	India	99.2	98.6	100	100	98.6	99.2	99.2	96.9
ChiLCV-IN	HM007109	Chilli	India	87.9	74.0	91.5	96.0	90.5	88.8	89.5	82.8
ChiLCV-IN	HM007102	Chilli	India	88.4	80.5	88.9	95.7	90.8	93.2	92.5	82.8
ChiLCV-IN	FJ345401	Chilli	India	87.3	74.0	85.1	96.0	90.3	93.2	88.0	80.8
ChiLCV-IN	EF194765	Chilli	India	86.7	63.1	87.6	95.3	89.4	97.7	88.8	78.7
ChiLCV-IN	HM007098	Chilli	India	87.5	71.6	88.9	94.9	82.5	92.5	88.0	50.5
ToLCKV-IN	HM007094	Chilli	India	84.5	61.0	85.5	97.6	85.0	85.0	77.6	73.7
AEV	FJ177031	Chilli	India	80.2	57.3	84.7	96.4	79.7	80.5	72.3	41.4
ChiLCGV	KJ957157	Chilli	India	81.3	54.1	72.7	90.2	71.9	90.2	82.8	40.7
ChiLCKaV	HM007106	Chilli	India	83.5	53.7	90.6	95.7	77.8	91.0	89.5	31.3
ChiLCNV	JN663864	Chilli	India	84.3	54.4	89.8	97.2	77.5	94.0	86.5	33.3
ChiLCNV	HM007096	Chilli	India	83.6	53.8	88.4	99.2	76.4	94.0	87.3	30.3
ToLCLwV	HF912280	Chilli	Oman	79.6	59.8	85.5	82.4	73.7	94.0	85.2	28.2
PepLCBDV	HM007111	Chilli	Bangladesh	82.0	57.0	88.9	95.3	77.2	89.5	86.5	31.3
ChLCAV	KM880103	Chilli	India	85.2	64.7	73.7	89.8	89.1	94.7	90.2	78.7
ChiLCAV	MH355641	Chilli	India	84.3	64.7	69.4	87.9	85.3	94.7	90.2	78.7
ChiLCAV	JN663865	Chilli	India	88.0	61.6	88.4	98.4	89.1	93.2	86.5	77.7
PepLCPKV	DQ116878	Chilli	Pakistan	85.6	73.1	86.4	95.3	90.5	92.5	82.8	81.8
PepLCPKV	HG932561	Chilli	Pakistan	91.7	71.8	89.2	99.2	89.1	94.7	88.0	80.8
ToLCJV	HM007117	Chilli	India	87.7	72.0	90.6	96.4	90.5	90.2	88.8	81.8

Begomovirus species[#]	Accession No	Host	Location	Genome	IR	Gene (percentage amino acid sequence identity)					
						V2	CP (AV1)	Rep (AC1)	TrAP (C2)	REn (C3)	C4
ChiLCBV	HM007119	Chilli	India	75.0	44.2	70.3	90.6	70.7	67.9	71.6	30.3
ChiLCBV	JN555600	Chilli	India	75.7	38.0	57.6	91.0	70.7	67.1	77.6	34.3
ChiLCSLV	JN555601	Chilli	Sri Lanka	75.6	27.5	56.7	91.0	69.7	68.6	77.6	32.3
ChiLCVV	HM007121	Chilli	India	76.1	43.5	85.5	95.3	71.3	68.6	64.1	31.3
ChiLCPKV	DQ114477	Chilli	Pakistan	79.8	54.1	84.7	95.3	76.1	85.0	76.8	30.3
ChiLCPKV	DQ116879	Chilli	Pakistan	79.9	55.4	85.5	95.7	74.0	84.3	76.8	31.3
PaLCuV	JN663850	Chilli	India	77.9	51.7	84.7	81.2	76.1	82.8	79.1	27.2
ChiLCINV	KT948070	Chilli	India	79.1	50.9	85.9	94.9	74.5	77.0	76.1	21.1
ChiLCINV	FM877858	Chilli	India	78.2	52.2	74.6	96.0	72.2	77.7	76.8	31.3
ToLCNDV	DQ116880	Chilli	India	73.1	36.7	66.1	93.3	77.0	51.4	62.5	38.3
ToLCNDV	HM007113	Chilli	India	72.9	36.4	65.2	92.5	77.2	52.8	65.4	40.4
ChLCBiV	KC465466	Chilli	India	73.2	46.8	83.0	92.1	70.3	52.8	58.8	32.3
ChiLCOMV	HG941644	Chilli	Oman	88.2	70.0	85.9	96.8	89.1	94.0	85.8	80.8
ChiLCOMV	JN604491	Chilli	Oman	87.7	68.0	86.7	95.7	90.0	94.7	85.0	80.8
ChiLCV-OM	JN604493	Chilli	Oman	88.2	69.7	88.9	96.8	89.4	94.0	86.5	79.7
ChiLCV-OM	JN604495	Chilli	Oman	88.2	70.0	86.7	96.8	89.4	93.2	85.8	80.8
ChiLCV-OM	JN604496	Chilli	Oman	88.0	69.3	867	96.8	89.1	94.0	86.5	79.7
MYMIV	DQ389153	Cow pea	India	62.4	24.5	38.6	74.7	69.8	42.6	41.7	56.5

IR- Intergenic region

#The species are indicated as, *Chilli leaf curl virus* (ChiLCV), *Chilli leaf curl India virus* (ChiLCINV), *Ageratum enation virus* (AEV), *Chilli leaf curl Vellanad virus* (ChiLCVV), *Tomato leaf curl Karnataka virus* (ToLCKV), *Chilli leaf curl Gonda virus* (ChiLCGV), *Chilli leaf curl Kanpur virus* (ChiLCKaV), *Chilli leaf curl Nagpur virus* (ChiLCNV), *Chilli leaf curl Lahore virus* (ChiLCLV), *Pepper leaf curl Bangladesh virus* (PepLCBDV), *Chilli leaf curl Ahmedabad virus* (ChLCAV), *Chilli leaf curl Bhavanisagar virus* (ChiLCBV), *Chilli leaf curl Bijnour virus* (ChLCBiV), *Tomato leaf curl Joydebpur virus*, *Chilli leaf curl Kanpur virus* (ChiLCKaV), *Tomato leaf curl Liwa virus* (ToLCLwV), *Pepper leaf curl Pakistan virus* (PepLCPKV), *Chilli leaf curl Sri Lanka virus* (ChiLCSLV), *Chilli leaf curl Oman virus* (ChiLCOMV), *Chilli leaf curl virus from Oman* (ChiLCV-OM), *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Mungbean yellow mosaic Indian virus* (MYMIV).

isolates (KF515609, MK161454, HM007116, KF515609, MK882926, JN663846) from the India (Fig 4).

The IR region of ChiLCV Guntur isolate had more homology (more than 98% nucleotide sequence identity) with IRs of ChiLCV-IN isolates (MK161454, HM007116) (Table 1), the length of intergenic region (IR) was found to be 298 nts and is similar to those of other monopartite begomoviruses reported so for. The IR encompasses an absolutely conserved hairpin structure containing nonanucleotide sequence (TAATATTAC) that marks the origin of virion-strand DNA replication and two repeated sequences known as “iterons” (GGCC) were detected adjacent to the TATA box in ChiLCV Guntur isolate that are recognition sequences for binding of the rep promoter and share significant homology with iterons identified in DNA-A so far (Fig 5 – Fig 11).

4.3.4 Neighbor-net and Recombination analysis of DNA-A sequence

The neighbor-net analysis of complete genome of ChiLCV Guntur isolate along with other selected begomviruses infecting chilli using split tree program indicates that the sequences are arranged in a pattern like network lines and squares structure of phylogenetic incongruence in the begomoviruses suggesting that most parts of the genome of ChiLCV Guntur isolate characterized under this study may have arisen from diverse origins due to recombination (Fig 11). The above data is strongly supported by PHI test as the probability value for the presence of recombination in the genome of the begomovirus isolate is most significant ($P < 0.001$). Further to identify the potential recombination sites present in DNA-A component of ChiLCV Guntur isolate was analyzed using recombination detection program (RDP4), which is integrated with seven different recombination detection methods with a default RDP setting with 0.05 P- value cutoffs throughout and standard Bonferroni correction except that the option ‘Reference sequence selection’ was set at ‘internal references only. RDP analyses showed that the ChiLCV Guntur isolate had the evidence of both intra and inter specific recombination in DNA-A component of ChiLCV Guntur isolate with other selected monopartite begomoviruses. A recombination fragment of 1506 nucleotides was detected in DNA-A of isolate Guntur isolate with major and minor parents resembling *Chilli leaf curl virus* from Oman (ChiLCV-OM, JN604493) and *Chilli*

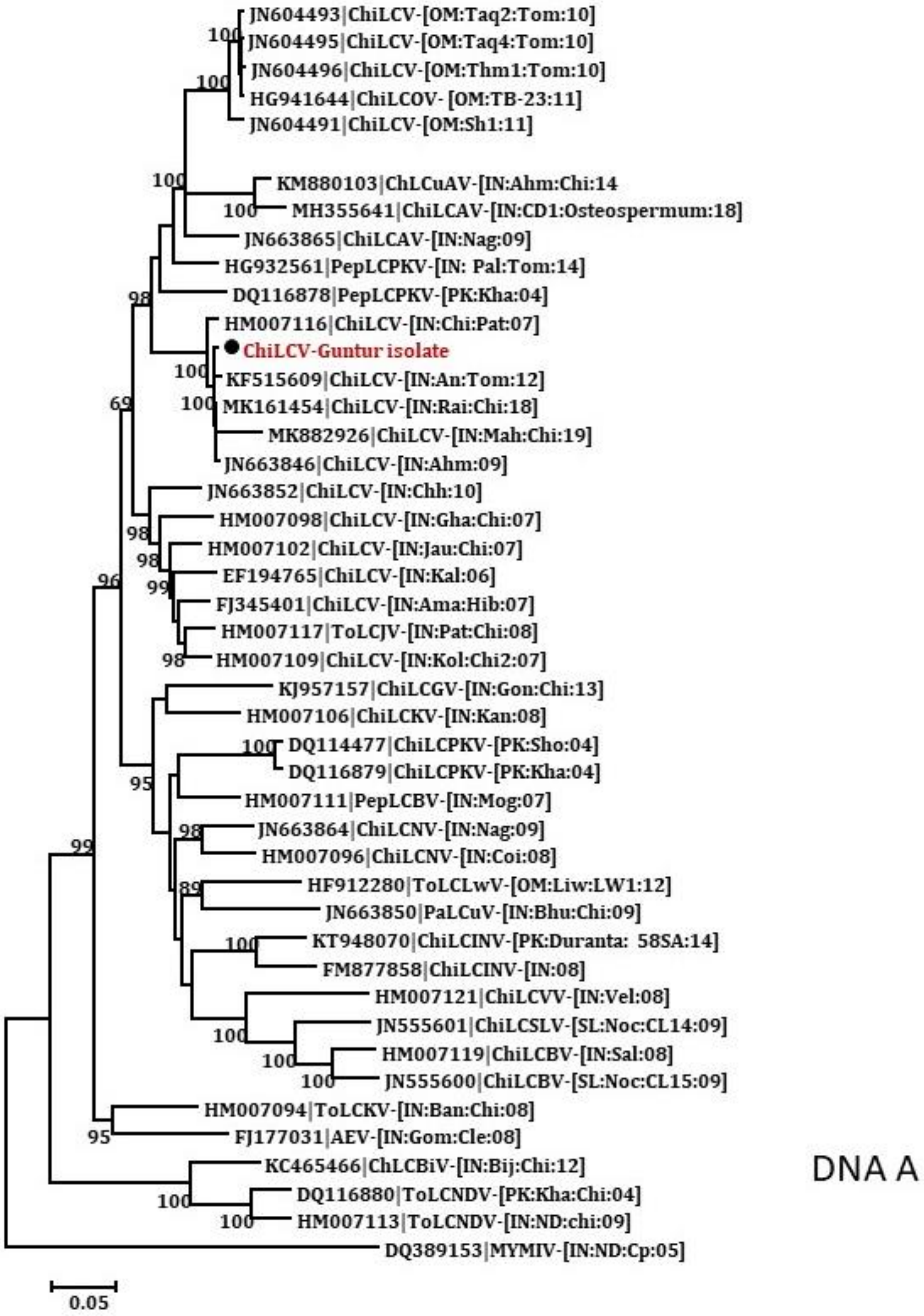


Figure 3: Phylogenetic trees constructed from aligned complete nucleotide sequence of ChiLCV Guntur isolate DNA A genome with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

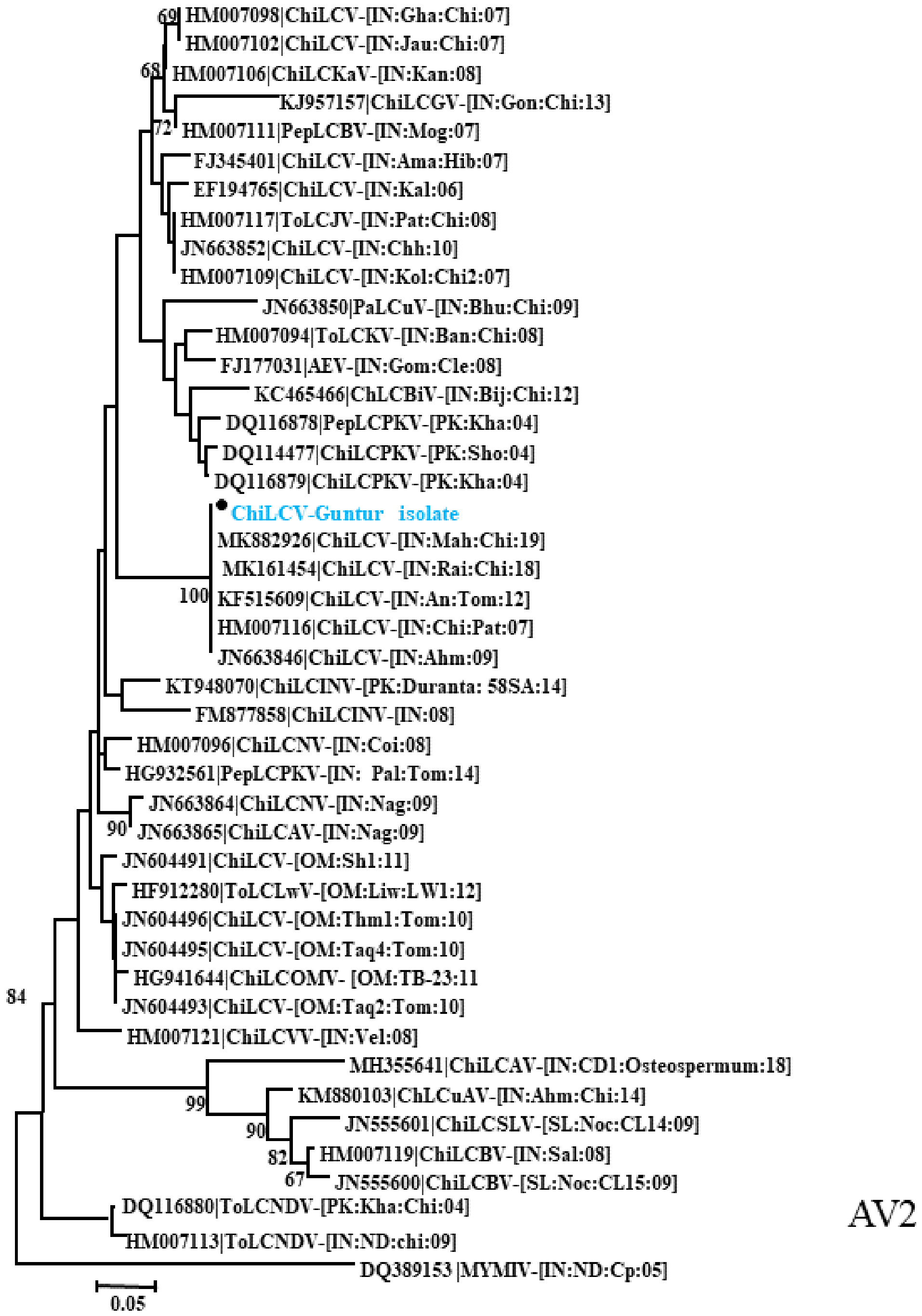


Figure 4: **Phylogenetic trees constructed from aligned amino acid sequence of pre - coat protein region (AV2) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.**

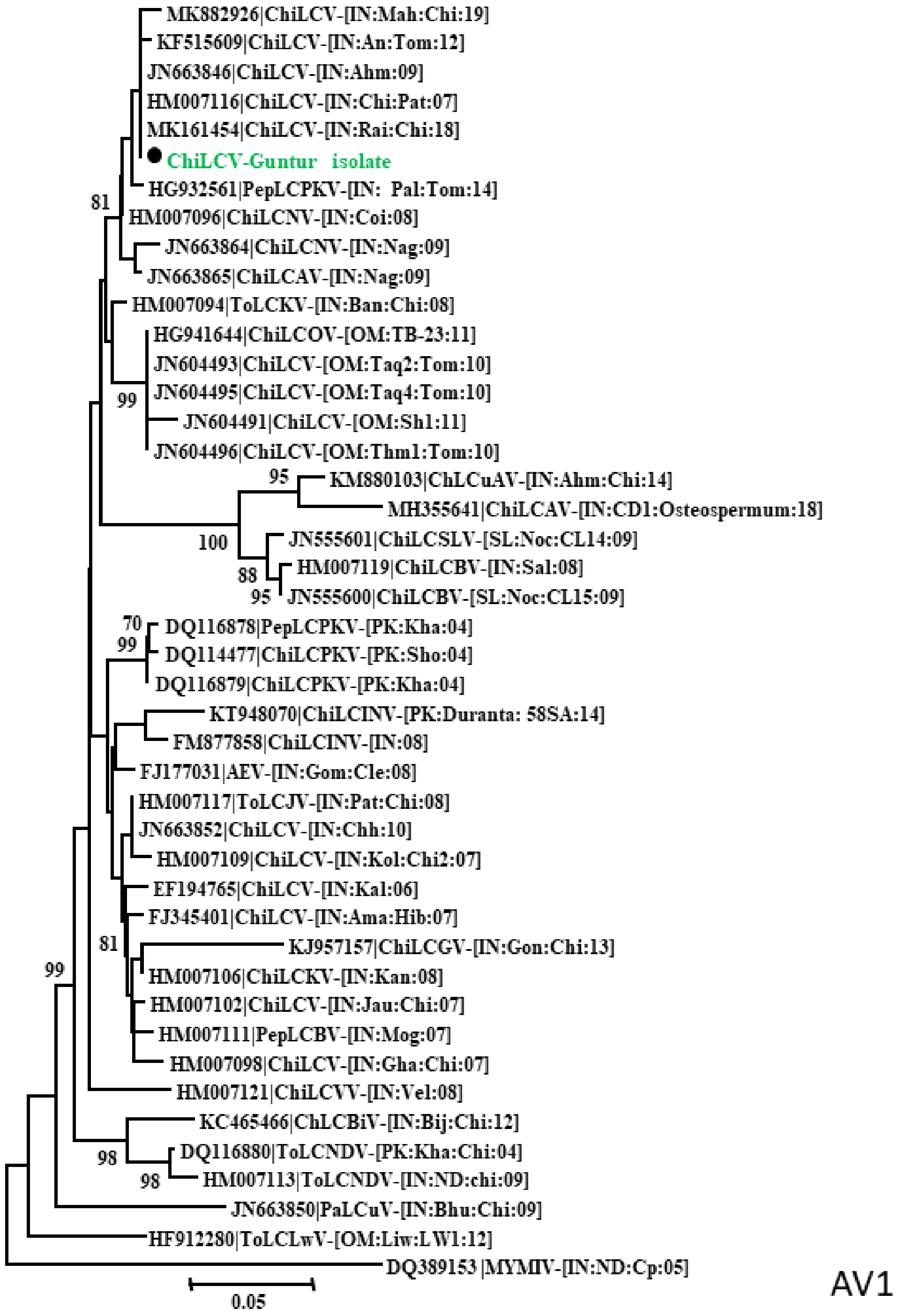


Figure 5: Phylogenetic trees constructed from aligned amino acid sequence of coat protein region (AV1) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

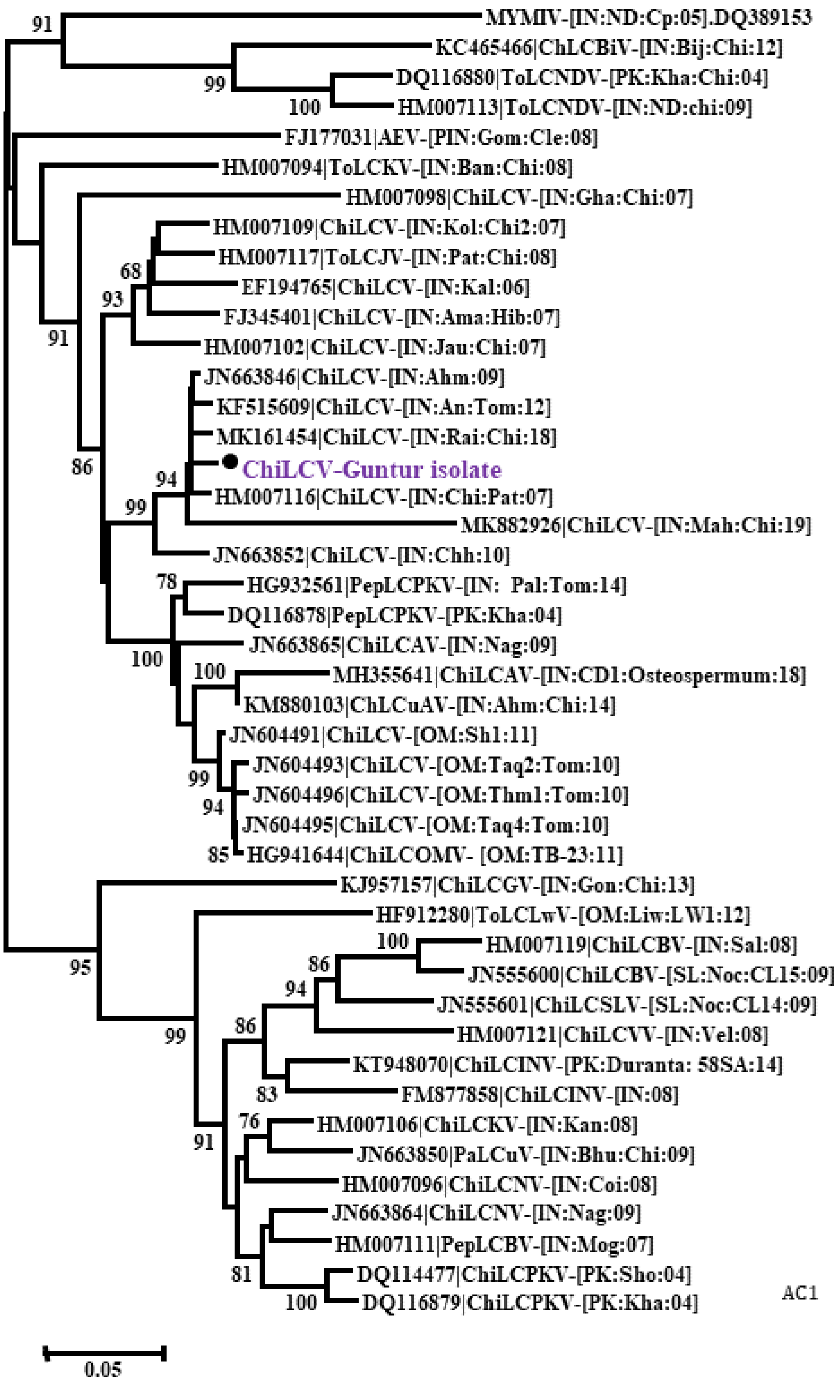


Figure 6: Phylogenetic trees constructed from aligned amino acid sequence of Rep protein (AC1) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

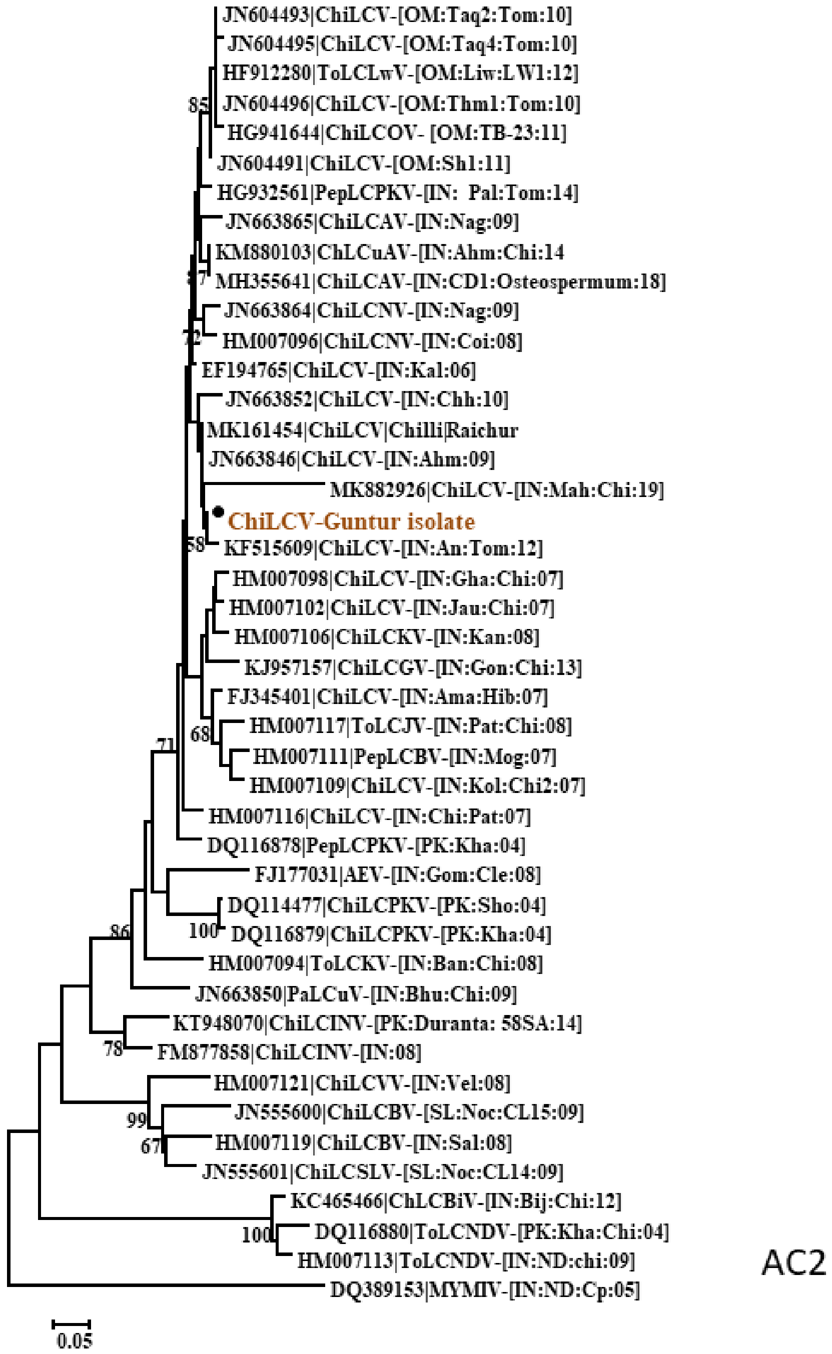


Figure 7: Phylogenetic trees constructed from aligned amino acid sequence of transcription activator protein (AC2) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

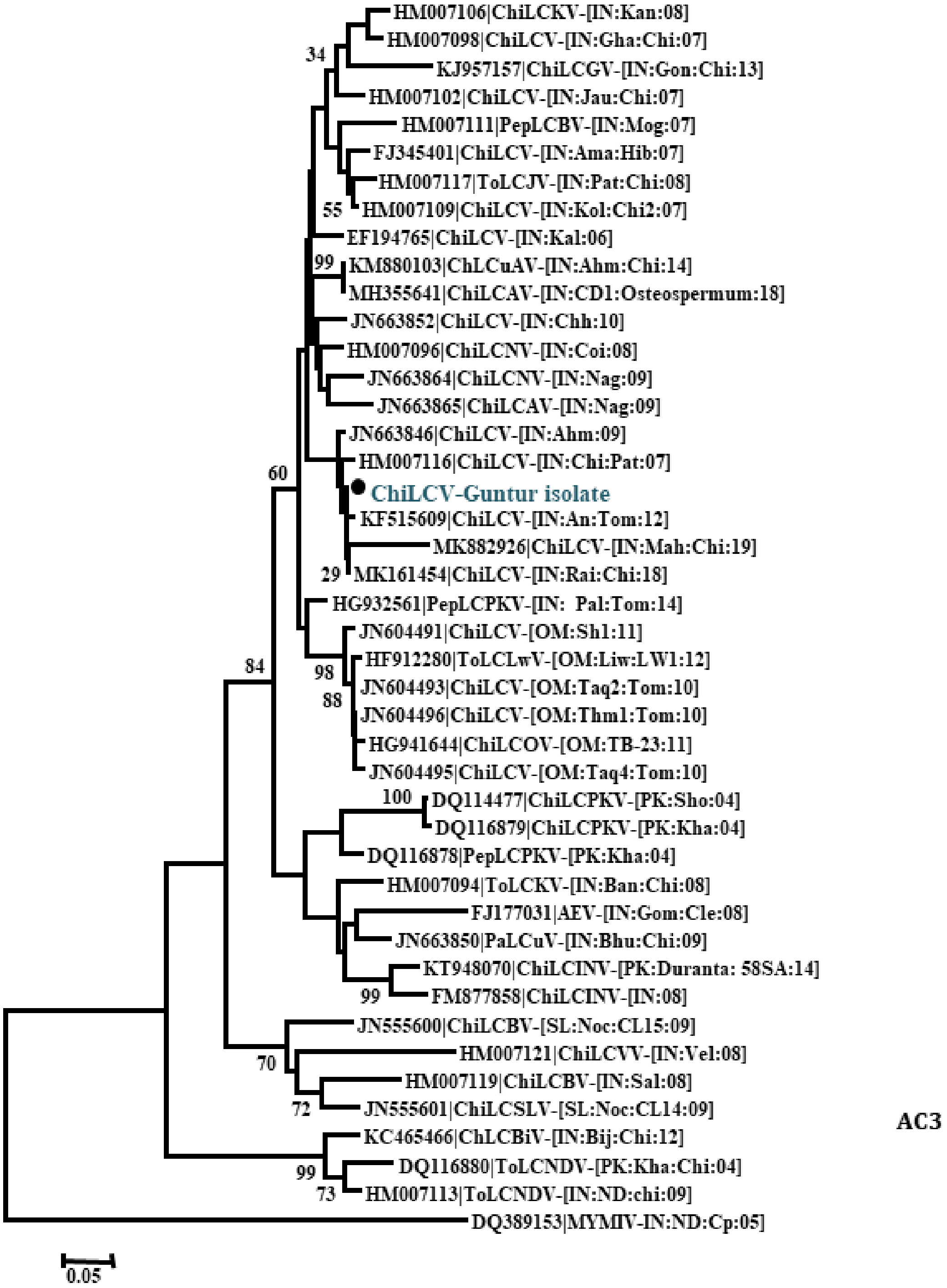


Figure 8: **Phylogenetic trees constructed from aligned amino acid sequence of Replication enhancer protein (AC3) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.**

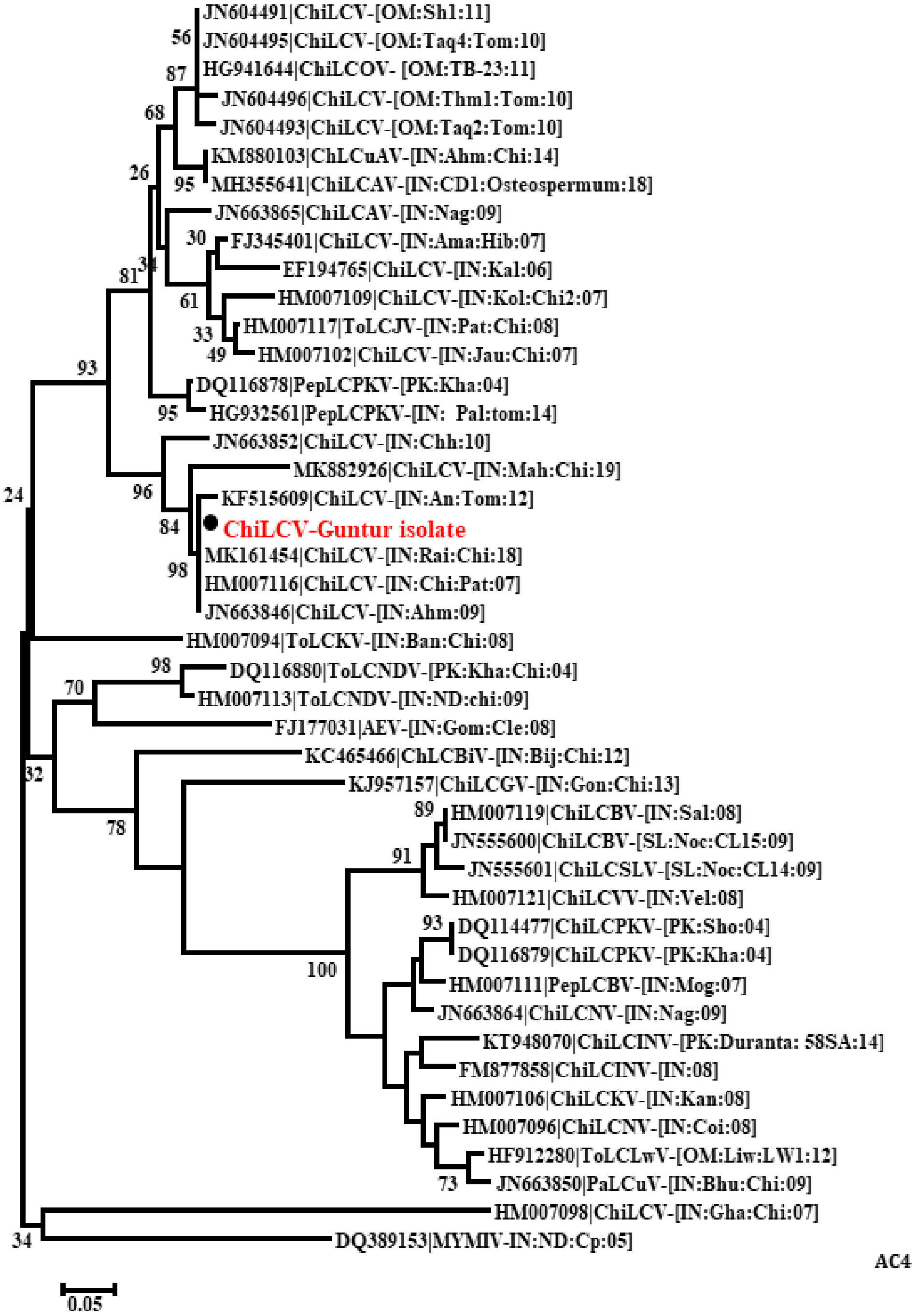


Figure 9: Phylogenetic trees constructed from aligned amino acid sequence of AC4 protein of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances, vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

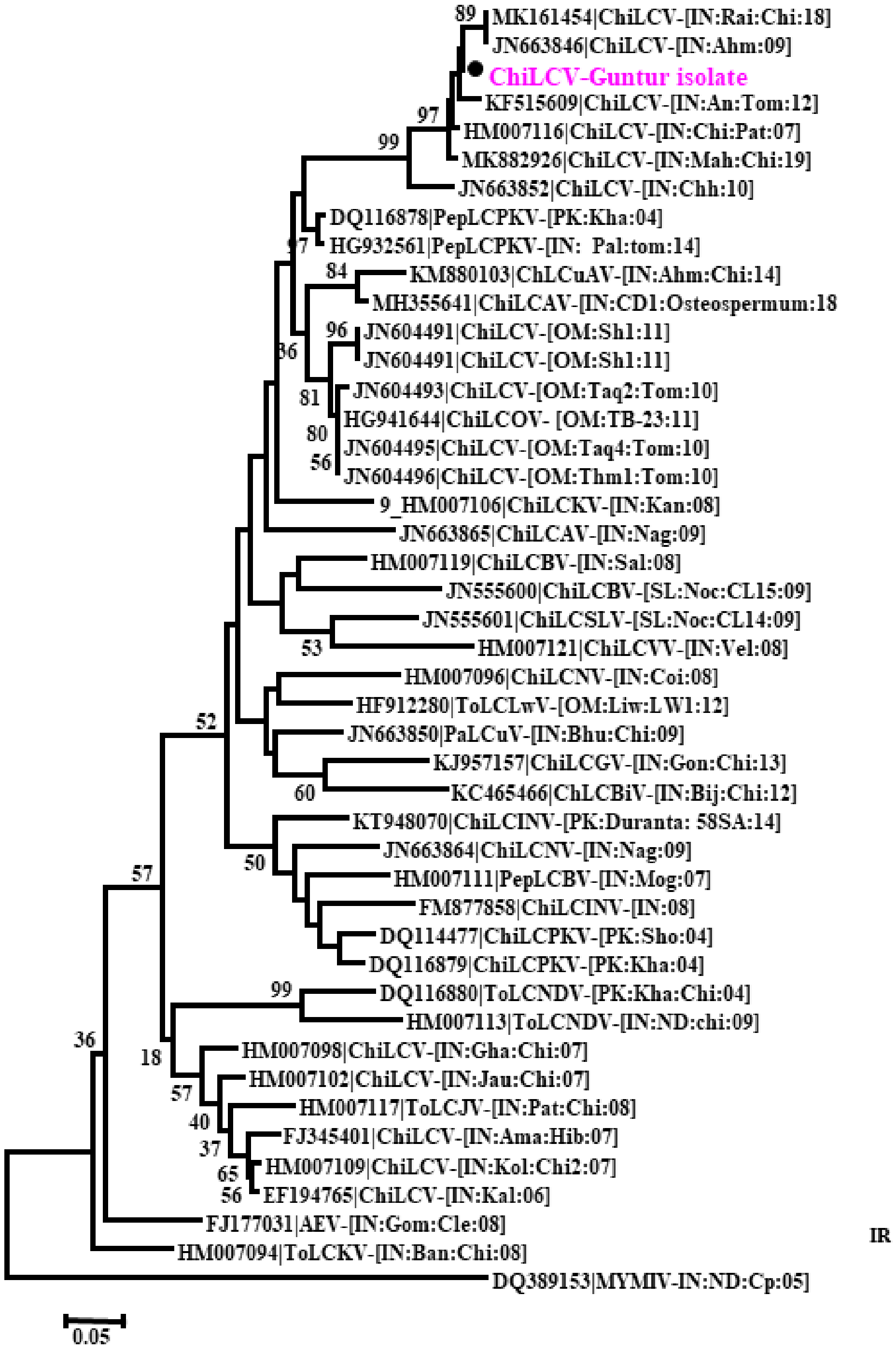


Figure 10: Phylogenetic trees constructed from aligned nucleotide sequence of Intergenic region (IR) of DNA A component of ChiLCV Guntur isolate with other begomoviruses using Neighbor-joining algorithm. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary. The trees are unrooted. A bootstrap analysis with 1000 replicates was performed and the bootstrap percent values more than 50 are numbered along branches.

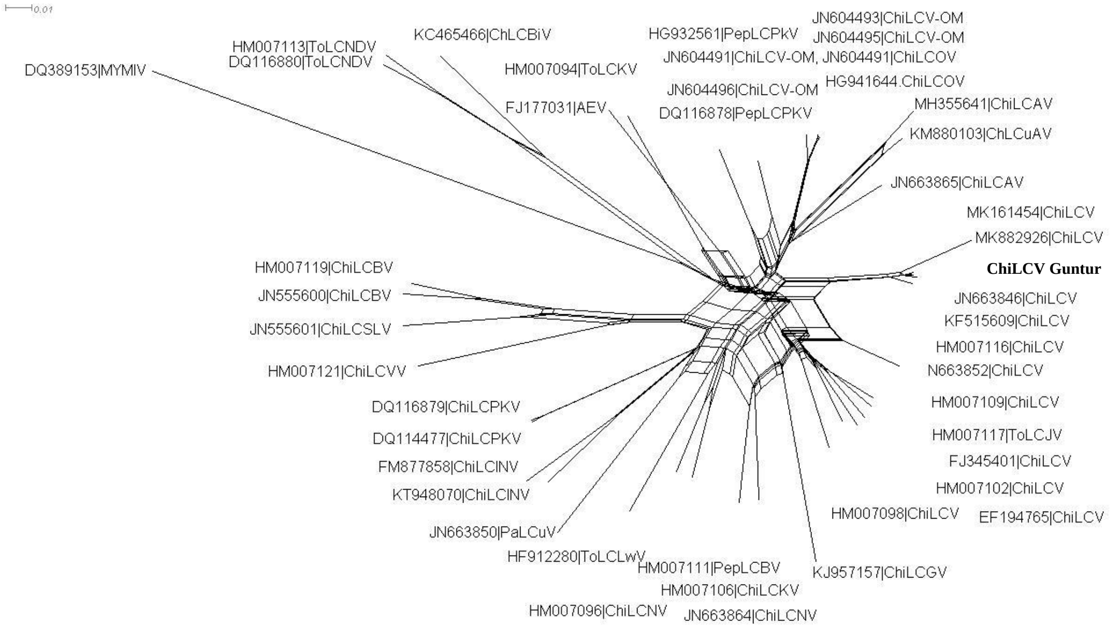


Fig. 11: **Neighbour net and recombination analysis of DNA-A component of ChiLCV Guntur isolate.**

leaf curl virus from India (ChiLCV-IN, EF194765), respectively infecting chilli. The recombinant breakpoints were determined to be 1167 and 2673 nts with average P-value of 1.992×10^{-10}.

Similarly, another recombinant breakpoint of 433 nts was observed in in DNA-A of ChiLCV Guntur isolate with ChiLCKaV (HM007106) and ToLCLwV (HF912280) as major and minor parents, respectively. The recombinant breakpoints were determined to be 1143 and 1576 nts with average P value of 1.00, which were detected in more than two methods implemented in RDP4 as major and minor parents for donors of DNA-A component of ChiLCV Guntur isolate evolution, under Indian conditions.

Recombination is one of the frequent diversification mechanisms in many begomoviruses (Kumar *et al*., 2015) and suggests that it has played a part in the origin of ChiLCV Guntur isolate. The evidence further suggests that, major portion of the DNA sequences has been descended from monoparatite begomoviruses (ChiLCV-OM, ChiLCV-IN, ToLCLwV and ChiLCKaV infecting chilli and tomato crops to make up *chilli leaf curl virus* infecting chilli. These results pinpoint the significant contribution of solanaceous infecting begomoviruses in intra- and inter-species recombination leading to the emergence of ChiLCD-associated begomovirus (Fig 12).

4.4 Physico-chemical characterization of coat protein (CP) of ChiLCV

Physicochemical properties are considered to be essential factors for predicting the function and structure of protein sequences. Hence, the physico-chemical properties of ChiLCV Guntur isolate-CP were computed with the help of Expasy-ProtParam tool. The calculated molecular weight was found to be 29.66 kDa. The isoelectric point (pI) is the pH at which the protein carries zero electric charge, the pI reveals that the protein carries no electric charge at 10.06 pH and this value plays a significant role in protein purification as the solubility is minimal, protein is stable and compact (Sahay and Shakya, 2010). The protein has 21 negatively 44 positively charged residues which indicates, the positive charged nature of the protein. The instability index (II) of protein was also computed and found to be 49.20. If the II is less than 40 then protein is considered to be stable and if the value is more than 40 then the protein is unstable (Guruprasad *et al*., 1990). This classifies the CP protein as unstable.

The aliphatic index (AI) represents the occupancy of aliphatic side chains (alanine, valine, isoleucine and leucine) in a protein (Ikai, 1980). The computed AI for ChiLCV Guntur isolate-CP was 65.78 indicating its high thermostability in wide range of temperatures. The sum of hydropathy values of a protein divided by number of residues in the sequence provides the grand average hydropathy (GRAVY) value and it represents the hydrophobicity of a protein. The positive value indicates hydrophobicity and negative value indicates hydrophilicity. The GRAVY value of ChiLCV CP was found to be -0.586 indicating its better interaction with the water (Table 2).

Table 2: Physico – chemical properties of *chilli leaf curl virus* Guntur isolate coat protein.

Sl. No.	Properties	Value
1	Molecular weight	29.66 kDa
2	Theoretical pI	10.06
3	Total number of negatively charged residues	21
4	Total number of positively charged residues	44
5	The instability index	49.2
6	Aliphatic index	65.78
7	Grand average of hydropathocity (GRAVY)	-0.586

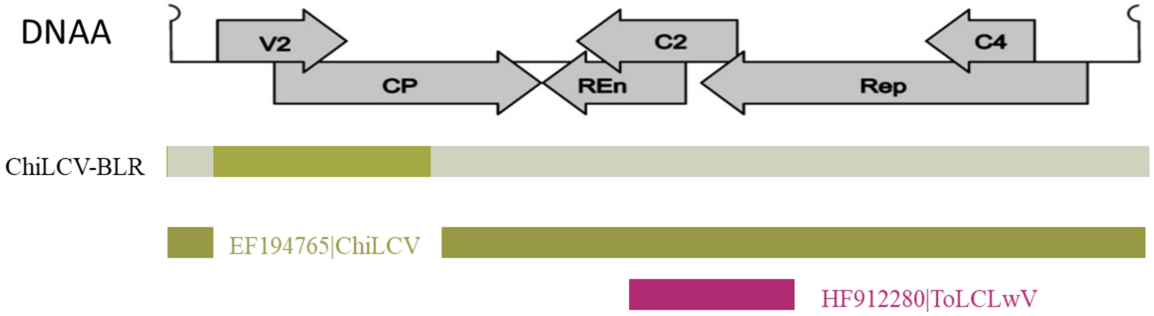


Figure 12: **Analysis of recombination for DNA A isolated from chilli The begomoviruses acronyms given are *Chilli leaf curl virus* (ChiLCV) and *Tomato leaf curl Liwa virus* (ToLCLwV). The box below at the top of the diagram indicates the approximate position recombination is occur in the genome of the begomovirus.**

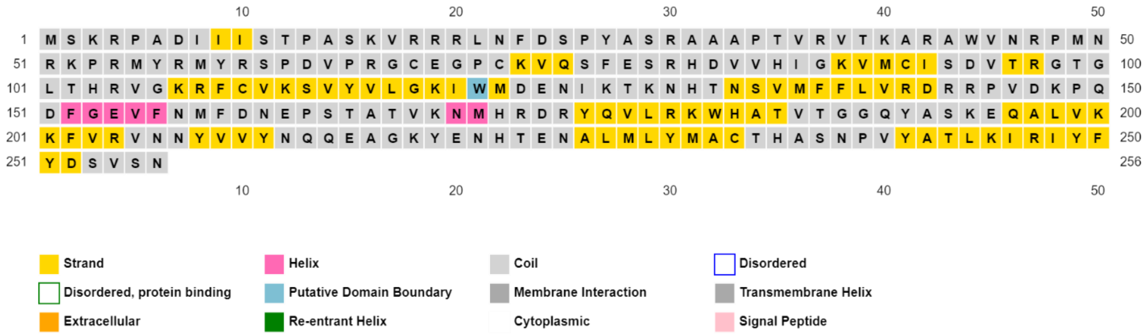


Figure 13: **Sequence annotation plot of *Chilli leaf curl virus* Guntur isolate coat protein. The different colour code represents different properties of amino acid residues.**

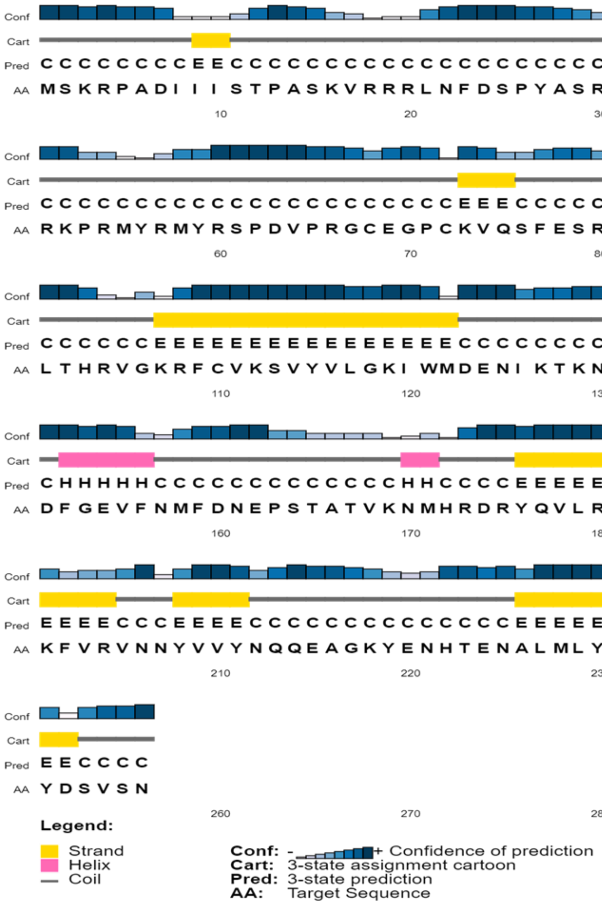


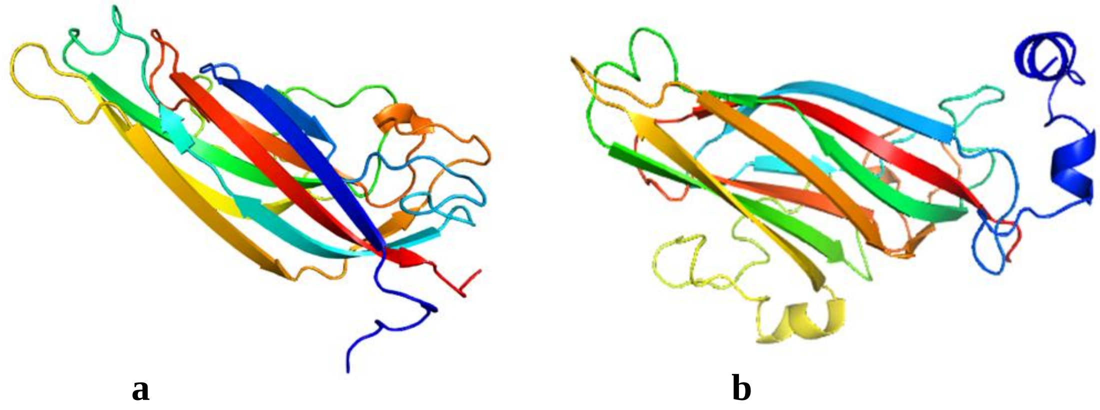
Figure 14: **PSIPRED cartoon of ChiLCV Guntur isolate CP: The diagrams annotate the query sequence with secondary structure cartoons and confidence value at each position in the alignment. The confidence is given as a series of blue bar graphs.**

4.4.1 Prediction of secondary, tertiary structure and active amino acid residues of ChiLCV Guntur isolate CP

The amino acid composition of a protein encodes information regarding folding and formation of stable three dimensional (3D) structure. The 3D structure has a lowest free energy, so, it can bind with other molecules (Deng *et al*., 2018). The secondary structure of ChiLCV Guntur isolate-CP was predicted with the aid of PSIPRED online tool. The sequence annotation plot (Fig 13) represents the annotated residues as per the predicted secondary structures. Through this graph the disordered residues and binding sites can be obtained. However, the CP protein in the current study was found to have good quality with no disordered residue and binding sites which was also well supported by the PSIPRED cartoon (Fig 14).

Homology modelling was employed to generate the 3D structure for ChiLCV Guntur isolate CP. The BLAST analysis of CP amino acid against PDB database confirmed the availability of template for prediction of 3D conformer. The protein with PDB id 6F2S **(**Fig 15**)** having amino acid identity of 82.79 per cent, e value 7e-141 and total score of 375 was used as reference sequence for building the 3D structure in SWISS-MODEL workspace. Three models were generated in the SWISS-MODEL workspace, however, the model with global model quality estimate (GMQE) value of 0.69 and QMEANDisCo global score of 0.79 ± 0.06 was downloaded and used for pre-processing **(**Fig 16**).** The generated model was compared structurally (Fig 17) using chimera and RMSD value of 0.416 was found between the models**.** The GMQE and QMEANDisCo global score value between 0 and 1 represent the model with good quality (Waterhouse *et al*., 2018). This suggests that the 3D model generated is of good quality.

However, other factors such as torsional angle and position of amino acids are also responsible for checking the quality of a model. Hence, Ramachandran plot was generated in PROCHECK. The Ramachandran plot provides information regarding the torsional angles - phi (φ) and psi (ψ) - of the residues (amino acids) contained in a protein. The Ramachandran plot obtained for CP revealed presence of 89.3 per cent amino acid residues in most favoured region, 9.2 per cent in additional allowed region, 0.5 per cent in generously allowed regions and one per cent in disallowed region (Fig 18). According to the Ramachandran plot, a good quality model would be expected to have over 90 per cent of the amino acids in the most favoured region. So, the model obtained from SWISS-MODEL was subjected to loop modelling to rectify the side chains and missing atoms through ModRfiner online tool. The refined model was superimposed with the 3D model obtained from SWISS-MODEL workspace in chimera and found RMSD value of 0.599 (Fig 19). The Ramachandran plot obtained for refined model showed presence of 91.3 per cent amino acids in most favoured region, 6.1 per cent in additional allowed region, two per cent in generously allowed regions and 0.5 per cent in disallowed region (Fig. 20). This model fulfilled all the requirements to be a good quality model.



6F2S

ChiLCV CP

Figure 15: **The three dimensional protein structure of a) template structure (6F2S) b) *Chilli leaf curl virus* Guntur isolate coat protein. The ChiLCV CP was generated with the aid of SWISS-MODEL workspace using 6F2S as a reference model**

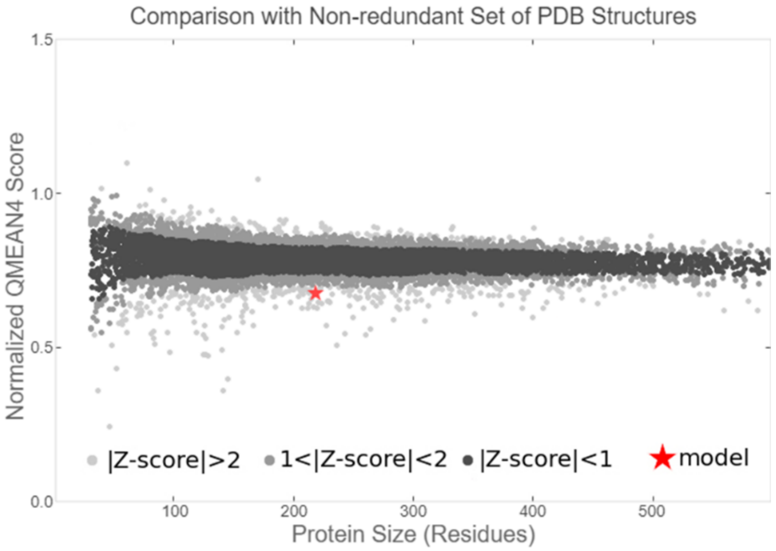


Figure 16: **Comparison plot of ChiLCV Guntur isolate CP: The x-axis shows protein length (number of residues). The y-axis is the "QMEAN" score. Every dot represents one experimental protein structure. Black dots are experimental structures with a "QMEAN" score within 1 standard devation of the mean (|Z-score| between 0 and 1), experimental structures with a |Z-score| between 1 and 2 are grey. Experimental structure that are even further from the mean are light grey. The actual model is represented as a red star.**

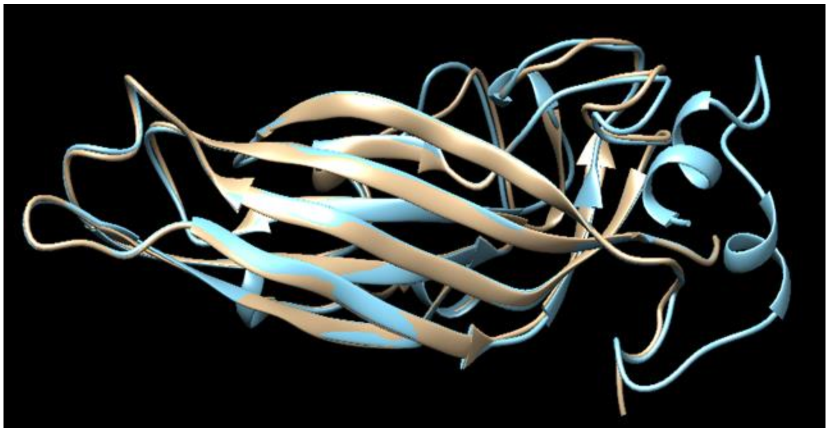
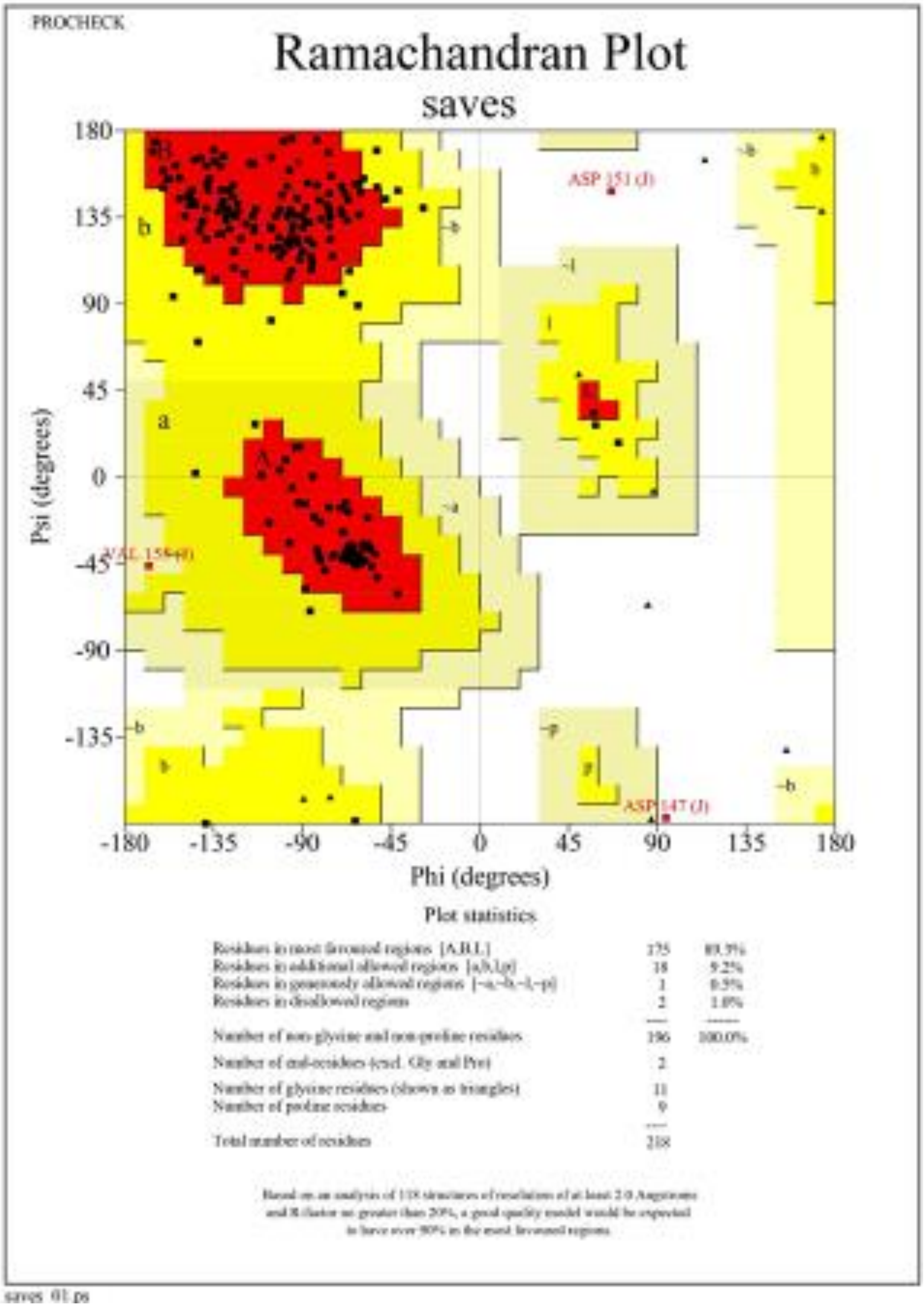


Figure 17: **Superimposition of protein structures of ChiLCV Guntur isolate CP and template 6F2S used for building homology model. The structure in golden yellow colour represents 6F2S and sky blue colour represents ChiLCV CP.**



Plot statistics

Residues in most favoured regions [A,B,L]	175	89.3%
Residues in additional allowed regions [a,b,l,p]	18	9.2%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.5%
Residues in disallowed regions	2	1.0%
	----	------
Number of non-glycine and non-proline residues	196	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	11	
Number of proline residues	9	

Total number of residues	218	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 18: **The Ramacahndran plot analysis of ChiLCV Guntur isolate CP. The homology model obtained from SWISS-MODEL was uploaded to PROCHECK online tool**

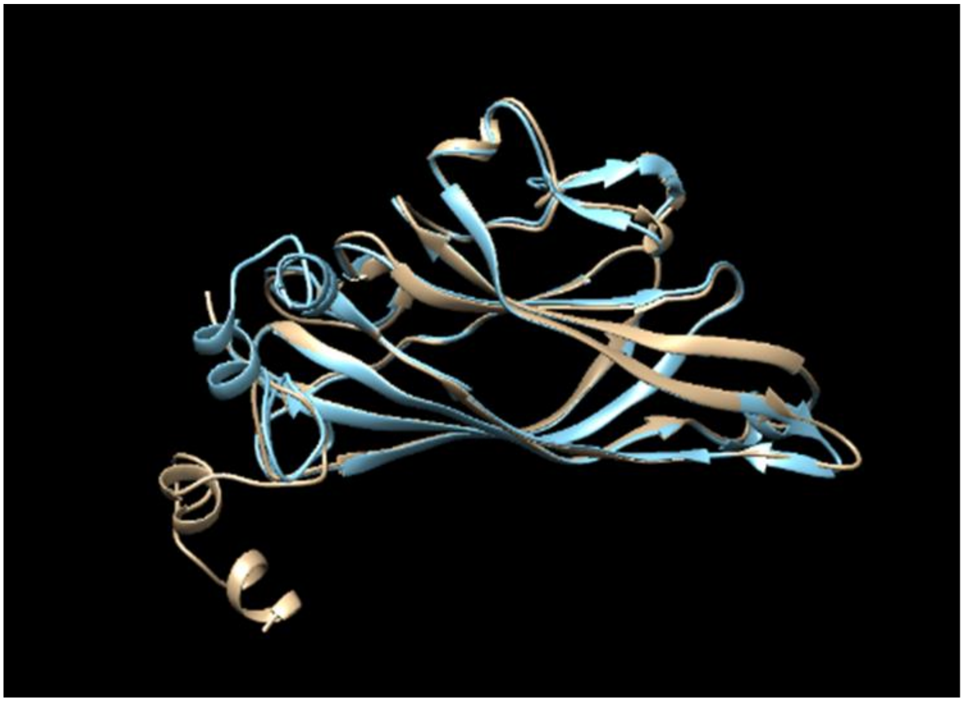
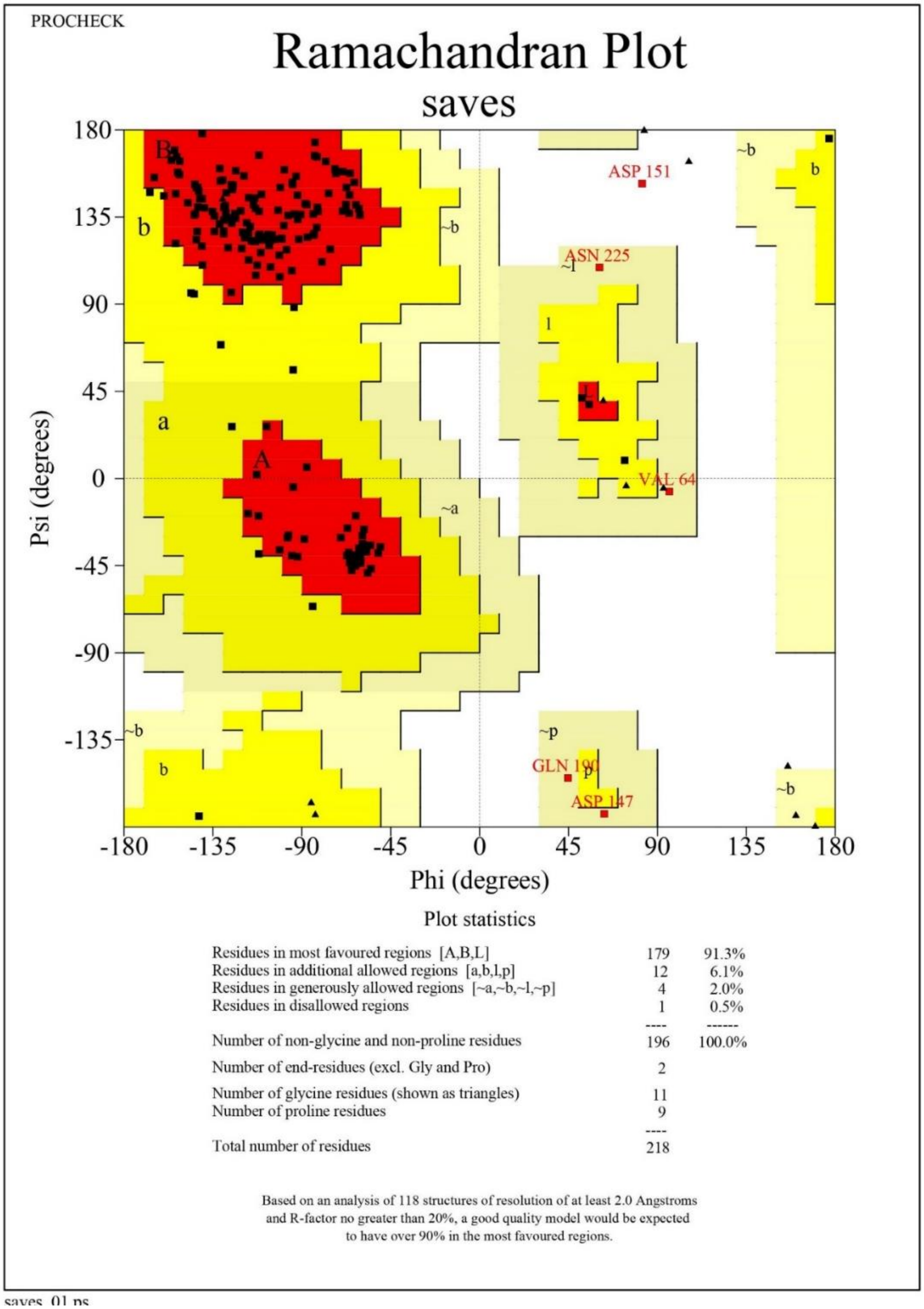


Figure 19: **Superimposition of protein structures ChiLCV Guntur isolate CP and refined ChiLCV CP. The structure in golden yellow colour represents ChiLCV Guntur isolate Guntur isolate CP.**



Plot statistics

Residues in most favoured regions [A,B,L]	179	91.3%
Residues in additional allowed regions [a,b,l,p]	12	6.1%
Residues in generously allowed regions [~a,~b,~l,~p]	4	2.0%
Residues in disallowed regions	1	0.5%
	----	------
Number of non-glycine and non-proline residues	196	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	11	
Number of proline residues	9	

Total number of residues	218	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 20: The Ramacahndran plot analysis of *Chilli leaf curl virus* Guntur isolate refined coat protein. The missing atoms and side chain refinement was done using ModRefiner and subjected to PROCHECK online tool.

V SUMMARY

Chilli (*Capsicum annum* L.) is one of the most important vegetable and spice crop grown intensively in tropics and subtropics. It suffers from various biotic stresses including many viral diseases. Among viral diseases, *chilli leaf curl virus* (ChiLCV) is one of the most widespread and destructive disease on chilli. In this study, biological transmission of ChiLCV to chilli and *Nicotiana spp*, molecular characterization, recombination events detection and *in-silico* analysis of coat protein of *chilli leaf curl virus* was carried out. The results of which are summarized below.

ChiLCV transmitted through whiteflies to chilli (Arka Lohith), *Nicotiana glutinosa, N. benthamiana and N. tabacum* produced typical leaf curl symptoms, indicating that the virus isolate can be transmitted by whitefly and it can infect other hosts in addition to chilli.

Molecular detection of ChiLCV by Polymerase Chain Reaction (PCR) using begomovirus specific primers produced an expected amplicon of 1400 bp in infected leaf samples confirming the virus isolate as begomovirus. The sample gave negative amplification to DNA B, indicating that, the virus isolate is monopartite and the virus isolate was designated as Guntur isolate.

Complete genome sequencing of DNA A homologue and its analysis along with sequences retrieved from NCBI GenBank using Species Demarcation Tool (SDT) for begomovirus revealed that it is having 97.5 to 99.2 per cent, 85.4 – 92.2 per cent and 87.9 – 90.8 per cent identity with ChiLCV, *pepper leaf curl virus* and *tomato leaf curl Joydebpur virus*, respectively. These results confirmed that the current isolate is ChiLCV. This was further supported by phylogenetic analysis. As per the begomovirus species demarcation criteria the current virus isolate is a variant of *chilli leaf curl virus* for which additional descriptor was added as ChiLCV-Guntur isolate.

The recombination events detected using RDP4 revealed that the virus under the current is a recombinant. The results also shown the significant contribution of *solanaceous* infecting begomoviruses in intra- and inter-species recombination leading to the emergence of ChiLCD-associated begomoviruses.

The computation of physico-chemical properties revealed the different functional properties of ChiLCV Guntur isolate coat protein. The ChiLCV CP was found to be negatively charged with the molecular weight of 29.66 kDa. The instability index of 49.20 indicated its unstable nature in *in-vitro* condition. The CP was also found to be thermostable and hydrophilic. The secondary structure of ChiLCV coat protein revealed its good quality with no disordered residue and binding sites which was also well supported by the PSIPRED cartoon. The tertiary structure of the protein was created using homology-based modelling and refined using various bioinformatics tools. It gives us an idea of how a functional protein looks like. The derived 3D structure can be utilized in studies such as protein- protein interaction and protein ligand interactions and molecular dynamic studies.

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