Mysore J. Agric. Sci., 58 (1) : 30-39 (2024)

Cloning and Molecular Characterization of Coat Protein Gene (*CP*) of *Tomato Leaf Curl New Delhi Virus* (G : Begomovirus, F : Geminiviridae) in Ridge Gourd and *In silico* Analysis of the BR1 Family of Coat Protein

Ananya and N. Nagesha
Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : ananya.sinha9416@gmail.com

Authors Contribution

Ananya : Conceptualization, experimentation and analysis of the results;
N. Nagesha : Guidance, Conceptualization, designing the methodology and editing

Corresponding Author :
Ananya

Received : November 2023
Accepted : December 2023

Abstract

Ridge gourd is cultivated in tropical, subtropical and few varieties like Pusa Nasdar and Satputia temperate regions across the world. It is a rich source of essential vitamins and minerals around the world. However, the production of ridge gourd has been severely affected by diseases among which viral diseases have major impact on its production. The viral disease caused by *tomato leaf curl New Delhi virus* (ToLCNDV), a member of the genus *Begomovirus*, family Geminiviridae-an economically important virus affecting the ridge gourd yield. It is transmitted by the whiteflies [*Bemisia tabaci* (Gennadius)] and causes ridge gourd yellow mosaic disease (RgYMD) in ridge gourds. The present study mainly aimed at the process of development of Agrobacterium coat protein gene construct and *in silico* analysis of *CP*. The total DNA from infected leaves of ridge gourd with ToLCNDV was amplified using *CP* specific primers with amplicon size of 771bp. The purified *CP*. gene was successfully cloned to the TA cloning vector. The *CP* gene was further moved into plant expression binary vector pBI121. The gene construct of the plant expression vector was transformed into *Agrobacterium tumefaciens. In-silico* analysis of the coat protein revealed that the protein contains nuclear export factors BR1 family. BLAST results analysis of coat protein with respect to the other coat proteins of ToLCNDV depicted 80-90 per cent resemblance. Phylogenetic tree revealed the common origin of this protein.

Keywords : Ridge gourd, ToLCNDV, *CP* gene, *Agrobacterium tumefaciens*, *In-silico* analysis, BLAST

Ridge gourd [*Luffa acutangula* (L.) Roxb.]-Loofah, (Family: Cucurbitaceae) is a popular vegetable crop in India and other Asian and Western countries like Europe. It contains a surplus amount of fiber, vitamins and minerals including Vitamin B2, Vitamin C, carotene, niacin, calcium, phosphorus, iron and small quantities of iodine and fluorine. Ridge gourd has been used in Indian traditional system as medicines in the form of diuretic, expectorant, laxative, purgative, hypoglycemic agent and bitter tonic. It harbors many phyto chemicals such as flavonoids, oleanolic acid, saponins, luffangulin, sapogenin and cucurbitacin (Manikandaselvi *et al*., 2016).

At present, the emergence of ridge gourd yellow mosaic disease (RgYMD) caused by *tomato leaf curl New Delhi virus* (ToLCNDV) is becoming the major constraint for ridge gourd production. An absolute complete devastation of the crop was observed at the seedling stage of virus infection. ToLCNDV is a member of the begomovirus family and infests various economically important plant species, including weeds, vegetable and ornamental species. The isolates of the virus are transmitted by the whiteflies (*Bemisia tabaci* (Gennadius)). In a study

done on coat protein by Likhith and Peter (2023), on the interaction between Coat Protein (CP) of *Tomato leaf curl virus* (ToLCV) and its host whitefly it was seen that during the process of transmission, CP interacts with the whitefly proteins in the digestive tract, midgut, haemolymph and salivary glands. This disease was first reported in tomatoes followed by other crops which belong to the family Solanaceae and Cucurbitaceae. Other cucurbitaceous crops species like cucumber, sponge gourd, watermelon, bitter gourd and pumpkin are also naturally infected by ToLCNDV with symptoms such as typical mosaic with light chlorotic areas on the leaf lamina, yellow, pale green intermingled (mosaic) with normal green tissues, reduction in the size of the leaf , stunting of plant, upward curling and blistering of leaves. In a study by Rajeshwari and Reddy (2014), diseased bottle gourd plants which were infected by Tomato Leaf Curl New Delhi Virus were transmitted readily and in a long lasting manner by the whitefly, *Bemisia tabaci*. ToLCNDV infection notably affects plant growth and thereby reduces the production and productivity. The farmers are managing the disease infection by controlling the white flies in the field through the application of chemical insecticides which may develop resistance to pesticides in the near future. Therefore, an alternative approach needs to be developed for the efficient control of the virus. The major approach for efficient control of viral diseases can be obtained by using the genes derived from the pathogen itself. This mechanism is called pathogen derived resistance (PDR). In PDR, the protection of the plants is obtained through the expression of nucleotide sequences derived from the genome of the plant viruses. Coat protein-mediated resistance (CP-MR) is one of the important PDR mechanisms in which the resistance is imparted to the transgenic plants that produce coat protein to impart resistance against the virus from which the *CP* gene is derived. From the past years, the transgenic plants are usually developed using coat protein mediated resistance. ToLCNDV is ssDNA virus it has two genome components, named DNA A and DNA B. The genes in the virion and complementary sense orientations on DNA A and DNA B are separated by an intergenic region containing a common region (CR) (Padidam *et al*., 1995). The ToLCNDV DNA A encodes a replication associated protein (Rep; from *AC1*), a replication enhancer protein [REn; from *AC2* open reading frame (ORF)], a transcriptional activator protein (TrAP; from *AC3*), a coat protein (CP; from *AV1*), an AV2 protein and an AC4 protein. DNA B encodes a movement protein (MP; from *BC1*) and a nuclear shuttle protein (NSP; from *BV1*) (Fondong, 2013). There can be re assortment and component capture, which are seemingly very common in begomoviruses and are most likely the major mechanisms in begomoviruses, adapted to infect new hosts (Lefeuvre and Moriones, 2015).

The characterization of BR1 family of *CP* gene of ToLCNDV has not been done so far in any cucurbits crop. However, mutagenesis studies on MSV coat protein (Liu *et al*., 1999) and TYLCV (Kunik *et al*., 1998) indicated that the NLS region resulted in the cytoplasmic accumulation of the mutant protein. In a study by Kumar *et al*. (2012) pairwise sequence alignment of NLS region from MSV and ToLCGV-[Vad] coat proteins resulted in an identity and similarity percentage of 25 per cent and 33.3 per cent with a score of '6 whereas TYLCV and ToLCGV-[Vad] yielded an identity and similarity percentage of 51.7 per cent and 58.6 per cent with a score of 49 suggesting that ToLCV coat protein is much more conserved with TYLCV rather than a representative protein member of BR1 family (pfam00844) in Pfam database. Thus BR1 family gives an idea about the conserved regions of the *CP* genes, which can be extrapolated to understand the infectivity of the virus.

There were no stable resistant varieties against ToLCNDV which is developed so far. Hence, in the present research paper, we are reporting the development of Agrobacterium with *CP* gene construct and *in-silico* analysis of *CP* genes. This gene construct can be further used for the development of resistant ridge gourd cultivars.

MATERIALS AND METHODS

Collection of ToLCNDV Infected Ridge Gourd Samples and DNA Extraction

Infected ridge gourd samples (Variety Arka Prasanna) were collected from Indian Institute of Horticultural Research, Hesaraghatta.

Fig. 1 : Infected ridge gourd samples

DNA Extraction

Total genomic DNA was isolated from the infected leaves by using CTAB method (Doyle 1990). The leaves were collected, grounded in autoclaved mortar and pestle by the addition of liquid nitrogen and homogenized with CTAB extraction buffer (preheated for 15 minutes at 65 °C). The sample was transferred to a 1.5 mL micro centrifuge tube and was incubated in a water bath for one hour at 65 °C. The tubes were centrifuged at 13000 rpm for fifteen minutes, pellet was discarded and in the supernatant chloroform: isoamyl alcohol (24:1) was added to the tube and mixed by inversion, spun for a minute and supernatant was transferred to a new 1.5 µL tube. 0.5 mL of chilled absolute alcohol along with 0.1 volume of 3M Sodium acetate or 7.5 M ammonium acetate was added to the new tubes and it was incubated in - 20 °C overnight, Next day, the tubes were again centrifuged at 13000 rpm for ten minutes. The supernatant was discarded and the pellet was washed with 70 per cent ethanol. The pellets were kept for drying for few minutes and then dissolved in 50 µL sterile nuclease free water and then stored at - 20 °C.

Bacterial Strain and Vector

The *CP* of ToLCNDV was cloned into the TA cloning vector, followed by binary vector pBI121 at the *Xba* I at 5' end and *Sac* I at 3' and mobilized into *Agrobacterium tumefaciens* strain GV3101 by a freeze-thaw method (Holsters *et al*., 1978). The *coat protein* gene (*CP*) was driven by the *cauliflower mosaic virus* (CaMV) 35 S promoter. The bacterial strain *E.coli* DH5α was grown overnight in Luria broth (LB) medium with appropriate antibiotics and collected in the log phase, when the absorbance at 550 nm was between 0.4 - 0.8.

PCR Analysis of *CP* Gene

Genomic DNA was isolated from the putatively transformed plants by CTAB method (Doyle 1990). Primers were designed using the available database from NCBI (Accession ID EU366163.1). The *CP* gene primers are primer 1 (sense) 5' TCTAGAATGGC GAAGCGACC 3' corresponding to the N - terminal sequence of *CP* gene and primer 2 (antisense) 5' GGTACCTTAATTTGTTACCG 3' corresponding to the C - terminal sequence of *CP* gene. PCR amplification was carried out with ten micro liter reaction mixtures for each sample. The PCR master mix was prepared fresh and in bulk for all samples - Takara PCR master mix (2x Premix) - 5.0 µL, Forward primer (10 pmol /µL) - 0.5 µL, Reverse primer (10 pmol/µL) - 0.5 µL, template DNA - 1.0 µL (100ng), nuclease free water - 3.0 µL. DNA was subjected to 35 cycles of 1 min at 92 °C, 1 min at 55 °C and 1 min at 72 °C. Amplified DNA fragments were separated on a 0.8 per cent agarose ethidium bromide gel and observed under ultraviolet light.

Cloning of *CP* Gene into TA Cloning Vector

Competent cells of *E.coli* DH5α were prepared and the tube containing competent cells was removed from the - 80 °C freezer and kept briefly in ice for thawing (Chang *et al*., 2017). Ligation mixture (10 µL) was added to 100 µL of competent cells and incubated in ice for 30 min. The cells were subjected to heat shock at 42 °C for 1 min and snap - chilled on ice for 10 min. Sterile Luria Bertani (LB broth - HiMedia) (1 mL) was added to cells and grown at 37 °C for 2h in a shaking incubator at 120 rpm. After incubation the cells were spun at 6,000 rpm and dissolved in 100 µL of LB broth and plated on LB agar (HiMedia) plates containing ampicillin (50 µg/mL), 5-bromo-4-chloro-3-indolyl-beta-D- galacto-pyranoside (X-gal) (20 mg/mL)

and Isopropyl β-D-1-thiogalactopyranoside (IPTG) (100 mM) and incubated overnight at 37 °C. The TA cloning vector (pTZ57R/T) used in cloning was compatible for blue-white screening when plated on X-gal and IPTG. The recombinant clones with a foreign DNA insert ligated at the 3'- ddT overhangs disrupt the reading frame of lac-Z and produce white colonies whereas, the self-ligated (circularized) vector produces blue colonies due to enzymatic activity of lac-Z on the substrate X-gal. Colony PCR of transformed recombinant vectors was done and the colonies were picked for plasmid isolation. Further, PCR amplification of plasmid DNA was done for confirmation. Further, the orientation of the *CP* gene in the vector was confirmed by restriction digestion.

Cloning and Transformation of *CP* Gene into pBI121 Plant Expression Vector

Single colony of pBI121 and transformed pTZ57R/T + *CP* was inoculated and grown overnight in 10 mL LB broth at 37 °C with appropriate antibiotics. The plasmid was isolated and the quantity and quality of isolated plasmid was estimated using Nanodrop. The vector pBI121 and transformed pTZ57R/T + *CP* were double digested with *Xba* I and *Sac* I restriction enzymes. The reaction mixture was incubated at 37 °C for 1 hour followed by 65 °C for heat inactivation. The digested products were run on 1.5 per cent agarose gel stained with ethidium bromide and visualized in UV- transilluminator. Elution of DNA from agarose gel was done and the plasmid pBI121 ~13kb and *CP* gene of ~771bp (Digested from vector pTZ57R/T + *CP*) DNA fragment were excised from the agarose gel under UV-transilluminator. Further, ligation of digested products (vector and insert) was done using ligase enzyme (200 U/ µL). The reaction mixture was incubated at 16 °C for 16 hours. Transformation of *E. coli* cells to express coat protein gene was done and the Colony PCR of transformed recombinant vector to pick the recombinants was performed further. Amplified PCR product was run on 1.0 per cent (w/v) agarose gel and visualized in UV-transilluminator.

Transformation of pBI121 + *CP* Vector into *Agrobacterium Tumefaciens*

The competent cells of *Agrobacterium tumefaciens* were prepared by taking a single colony of Agrobacterium strain GV3101 from a freshly streaked YEP agar plate which was inoculated into 5 mL YEP broth containing rifampicin (50 µg/mL) and was subsequently grown under controlled conditions for 48 hours until the OD600 reaches 0.5 - 0.6 and the transformation was carried out by heat shock method with the help of competent cells. The competent cell and plasmid DNA mixture was dipped in liquid nitrogen for 5 minutes followed by heat shock which was given to the transformation tube by placing the bottom half of the tube into a 37 °C water bath for 5 min. After that the tubes were kept back on ice for 2-5 min. YEP broth (1 ml) was added to the tube and incubated at 28 °C in the shaker incubator at 160 rpm for 4 - 6 hrs. Then culture tubes were centrifuged at 5000 rpm 2-3 min and the pellet were re-suspended in the 100 µL broth. The plating was done with antibiotic Kanamycin (50 µg/mL) and rifampicin and incubated at 28 °C for 48 h. Further, colony PCR, plasmid isolation and confirmation of transformation by plasmid PCR was done.

Analyses of Putative Functions Domains in Coat Protein

The functional domain of the coat protein was extracted from *https://www.genome.jp/tools- bin/search_motif_lib* (from NCBI). Analysis of the function was done.

Comparison of Geminivirus Coat Protein/Nuclear Export Factor BR1 Family with other Viruses

Using https://www.ebi.ac.uk/Tools/services/rest/fasta/result/fasta-I20230612-102928-0217-18925628-p2m/visual-svg (from NCBI), the comparison of coat protein/nuclear export factor BR1 family of most of the ToLCNDV infected plants was done. This gives an automated result containing the amino acid sequences. The coloured data can be analyzed manually. The list includes the coat protein/nuclear

export factor BR1 family of most of the ToLCNDV infected plants already fed in the database. The comparison of our data was done with this.

Results and Discussion

PCR Analysis of *CP* Gene in Infected Ridge Gourd Samples

The infected ridge gourd samples amplified against 771 bp (Plate 1) which depicts the presence of the viral genome within the sample. This amplification confirmed the presence of ToLCNDV in ridge gourd, Arka Prasanna. Naganur *et al*. (2023) performed the immunocapture - PCR and immunocapture - LAMP assays to capture the virus particles directly from the supernatant obtained from ToLCNDV infected plant sap of ridge gourd. They produced and reported an amplicon of the size of 771 nt from ToLCNDV-Rg-infected ridge gourd samples but not from healthy samples and water control. Similarly, 771 nt size amplicons were observed in begomovirus-infected bitter gourd, bottle gourd, cucumber, pumpkin, ridge gourd, snake gourd and watermelon samples, but not in chilli, capsicum, cowpea and french bean and healthy samples using ToLCNDV-CP specific primers.

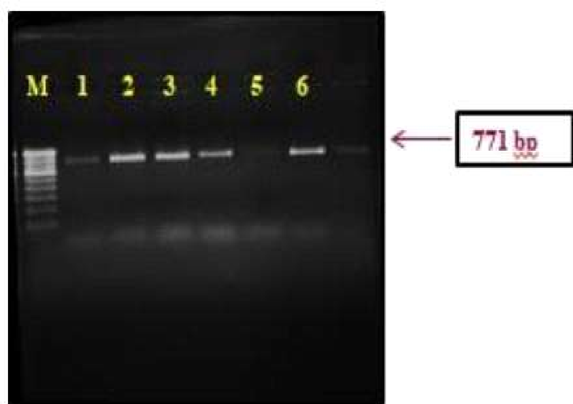


Plate 1 : Amplification of antiviral *CP* gene of using gene specific primers

Lane M : 100 bp marker; Lane 1-6 : amplified *CP* gene from infected plants

Analysis of *CP* Gene in pBI121

PCR analysis of the construct prepared in *Escherichia coli* produced an expected amplicon of approximately 771 bp using ToLCNDV *CP* gene-specific primers (Plate 2). Restriction digestion of the construct using

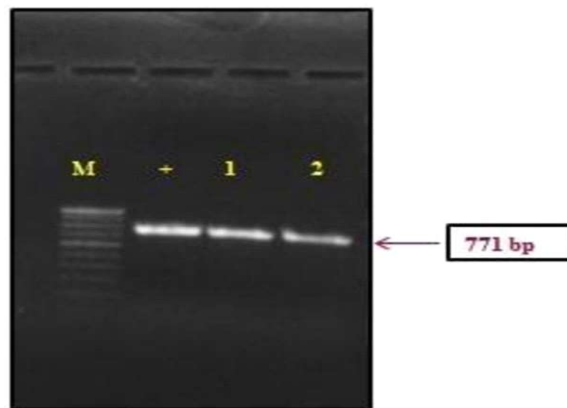


Plate 2 : Confirmation of positive transformants by PCR amplification using gene specific primers

Lane M : 100 bp Marker; Lane + : Positive transformants, Lane 1, 2 : Confirmed transformants

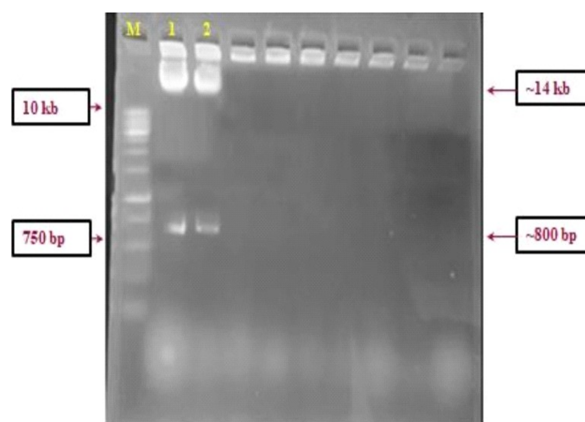


Plate 3 : Digestion of transformed pB4NU + *CP* of ToLCNDV with *Xba* I and *Sac* I. yields ~ 14kb vector and ~ 800 bp gene. Lane M : 1 kb Marker; Lane 1, 2 : vector pB4NU + *CP* of ToLCNDV.

Plate 4 : PCR confirmation of *CP* gene in plant expression vector pBI121

Lane M : 100 bp Marker; Lane 1 : Amplified *CP* gene

Xba I and *Sac* I restriction enzymes released a band of approximately 771 bp (ToLCNDV RgCP) gene from the 14.75-Kb vector confirming the recombinant nature of the plasmid (Plate 3). Further, PCR confirmation of insertion of *CP* gene into plant expression vector pBI121 was done (Plate 4).

Analysis of Putatively Transformed *Agrobacterium* GV3101 Strain with *CP* Gene Construct

The plasmid gene construct of pBI121 with *CP* gene was transferred to *Agrobacterium* and it was further confirmed by colony PCR. The presence of *CP* gene in *Agrobacterium* with the amplicon size of 771bp was confirmed (Plate 5).

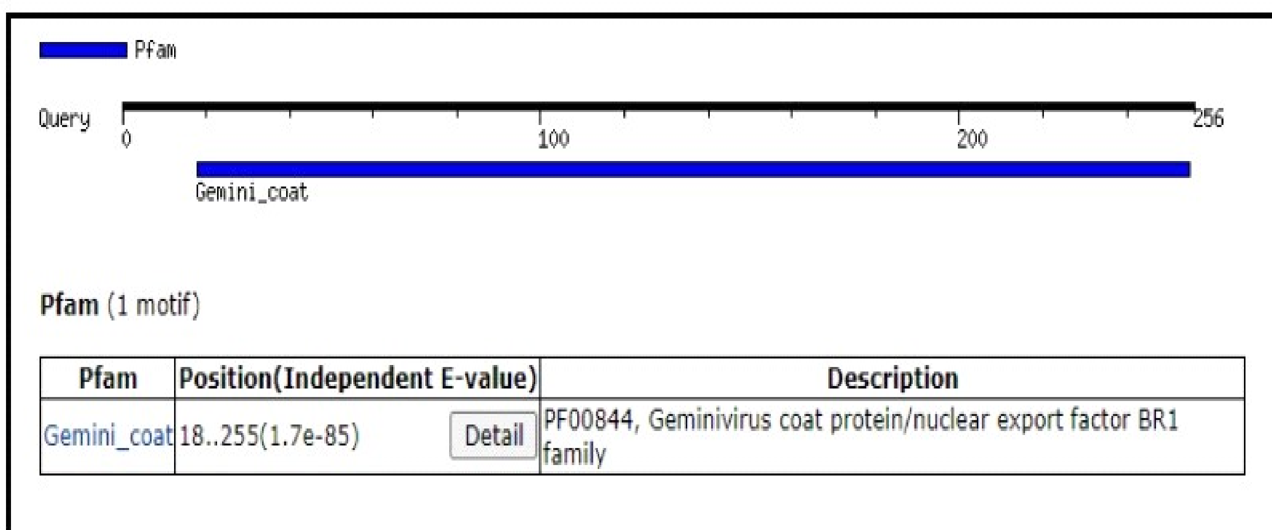
Plate 5: Colony PCR of transformed *Agrobacterium tumefaciens* GV3101
Lane M: 100 bp Marker; Lane 1-6: Confirmed transformants

In-silico Analysis of the *CP* Gene Sequence

Functional Domains in Coat Protein

Geminivirus Coat Protein/Nuclear Export Factor BR1 Family

The member BR1 has been found to be the protein predominantly present in the *CP* gene of ToLCNDV of ridge gourd (Fig.2) and the amino acid sequence of the protein is depicted in Fig. 3. This facilitates the export of both ds and ss DNA from the nucleus. It has been shown that the 104 N-terminal amino acids of the *maize streak virus* resembles the ToLCNDV coat protein and binds DNA non- specifically. This family also includes various geminivirus movement proteins that are nuclear export factors or shuttles. ToLCV coat protein possesses DNA binding properties to function similar to BR1 nuclear shuttler. In this study, the amino acid combinations that are necessary to impart virulence were investigated through the evolutionary relationship and Pfam (Pfam is a database of protein families which includes the annotations and multiple sequence alignments of those proteins, which are generated using hidden Markov models). The predicted Nuclear localization signals (NLS) of ToLCV coat protein shares more similarity with experimentally known TYLCV coat protein NLS as cited in literature. Further, sequence and structure based approaches are needed for the



Pfam	Position(Independent E-value)		Description
Gemini_coat	18..255(1.7e-85)	Detail	PF00844, Geminivirus coat protein/nuclear export factor BR1 family

Fig. 2 : The member BR1 has been found to be the protein pre-dominantly present in *CP* gene of ToLCNDV of ridge gourd

Motif in the sequence

Pfam ID:
Gemini_coat
Description:
PF00844, Geminivirus coat protein/nuclear export factor BR1 family
Appearance:

Position	18..255
Alignment Query Database	RRRLNFDSpYGARAVVPIARVT-KAKAWTNRPMNRKPRMYRMYRSPdvprgcegPCKVQSFE-----SRHDVSHIGKVMCVSDVTRGTGLTHRVGKRFCVKSVYVLGKIWMDENIKTKNHTNSVMFFLVRDRRPTG-SPQDFGEVFNMFDNEPSTATVKNMHRDRYQVLRKWHATVTGGT----- Rrrlnfds.rrsraraplkrstnkkksrskrpmrrkgrvvrvarsp........plkvqsfeinqegsefvvsntgkvslisdpsrGkgetnRvrkriklkslaikgtvaidenaakknmtgvvvfwLVrDrkPtgstpgtfdeiFgaydnepstltvkralkdRfvVlrkwkvvvssegdtrvv
i-Evalue	1.7e-85

Sequence:
MAKRPADIIISTPASKVRRRLNFDSPYGARAVVPIARVTKAKAWTNRPMNRKPRMYRMYR
SPDVPRGCEGPCKVQSFESRHDVSHIGKVMCVSDVTRGTGLTHRVGKRFCVKSVYVLGKI
WMDENIKTKNHTNSVMFFLVRDRRPTGSPQDFGEVFNMFDNEPSTATVKNMHRDRYQVLR
KWHATVTGGTYASREQALVRKFVRVNNYVVYNQQEAGKYENHTENALMLYMACTHASNPV
YATLKIRIYFYDSVTN

Fig. 3 : The amino acid sequence of the protein BR1

identification of the ToLCNDV coat protein through its protein topological interactions with viral DNA. Here, we anticipate the conserved region - BR1 of ToLCNDV coat protein is prominent for DNA binding and functional sequence pattern can be targeted by RNA interference to develop disease-resistant transgenic ridge gourd plants. Similar studies by Kumar *et al*. (2012) showed that the ToLCV coat protein must possess a NLS region containing BR1 motif in its sequence in order to be translocated to nucleus.

Comparison of Geminivirus Coat Protein/Nuclear Export Factor BR1 Family with other Viruses

The comparison showed the presence of the BR1 family amongst different isolates of ToLCNDV. The origin remains the same amongst all the isolates (Fig. 4, 5). This depicts that over a period of years, there have been successive mutations which have led to the evolution of different isolates of ToLC NDV. This also gives an idea about the infectivity of the virus. Further, there was a difference of one or two amino acids amongst different isolates. This can

coat protein [Tomato leaf curl New Delhi virus [India:Bangalore:OY135:2005]]
viruses | 3 leaves
coat protein [Tomato leaf curl New Delhi virus]
coat protein [Tomato leaf curl virus]
coat protein [Tomato leaf curl virus]
viruses | 46 leaves
viruses | 2 leaves
coat protein [Tomato leaf curl New Delhi virus]
viruses | 6 leaves
viruses | 9 leaves
viruses | 10 leaves
coat protein [Tomato leaf curl New Delhi virus-India [India/New Delhi/Chilli/2009]]
coat protein [Tomato leaf curl New Delhi virus]
viruses | 2 leaves
viruses | 2 leaves
coat protein [Tomato leaf curl New Delhi virus]
viruses | 3 leaves
viruses | 5 leaves
coat protein [Tomato leaf curl New Delhi virus - Gorakhpur]
viruses | 2 leaves
viruses and unknown | 2 leaves

Fig. 4 : Presence of BR1 family amongst different isolates of ToLCNDV

```
Reformat  Format: Compact Hypertext  Row Display: up to 10  Color Bits: 2.0 bit  Type Selection: the most diverse members

Q08595      2 RRGAYTPR.[13].GKGR.[3].SYRRRGP.[2].PLARR.[7].ARAFTYKT.[7].PDFTIH.[1].NNYKSSYISMP 83  Indian cassava mosaic virus
CAL30139    5 KSKGKRKR.[ 8].WKGA.[3].RRKTQYK.[2].PVRPP      ALCVLRQQ.[6].QSVVVT      NTPRIDLITCF 72  Oat dwarf virus
Q89238      8 RGKGKRKL.[ 8].WKGA.[3].RRKQAYK.[2].PVKPP      ALCVFRYN.[6].TNIVVG      NTPRVDLITCF 75  Wheat dwarf virus
O39520      8 KKRKRSGG.[ 1].SKAK.[3].SSSYIPR.[2].SSRRE      SLQVATFS.[5].SGIKFS      PGGAAYLVSNF 67  Bean yellow dwarf virus
P31616     12 RKRGRYGK.[ 8].SQRE.[3].LVNQPGR.[2].SNRRP      ALQVAEYL.[5].TGMTFS      PGGSTFLITNF 78  Tobacco yellow dwarf vir...
P14985      9 RKRPSSSS      AQAS.[3].RVYRPAV.[2].SLARR.[1].PLQVQDFV.[2].TDVAFN      RGGGCYLLTSY 65  Chloris striate mosaic v...
Q76QL8      6 RKRGDDSN.[ 7].KKPS.[2].GLKRAGS      KADRP      SLQIQTLQ.[4].TMITVP      SGGVCDLINTY 67  Maize streak virus - [Re...
Q67566      7 RKRTDEGS.[ 7].KKSA      AKGRTSS.[1].RAIRP      ALQIQSFV.[4].ATIAVP      TGGVCHLLSSY 67  Digitaria streak virus
Q89551      8 RKRSDETG.[ 7].VKQG.[2].SAARAGS.[2].RRTRP      SLQIQTLQ.[4].SMIEVP      SGGVCDLLGSF 71  Sugarcane streak virus -...
Q84369      7 RKRSDEVA.[ 7].KKQD.[3].PLPRAGP.[2].RRGLP      ALQIQTLT.[4].TMITVP      SGGICSLIGTY 71  Panicum streak virus - K...

Q08595     84 VKTRA.[1].SDNRVGDYIKLVNISFTGTVCIKNSQMESDGS.[6].GLFTCVLVRDKTPRI.[1].SAT.[4].PFP 153 Indian cassava mosaic virus
CAL30139   73 AAGKG     DDNRHTNQTIMYKFHLQGSVYVADASSKFVTP     LRLYHWLVYDAEPKQ.[1].MPG     IED 131 Oat dwarf virus
Q89238     76 AQGKA     DNNRHTNQTVLYKFNIQGTCYMSDASAPFIGP     VRLYHWLVYDAEPKQ.[1].MPD     ATD 134 Wheat dwarf virus
O39520     68 PQGAN     DNCRHTNKTVLYKFMAKNTIYLDSSHYMKVFR     APFHFWLVYDKSPGA.[1].VPS     TGD 126 Bean yellow dwarf virus
P31616     79 PQGAN     ENCRHTNRTITYKMAVKTWVALDGSMFSRVSK     FPIYFWLVYDKNPGE.[1].NPS     PSA 137 Tobacco yellow dwarf virus...
P14985     66 ARGSA     ENQRKTAETITYKVAVNLGCAISGTMQQYCIS     SRPVCWIVYDAAPTG.[1].AVT     PKD 124 Chloris striate mosaic virus
Q76QL8     68 ARGSD     EGNRHTSETLTYKIAIDYHFVADAAACRYSNT     GTGVMWLVYDTTPGG.[1].APT     PQT 126 Maize streak virus - [Reun...
Q67566     68 SRGSG     EGDRHTNETVTYKAAFDYHFSANAGPCAYSSI     GVGVLWLVYDAQPSG.[1].VPA     VTD 126 Digitaria streak virus
Q89551     72 SRGSD     EGNRHTNETVIYKVALDYHFIATAAACKYSSI     GTGVVWLVYDAQPSG.[1].PPT     VKD 130 Sugarcane streak virus - [...
Q84369     72 ARGSD     EGNRHTNETLTYKVALDYHFVATAAACKYSSI     GVGVMWLVYDAQPTG.[1].SPE     VKD 130 Panicum streak virus - Karino

Q08595    154 .[1].LFGS     INASYADLSIQDP.[3].RFTVIRQVSYPVNTEK.[ 7].KGTRRFG     GRYPIWTSFKDDG 217 Indian cassava mosaic virus
CAL30139  132      IFTM.[1].WNVLPSTWTIWRA.[3].RFVVKRKWHVDLVSDG.[15].VGKNIVD.[1].NKFIKGLRVSTEW 204 Oat dwarf virus
Q89238    135      IFTM.[1].WNLLPSTWTVQRA.[3].RFVVKRKWTVNLVTDG.[15].VGKNIVD.[1].SKFFKGLRVTTEW 207 Wheat dwarf virus
O39520    127      IFEG.[3].FPHNPWTWTVSRA.[3].RFVVKKTWSCVVESNG.[15].PCNQVKS.[1].NKFFKRLGVSTEW 201 Bean yellow dwarf virus
P31616    138      IFDS.[1].YQDQPGTWTVTRN.[3].RFVVKKTWSCMLESNG.[15].PCYQWRH.[1].TKFFKRLGVSTEW 210 Tobacco yellow dwarf viru...
P14985    125      IFGY.[3].LVNWPTTWKVARA.[3].RFIVKRRWVFTMESNG.[13].IPQSLPV.[1].NKFAKQLGVRTEW 197 Chloris striate mosaic virus
Q76QL8    127      IFAY.[3].LKAWPATWKVSRE.[3].RFVVKRRWLFNMETDG.[14].PCKRNIY.[1].HKFTSGLGVRTQW 200 Maize streak virus - [Reu...
Q67566    127      IFPH.[3].LQSFPYTWKVGRE.[3].RFVVKRRWCFTMETNG.[14].PCKRSIY.[1].HKFATGLGVKTEW 200 Digitaria streak virus
Q89551    131      IFPH.[3].LTAFPYTWKVGRE.[3].RFVVKRRWTFTMEVNG.[14].PCRKNIY.[1].HKFVTGLGVKTEW 204 Sugarcane streak virus - ...
Q84369    131      IFPH.[3].LSAFPYTWKVGRE.[3].RFVVKRRWCFTMETNG.[14].PCKKDIY.[1].HKFCTGLGVKTEW 204 Panicum streak virus - Ka...

Q08595    218 .[1].SGDSSGLYSNTYKNAILVYY.[4].DVSSQLEMY      CKYVTRYIG 260 Indian cassava mosaic virus
CAL30139  205      MNTGDGKIGDIKKGALYLVT.[4].GVTGDSAST.[11].CYFKSIGLQ 257 Oat dwarf virus
Q89238    208      MNTGDGKIGDIKKGALYLIS.[4].GVTGDSAST.[11].CYFKAIGIQ 260 Wheat dwarf virus
O39520    202      KNSATGDVGDIKEGALYIVG     APSQKSDVY.[ 6].VYFKSVGNQ 245 Bean yellow dwarf virus
P31616    211      KNSSTGDVADIKEGALYLVC     APGGGATVR.[ 6].MYFKSVGNQ 254 Tobacco yellow dwarf virus (strain Australia)
P14985    198      KNAEGGDFGDIKSGALYLVM     APANGAVFV.[ 6].VYFKSVGNQ 241 Chloris striate mosaic virus
Q76QL8    201      KNVTDGGVGAIQRGALYMVI     APGNGLTFT.[ 6].LYFKSVGNQ 244 Maize streak virus - [Reunion2]
Q67566    201      KNTTDGGVGSIKKGALYFVI     APGSGIDFT.[ 6].MYFKSVGNQ 244 Digitaria streak virus
Q89551    205      KNTTGGEVGDIKKGALYIVI     APGNGLDFT.[ 6].LYFKSVGNQ 248 Sugarcane streak virus - [Natal]
Q84369    205      KNVTDGKVGAIKKGALYIVI     APGNGLEFT.[ 6].LYFKSVGNQ 248 Panicum streak virus - Karino
```

Fig. 5 : Comparison of amino acid sequence Geminivirus coat protein/nuclear export factor BR1 family with other viruses with respect to amino acid sequence

be interpreted as the evolutionary mechanism for the production of slightly different protein as a result of mutation to combat resistance.

The systemic spread of the ToLCNDV virus is assisted by the *CP* gene. The Coat protein of ToLCNDV are involved in the movement of the virus and their absence results in reduced levels of ssDNA that affects the systemic infection and symptom development in plants (Padidam *et al*., 1995). ToLCNDV has been reported in Cucurbitaceae family in crops such as cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon [*Citrullus lannatus* (Thunb.)], bottle gourd [*Lagenaria siceraria* (Molina) standl.], sponge and ridge gourds (*Luffa* spp), bitter gourd (*Momordica charantia* L.), wax gourd [*Benincasa hispida* (Thunb.) Cogn.] and pumpkin (*Cucurbita maxima* Duch.) (Chang *et al.,* 2010, Jyothsna *et al.,* 2013 and Bandaranayake *et al.,* 2014). The coat protein is also known for exclusively affecting the production/accumulation of MP (movement protein) in the plant cell and plays a significant role in the fast long-distance transfer of TMV throughout the plant (Hilf & Dawson, 1993 and Bendahmane *et al.,* 2002). The CP of ToLCNDV also interacts with a midgut protein of *B. tabaci* and probably facilitates virus transport both from the digestive tract to the haemolymph and from the hemolymph to the salivary glands (Rana *et al*., 2016). ToLCNDV results in leaf curl disease in *Luffa acutangula* posing an important threat to its production. An effective control measure against ToLCNDV was lacking and no resistant variety of *Luffa acutangula* is available, the genetic transformation of *Luffa acutangula* was attempted in this study for providing resistance.

Transgenic approaches aim at enabling the production of durable and safe virus-resistant plants. However,

extensive field studies are needed for further assessment of this promising transgenic line. The *CP* gene was cloned into plant expression vectors pBI121. Subsequently, the confirmed pBI121 having *CP* gene construct was transformed into *Agrobacterium* tumefaciens. This is the first report of construction of the *CP* gene of ToLCNDV in pBI121 vector. It will be further used for transgenic plant development. The findings related to the *in-silico* approaches related to the *CP* gene will be further used for finding the exact virulence domain within the *CP* gene. This can be used further to establish a targeted approach in mitigating the virus.

Acknowledgement : The research was supported under the PhD thesis work 'Development of transgenic ridge gourd [*luffa acutangula* (l.) roxb] against *tomato leaf curl new delhi virus'* (g: begomovirus, f:geminiviridae) funded by Ph.D. supervisor Dr. Nagesha, N., Dept. of Plant Biotechnology, UAS, Bangalore and Advisory committee member Dr. Mahesha, B, Scientist (Plant Pathology), Division of Plant Protection, Indian Institute of Horticultural Research, Bangalore - 560 089. I am also greatly thankful to Department of Biotechnology, Govt. of India, New Delhi for providing fellowship (DBT-JRF).

References

Au, T. K., Collins, R. A., Lam, T. L., Ng, T. B., Fong, W. P. and Wan, D. C., 2000, The plant ribosome inactivating proteins luffin and saponin are potent inhibitors of HIV-1 integrase. *FEBS lett.,* **471** (2-3) : 169 - 172.

Badgujar, S. B. and Patil, M. B., 2008, Estimation of chemical constituents of *Luffa acutangular. Nat. Prod. Rad.,* **7** (1) : 79 - 81.

Bandaranayake, W. M. E. K., Wickramarachchi, W. A. R. T., Wickramasinghe, H. A. M., Rajapakshe, R. G. A. S. and Dissanayake, D. M. K. K., 2014, Molecular detection and characterization of begomoviruses associated with cucurbitaceae vegetables in Sri Lanka. *J. Natl. Sci. Found. Sri.*, **42** (3) : 265 - 271.

Bendahmane, M., Szecsi, J., Chen, I., Berg R. H. and Beachy, R. N., 2002, Characterization of mutant *Tobacco mosaic virus* coat protein that interferes with virus cell-to-cell movement. *Proc. Natl. Acad. Sci. U. S. A.,* **99** : 3645 - 3650.

Chang. H. H., Ku, H. M., Tsai, W. S., Chien, R. C. and Jan, F. J., 2010, Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. *Eur. J. Plant Pathol.,* **127** : 219 - 228.

Chang, A. Y., Chau, V., Landas, J. A. and Pang, Y., 2017, Preparation of calcium competent *Escherichia coli* and heat-shock transformation. *JEMI methods*, **1** : 22 - 25.

Doyle, J. J., 1990, Isolation of plant DNA from faesh tissue. *Focus*, **12** : 13 - 15.

Fondong, V. N., 2013, *Geminivirus* protein structure and function. *Mol. Plant Pathol*., **14** : 635 - 649.

Hilf, M. E. and Dawson, W. O., 1993, The *Tobamo virus* capsid protein functions as a host-specific determinant of long-distance movement. *Virol.,* **193** (1) : 106 - 114.

Holsters, M., De Waele, D., Depicker, A., Messens, E., Van Montagu, M. and Schell, J., 1978, Transfection and transformation of *Agrobacterium tumefaciens*, *MGG.,* **163** : 181 - 187.

Jyothsna, P., Haq, Q. M. I., Singh, P., Sumiya, K. V., Praveen, S., Rawat, R., Briddon, R. W. and Malathi, V. G., 2013, Infection of *tomato leaf curl New Delhi virus* (ToLCNDV), a bipartite begomovirus with beta satellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and beta satellites. *Appl. Microbiol. Biotechnol.,* **97** : 5457 - 5471.

Kumar, S. P., Patel, S. K., Kapopara, R. G., Jasrai, Y. T. and Pandya, H. A., 2012, Evolutionary and molecular aspects of Indian tomato leaf curl virus coat protein. *Int. J. Plant Genomics*.

Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V. and Gafni, Y., 1998, Nuclear import of the capsid

protein of tomato yellow leaf curl virus (TYLCV) in plant and insect cells. *Plant J.*, **13** (3) : 393 - 399.

LEFEUVRE, P. AND MORIONES, E., 2015, Recombination as a motor of host switches and virus emergence : *Geminiviruses* as case studies *Curr. Opin. Virol.,* **10** : 14 - 19.

LIKHITH, R. AND PETER, A., 2023, Comparative *in silico* Analysis of Coat Protein (CP) of *tomato leaf curl virus* (ToLCV) and *tomato yellow leaf curl virus* (TYLCV) and their molecular docking with GroEL protein of Hamiltonella an Endosymbiont of their Vector *Bemisia Tabaci*. *Mysore J. Agric. Sci.*, **57** (2).

LIU, H., BOULTON, M. I., THOMAS, C. L., PRIOR, D. A. M., OPARKA, K. J. AND DAVIES, J. W., 1999, *maize streak virus* coat protein is karyophyllic and facilitates nuclear transport of viral DNA. *Mol. Plant Microbe*, **12** (10) : 894 - 900.

MANIKANDASELVI, S., VADIVEL, V. AND BRINDHA, P., 2016, Review on *Luffa acutangula* L. : Ethnobotany, phytochemistry, nutritional value and pharmaco logical properties. *Int. J. Curr. Pharm. Rev. Res.,* **7** (3) : 151 - 155.

NAGANUR, P., SHANKARAPPA, K. S., MESTA, R. K., RAO, C. D., VENKATARAVANAPPA, V., MARUTHI, M. N. AND REDDY, L. R. C. N., 2023, Detecting *tomato leaf curl new delhi virus* causing ridge gourd yellow mosaic disease and other begomoviruses by antibody-based methods. *Plants*, **12** (3) : 490.

PATIL, C. V., RAMDAS, S. V., PREMCHAND, U. AND SHANKARAPPA, K. S., 2017, Survey, symptomatology, transmission, host range and characterization of begomovirus associated with yellow mosaic disease of ridge gourd in southern India. *Virus Dis.*, **28** (2) : 146 - 155.

PADIDAM, M., BEACHY, R. N. AND FAUQUET, C. M., 1995, *tomato leaf curl geminiviruses* from India has a bipartite genome and coat protein is not essential for infectivity, *J. Gen. Virol.,* **76** : 25 - 35.

RAJESHWARI, R. AND REDDY, M. K., 2014, Biological characterisation of *tomato leaf curl New Delhi virus* infecting bottle gourd (*Lagenaria siceraria*) from Karnataka. *Mysore J. Agric. Sci.*, **48** : 387 - 393.

RANA, V. S., POPLI, S., SAURAV, G. K., RAINA, H. S., CHAUBEY, R., RAMAMURTHY, V. V. AND RAJAGOPAL, R., 2016, A *Bemisia tabaci* midgut protein interacts with begomoviruses and plays a role in virus transmission. *Cell Microbiol.,* **18** : 663 - 678.