Cloning, expression of recombinant nucleocapsid protein of capsicum chlorosisvirus (CaCV) and its detection methods

V. Laxmi Devi1,2*, Amruta S. Bhat3 and P. Dinesh Sankar Reddy4&5

1Ph.D. Scholar, JNTUA, Anantapur, Anantapuram-515002, Andhra Pradesh,
2COHB, UHS Campus GKVK Bengaluru-560065,
3Regional Horticulture Research and Extension Centre, UHS Campus GKVK Bengaluru-560065,
4Department of Chemical Engineering, JNTUA, Anantapur-515002,
4Department of Chemical engineering, National Institute of Technology, Thadepalligudem, Andhra Pradesh-534101.

1 lakshmidevi.biochem@gmail.com, 3 amruta.bhat@uhsbagalkot.edu.in,
4 pdsreddy@gmail.com

Abstract:

Capsicum (Capsicum annuum L.) is one of the most cultivated vegetable crop in the world, belongs to solanaceae family. It is infected by different species of orthotospoviruses includes capsicum chlorosis virus is one among them.Capsicum chlorosis virus (CaCV), belonging to the genus orthotospovirus, causing disease on several economically important crops in India.In the year 2014, the capsicum plants showed diverse kind of symptoms were collected in Bengaluru (Byatha) Karnataka state, India. To confirm the identity of the virus infected capsicum samples were subjected to DAC-ELISA using polyclonal antibody of groundnut bud necrosis virus. The ELISA positive samples were subjected to RT-PCR using nucleocapsid protein (NP) gene specific primers were amplified and sequenced. Pairwise analysis of NP gene showed 95- 98.55% nucleotide identity with CaCV isolates from GenBank database. Further the NP gene from CaCV isolate was cloned into pRSETA vector and expressed inE. coli BL21 (DE3) pLys S cells. The Expressed and purified recombinant protein (~32 kDa) from NP gene of CaCV was conformed in western blot.

Keywords: *Capsicum chlorosis virus* (CaCV), Recombinant protein, ELISA, DIBA and IC-RT-PCR

Introduction

Capsicum chlorosis virus (CaCV), belongs to the genus *orthotospovirus*, affecting several economically important crops such as tomato (Kunkalikar et al., 2007), chilli pepper (Krishnareddy et al., 2008; Haokip et al., 2016), Amaranthus sp. (Sharma and Kulshrestha, 2014) and groundnut (Vijayalakshmi et al., 2016) in India. Similarly CaCV infection in different crops are well documented throughout the world (McMichael *et. al*., 2002; Knierim *et. al.,* 2006; Chiemsombat *et. al.,* 2008;Chen *et.al.,* 2007; Zheng *et. al.,* 2011; Chen *et. al.,* 2012; Melzer *et. al.,* 2014; Orfanidou *et. al.,* 2018; Bayat *et. al.,* 2018; Chiaki *et. al.,* 2019).

The virus particle is an envelope, quasi-spherical structure of 80–120 nm in diameter and has a segmented RNA genome of three single-stranded RNA molecules. The SRNA encodes a nucleocapsid protein (NP) in viral-complementary sense and the non-structural protein (NSs) in viral sense orientation. The MRNA encodes a non-structural protein (NSm), in the sense orientation and a glycoprotein precursor in complementary sense. The LRNA encodes an RNA-dependent RNA polymerase (RdRp) in complementary sense orientation (Plyusnin et al., 2012).

Capsicum *(Capsicum annuum* L.*)* is one of the most cultivated vegetable crops in the world, which belongs to *solanaceae* family. It's commonly known as bell pepper or sweet pepper. It is extensively cultivated in states of Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu and Bihar in as autumn crop and hilly areas of Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir, Arunachal Pradesh and Darjeeling district of West Bengal during summer months (Singh et al., 1993; Sreedhara *et al.,* 2013). Geographically, capsicum cover an area of 29,720 ha with 1,66,880 tonnes production and productivity of 5.61 tonnes /ha in India (Anonymous, 2014). In Karnataka, capsicum was grown in an area of about 4.13 thousand ha with a production of about 81.67 thousand tons (Anonymous, 2018). The major viruses infecting capsicum are cucumber mosaic virus (CMV), pepper mottle virus (PeMV), potato virus Y (PVY), tobacco mosaic virus (TMV) alfalfa mosaic virus (AMV) and several members of orthotospovirus. Among various genera infecting capsicum, orthotospovirus have fast emerged as a serious threat for it cultivation under both field and protected cultivation (Varma *et al.,* 2002). To date fivedifferent orthotospovirus species have been reported to occur in India on different crops (Kunkalikar et al., 2010) and *Tomato spotted wilt virus* (TSWV) has been reported on chrysanthemum in the India very recently (Renukadevi et al., 2015). Of these, Capsicum chlorosis virus is an emerging threat for capsicum cultivation. Our studies revealed, the capsicum chlorosis virus identification from infected plant samples as well as cloning, over expression and purification of CaCV NP recombinant proteinfrom CaCV NP gene.

Materials and methods

Survey and collection of samples

During the year 2014, samples from capsicum plants showing typical symptoms to orthotospovirus infection were collected from the Byatha village in Karnataka. These samples were stored at -80°C until further use.

Serological detection

Naturally infected field capsicum samples collected from Byatha and tested for the presence of serogruop IV orthotospoviruses using polyclonal antibody raised against GBNV nucleocapsid protein reported to detect all serogroup IV orthotospoviruses (Mandal and Jain, 2010) as primary antibody and goat anti-rabbit IgG labeled with alkaline phosphatase as secondary antibody by direct antigen coating (DAC) – ELISA [Clark and Bar-Joseph, 1984]. Proper negative (bufer and healthy plant) and positive controls purified recombinant nucleocapsid (N) protein of GBNV was maintained for comparison.

RT-PCR, cloning and sequencing:

Total RNA was extracted from symptomatic (and healthy leaf samples using Trizol reagent (Sigma-Aldrich, USA) according to manufacturer's protocol and then it was subjected to RT-PCR amplification. The reaction was carried out with 5µg total RNA denatured along with primers specific to CaCV (20pmol/µL) (Krishna Reddy et al., 2008) at 72^0C for 5 min, followed by addition of 4µL of 5X first strand buffer, 0.2µL ribonuclease inhibitor (40 U/µL), 2 µL of 10mM dNTPs and 01µL MMLV-RT (200 U/µL) (Fermentas) in a total reaction of 20µL. The reaction was performed at 42^0C for 60 min, followed by incubation at 75^0C for 5 min.

Further the c-DNA was used for the amplification of PCR was carried out in a thermal cycler (Applied Biosystems) using the reverse primer and forward primer with the following conditions: initial denaturation at 94 °C for 4 min followed by 35 cycles of 94 °C for 45 s, 55 °C for 1 min, 72 °C for 1.5 min and final elongation at 72 °C for 10 min. Further, PCR amplified products were purified from agarose gel by using standard protocols and ligated into pTZ57R/T vector using Ins T/A clone PCR product cloning kit (ThermoScientifc, USA) according to the manufacturer'sinstructions were transformed in to DH5α competent cells. The transformed colonies were screened by blue white selection and selected colonies were tested by colony PCR for the presence of DNA fragment of interest and restriction analysis. The confirmed clones were purified by alkali lysis method (Sambrook et al. 1989). Independent three clones were sequenced using M13 universal primers, in both forward and reverse directions in an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) at Scigenome Laboratories.

Sequence analysis:

A sequence similarity search for obtained sequence was carried out at NCBI BLAST program (http://blast.ncbi.nlm.nih.gov). GenBank sequences showing maximum blast scores corresponding to CaCV infecting different crops were retrieved and used for further analysis. The nucleotide (nt) and amino acid alignment was performed using CLUSTALW program. Phylogenetic trees were constructed by neighbour-joining (N-J) method using MEGA 7.0 (Kumar et al., 2016) with 1,000 bootstrap replications.

Cloning, over expression and purification of CaCV recombinant protein:

The complete CaCV N gene was amplified by RT-PCR using the primer pairs (CaCV Sense primer: 5' ATGCTAGCATGTCTAACGTCAGGC 3', CaCV Antisense primer: 5' GCGAATTCTTACACTTCTATAGATGTA 3'- the underlined nucleotides represent *NheI* and *EcoRI* restriction sites) designed with the help of primer3 software and oligo analyzer (1.0.2) from the conformed clone. The NP gene was amplified using Deep Vent DNA polymerase (New England Biolabs, Inc, MA) under the following conditions: 94◦C, 3 min (1 cycle); 94°C, 30 sec denaturation; 50°C, 30 sec annealing and 72 °C, 1 min extension (30 cycles) followed by an extension step at 72°C, 10 min. The amplified product was gel purified and digested *NheI* and *EcoRI* and cloned into *NheI* and *EcoRI* digested pRSET A (Invitrogen) expression vector and transformed into *E. coli* BL21 (DE3) pLys S cells. Positive clones were confirmed by either colony PCR or restriction digestion with *NheI* or *EcoRI* and further confirmed through nucleotide sequencing of inserted clone.

A smaller scale culture of *E. coli* BL21 (DE3) pLys S cells having pREST A containing the NP clone was established. A single colony from *E. coli* BL21 (DE3) pLys S incubated plate at 37 °C overnight was inoculated into a 25ml Luria broth culture and grown overnight. One percent of overnight culture was inoculated in 2 X YT medium containing 50 mg/ml of ampicillin& 34 mg/mlof chloramphenicol added to the medium was incubated with shaking at 37 °C until OD600 reached 0.6. Two ml of the culture was removed for un induced control and rest of the culture was induced by the addition of different concentrations of IPTG (0.1, 0.3, 0.5, 0.8 and 1 mM IPTG), and the culture was grown for 4-5 h at 30°C or 10-12 h at 18°C. The flask was placed on ice for 5 min and the cells were harvested by centrifugation at 5000 × g for 5 min at 4 °C. The cell pellet saved for further analysis. The cell pellet was resuspended in lysis buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, and 1% Triton-X 100, and 10% glycerol). The cell suspension was sonicated using a Vibra cell sonicator in an ice bath and subjected to centrifugation at 13000 rpm for 10 min to remove debris. The recombinant NP from the soluble fraction was purified using Ni NTA column chromatography (Qiagen Inc, Chatsworth, CA) according to manufacturer's instructions. The protein eluted with 250 mM imidazole containing buffer having 50mM Tris and 300mM NaCl fraction collected from Ni NTA column were dialyzed against elution buffer (250 mM imidazole, 50mM Tris and 300mM NaCl, pH-8.0) at 60V for six hours at temperature of 4°C and the protein expression analyzed by 12 % SDS-PAGE and western blot analysis.

Western blot analysis:

The protein (20ug) was loaded on 12% gel and SDS-PAGE was performed using a mini-PRO TEAN Electrophoresis Cell (Bio-Rad, USA). After electrophoresis, the protein band was electro transferred on to a nitrocellulose membrane (Hybond-C, Amersham) (Towbin et al. (1979)) with a semi-dry trans blot apparatus (Bio-Rad semi-dry transblot apparatus, USA) at 140 mA for 45 min. Protein bands were electrophoretically blotted on to the nitrocellulose membrane (Hybond-C super, Amersham) as described (Towbin et al., 1979) for western blot analysis. The unoccupied sites of the blot were blocked by blocking buffer (5% skimmed milk powder) for 1 h to eliminate nonspecific binding sites and then washed thrice in PBS. The blot was then incubated with 1:10000 dilution of GBNV polyclonal antibodyat 37^{o}C for 2 hours. The blot was washed thrice with PBST (PBS containing 0.1% v/v Tween 20), followed by PBS to remove the unbound antibodies and further incubated with goat anti-rabbit IgG (secondary) conjugated with horseradish peroxidase. The unbound antibodies were once again removed by washing and the blot was developed by incubating with di amino benzidine (DAB) in the presence of hydrogen peroxide, pH 4.8. The membrane was washed in autoclaved double distilled water and dried after colour development.

Immuno capture RT-PCR (IC-RT-PCR):

IC-RT-PCR was performed as described by Nolasco et al. (1993). Healthy and CaCV infected leaf extracts were prepared by grinding tissue in extraction buffer (500 mM Tris-HCl, pH 8.3, containing 2% polyvinyl pyrrolidone, MW 40,000; 0.01 M Na2SO3; 3 mM NaNO3; 140 mM NaCl and 0.05% Tween 20) at 1:10 dilution.

The extracts were clarified by centrifugation at 10,000 rpm for 5 min. Immunocapture of virions was carried out using a coating and trapping method adapted from Clark and Adams (1977). Clarified CaCV infected and healthy leaf extract at 1:10 dilution was added to sterile PCR tubes (50 µl/tube) that had been previously coated with GBNV polyclonal antibody (1:1000) diluted in coating buffer. After an overnight incubation at 4°C, the tubes were washed thrice with PBS-T and once with sterile distilled water. cDNA was synthesized in a 20 µl reaction mix using M-MuLV reverse transcriptase (Fermentas). PCR was performed using a 5 µl aliquot of the cDNA mix as template and 10 pmoles each of the NP-F and NP-R primers under the conditions as described above for the NP amplification. The amplified products were analyzed by 1% agarose gel (Sambrook et al., 1989).

Dot immune biding assay (DIBA):

Crude extract was prepared by the grinding the sample collected from healthy and infected capsicum plants showing chlorotic and necrotic ring spots on leaves and fruits in a phosphate buffer saline, pH 7.5 (1g/5ml) with the help of sterile pestle andmotor. The extracts were clarified by centrifugation at 8000rpm for 10 minutes at 4^0C. The collected supernatant from both infected and healthy leaf tissues as well as recombinant protein was directly spotted on the nitrocellulose membrane strips (Hybond–C super, Amersham) without any dilution. The unoccupied sites of the strip were blocked by blocking buffer (5% skimmed milk powder) for 1 h to eliminate nonspecific binding sites and then washed thrice in PBS. The strip was then incubated with 1:10000 dilution of GBNV polyclonal antibodyat 37^oC for 2 hours. The strip was washed thrice with PBST (PBS containing 0.1% v/v Tween 20) and wash with PBS to remove the unbound antibodies followed by incubation with goat anti-rabbit IgG (secondary) conjugated with horseradish peroxidase. The unbound antibodies were once again removed by washing and the blot was developed by incubating with di amino benzidine (DAB) in the presence of hydrogen peroxide, pH 4.8. Immediately after colour development, the membrane strip was washed in autoclaved double distilled water and dried (Hemalatha et al., 2008).

Results:

Survey and detection of the virus:

During the year 2014, the orthotospovirus infection was observed in the capsicum field in Byatha of Karnataka, India and typical symptoms of orthotospovirus infections observed were chlorotic and necrotic ring spots on leaves, stem necrosis, drying of twigs, bud necrosis and fruits showing chlorotic spots (Figure 1). Totally 5 orthotospovirus suspected samples were collected from Byatha and tested by DAC-ELISA for presence of serogroup IV orthotospoviruses. Total five samples were positive for serogroup IV orthotospoviruses. The ELISA positive samples using RT-PCR analysis with specific N-gene primes, followed by cloning and sequencing.

Figure 1 a) CaCV infected capsicum plant showing chlorosis on leaves **b) CaCV i**nfected fruit showing chlorotic spots

RT-PCR, cloning and CaCV N gene analysis:

RT-PCR amplification was carried out using the ELISA positive samples using the CaCVF/R primers resulted in an amplification of 895bp (Figure 2), was cloned and sequenced and sequence was deposited in GenBank under the accession number MN707988. The N gene sequence consisted of 828 nucleotides in viral complementary sense encoding 275 amino acids. 3′ UTR consisted of 67 nucleotides with terminal 10 nucleotide repeat region. Blast and pairwise analysis showed 95- 98.55% with the other available CaCV isolates in the GenBank database.

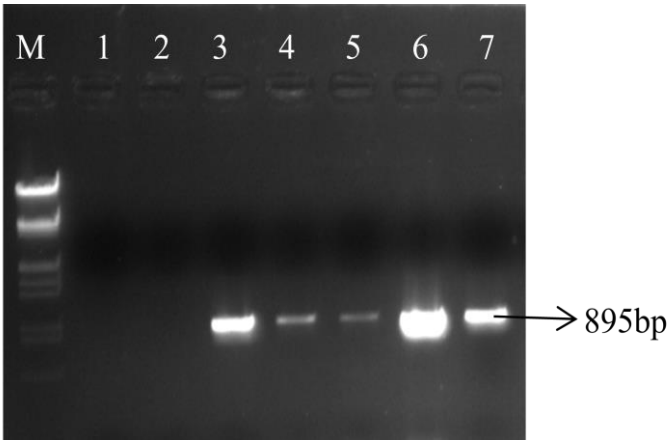

Figure 2: Agarose gel analysis of RT-PCR products from *capsicum chlorosis virus infected capsicum leaves Ngene* .Lane M : (Lambda DNA/EcoRI plus HindIII Marker), Lane 1: Negative control, Lane 2: Healthy Capsicum plant and Lane 3-7: Infected capsicum plants

The phylogenetic tree analysis of N gene sequences of CaCV isolate (Capsicum Byatha) with other CaCV isolates retrieved from GenBank and outgrouped with serogroup IV orthotospoviruse (GBNV, WBNV and WMSoV) isolates. The results reviled that the isolates formed majorly two clusters. In one of the major cluster the present study isolate capsicum Byatha (MN707988) shared with chilli Bangalore (EF625227) and Coimbatore (KU941832) (Figure 3).

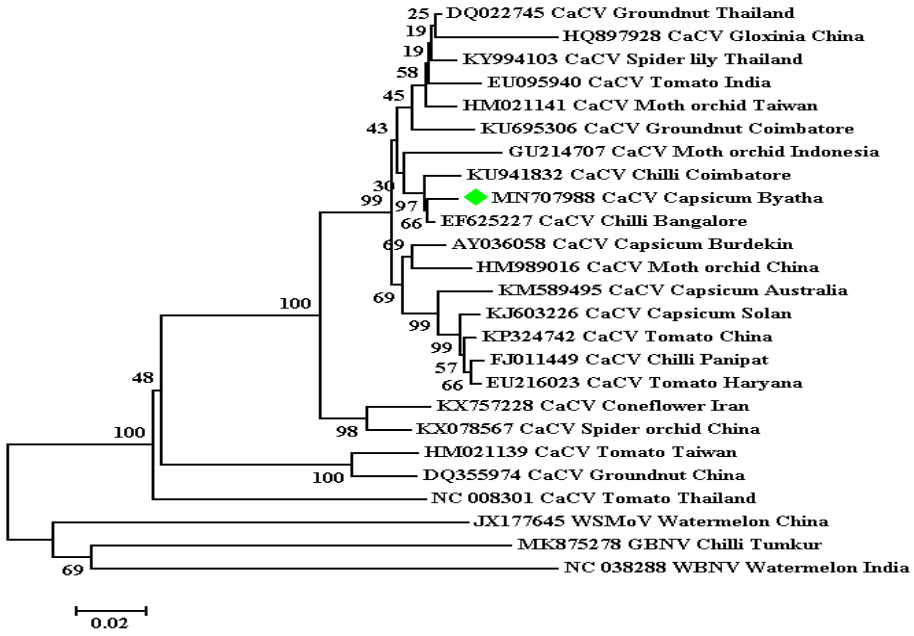

Figure 3: Phylogenetic tree analyses of CaCV N gene capsicum isolate with other isolates of orthotospoviruses using neighbor- joining method with 1,000 bootstrap replications.

Cloning, over expression and purification of CaCV recombinant protein:

The CaCV NP gene amplified using the CaCV sense and antisense primers with restriction sites, The amplified PCR product, 840bp (Figure 4) from the gel was eluted using GeneJET gel extraction kit (Thermo Fisher Scientific) as per manufacturer's protocol. The purified product from the gel was digested with *NheI* and *EcoRI* (Restriction Enzymes) cloned into *NheI* and *EcoRI* digested pRSETA (Invitrogen) expression vector (Figure 5). Identifiedclones were transformed into *E.coli* BL21 (DE3) pLysS, was screened and digested by restriction digestion using *NheI* and *EcoRI* (Restriction Enzymes). Amongdifferent concentrations of IPTG to enhance the protein expression, 0.3mM IPTG concentration was efficient for inducing the CaCVrNP as well as the protein observed in solubilization from. From The SDS-PAGE analysis indicated that the recombinant nucleocapsid protein molecular weight of (~32kDa) expected in soluble fraction in the *E. coli* BL21 (DE3) pLysS (Figure 6).

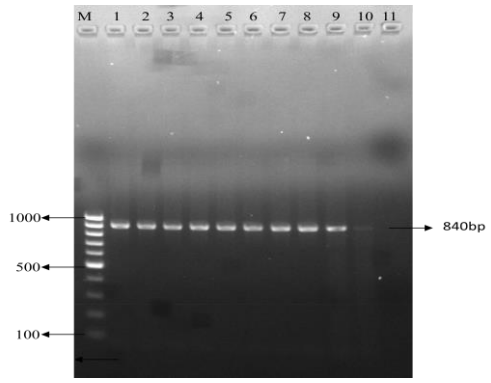

Figure4

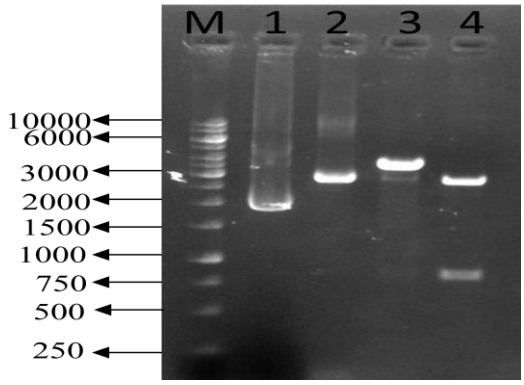

Figure5

Figure 4: Gel analysis of RT –PCR product CaCV capsicum isolates replica Lane: 1-10 and Lane 11: healthy, **Figure5:** M: Lader (1000bp), Lane1: Undigested pRSETA vector, Lane2: Digested pRSEA vector, Lane3: pRSETA-CaCV NP clone and Lane 4: digested pRSETA-CaCV NP

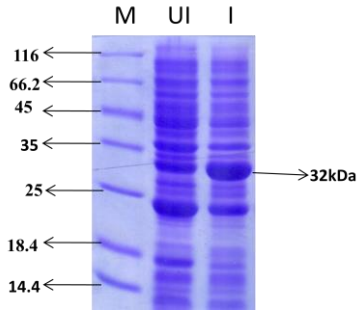

Figure 5

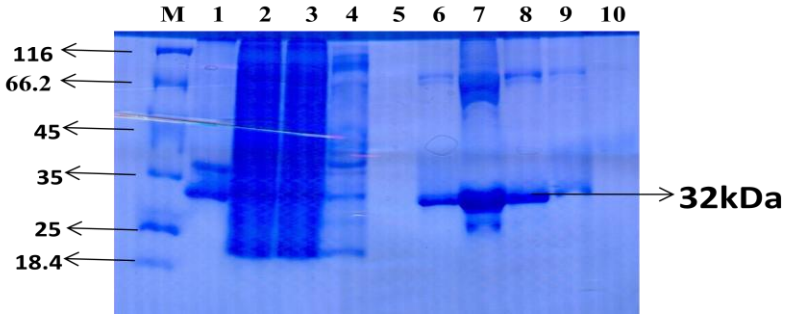

Figure 6

Figure 5 SDS-PAGE analysis for the expression of pSETA(+)-CaCV NP in BL21 *E. coli* BL21 (DE3) pLysS cells in Tris and Phosphate buffer, Lane M: Stained marker; Lane UI: Induced supernatant; I: Induced pellet

Figure 6 Lane M: Thermo scientific Pierce™ Unstained Protein MW Marker, Lane 1: soluble form, Lane 2: Flow through, Lane 3, 4: wash 1 and 2, Lane 5-10: Recombinant protein Elutes

Western blot analysis:

Western blot analyses revealed a strong band at position correspond to ~32kDa, which is the expected molar mass of CaCV rNP (Figure 7).

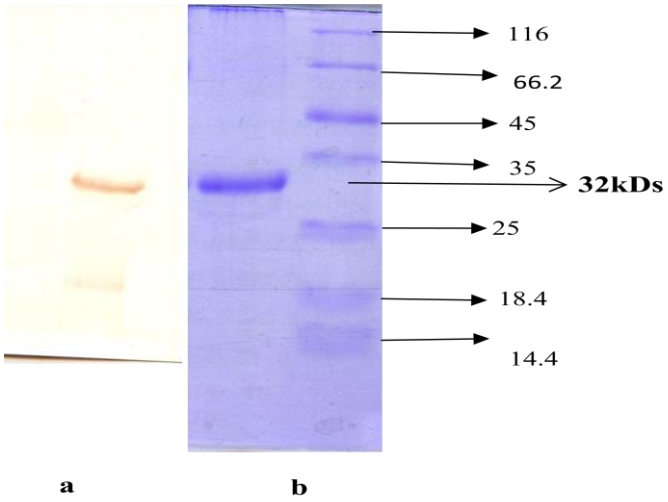

Figure 7: a) Western Blot **b)**Purified CaCV N gene Recombinant protein SDS-PAGE analysis

IC-RT-PCR:

IC-RT-PCR was successfully developed and tested for detection of CaCV infected different isolates. The PCR product (828bp) was amplified from all infected samples but no such amplification was observed from healthy plants (Figure 8).

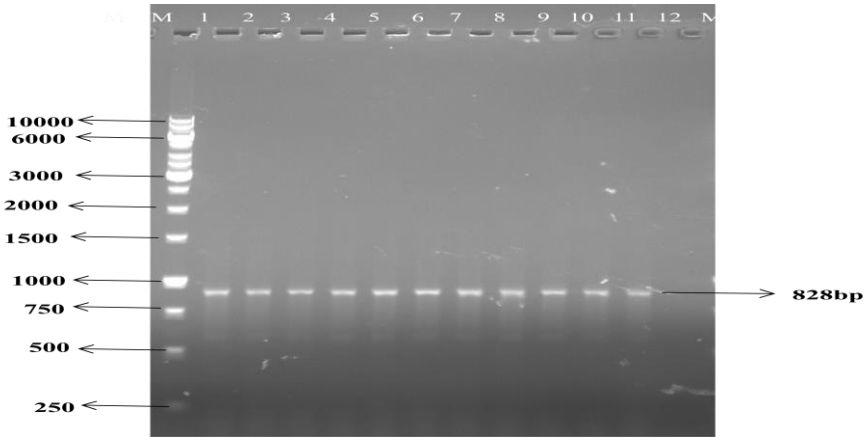

Figure 8: Agarose gel analysis of IC-RT-PCR products from *capsicum chlorosis virus* infected capsicum leaves

Dot immune biding assay (DIBA):

DIBA also indicated a strong purple colour development in the blotted areas of nitrocellulose membrane which was observed with the CaCV infected field capsicum as well as recombinant coat protein of CaCV and no blot on healthy samples of capsicum (Figure 9).

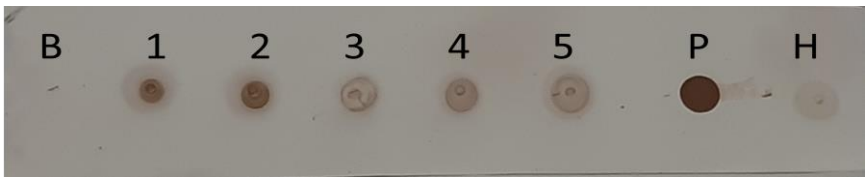

Figure 9: DIBA results of CaCV infected capsicum samples (1-5), P: CaCV rNP protein, H: healthy sample

Discussion:

Orthotospoviruses are serious disease causes in economic losses in tomato, chilli and capsicum and many other important crops worldwide (Whitfield et al.2005; Pappu et al.2009; Kunkalikar et al.2011 and Turina et al. 2016). Capsicum chlorosis virus was first reported in India on tomato (Kunkalikar *et. al.,* 2007), chilli (Krishanareddy *et. al.,* 2008), Amaranthus. sp (Sharma and Kulshrestha2014), bell pepper in Himachal Pradesh (Yogitha Bhora *et. a.,* .2016), chilli in Tamil Nadu (Haokip *et. al,* 2016) and groundnut in Tamil Nadu (Vijayalakshmi et al.2016) in different locations. Earlier it was reported other than India in tomato and capsicum in Australia and Thailand, peanut in Thailand, tomato in China and orchids in China (McMichael *et. al.,* 2002; Hassani-Mehraban et al. 2016; Knierim et al. 2006). CaCV was first reported in Australia and different from the Indian isolates by host range (Chiemsombat *et. al.,* 2008) and genome sequence (Gamage *et. al.,* 2015) but CaCV host range is similar to the GBNV host range (Jones and Sharma 2005; Kunkalikar *et. al.,* 2007). The variation in symptom due to the virus isolate, cultivar and environmental conditions (Bayat 2018., Lamas Lamas 1998). The report of more than 20 per cent disease incidence in several commercial fields of chilli pepper near Bangalore in Karnataka state constituting of chlorotic and necrotic spots and rings on leaves, apical necrosis and leaf distortion symptoms observed and identifies as a capsicum chlorosis virus (Krishna Reddy et al., 2008) and also the repot of the disease in chilli and tomato that exhibited spotted wilt like symptoms varied from 10 to 15% across different locations and attributed them to Capsicum chlorosis virus on the basis of molecular studies (Kunkalikar et al., 2010) and in recently bell pepper crop is reported by a novel tospovirus i.e capsicum chlorosis virus causing losses up to 81% (Yogita Bhora et. al., 2019). The report of CaCV on groundnut infected plants showing the symptoms were observed in 40 to 45day old groundnut plants and included chlorotic and necrotic spots, rings on the leaves, and necrosis on the leaflets (Vijayalakshmi *et al.,* 2016). An inadequate information on CaCV from the literature, the producedrecombinant CaCV NP gene from south India and shall be useful for the production of polyclonal and monoclonal antibodies.

During the field survey (2014) in Karnataka for orthotospovirus like infection were found in the Byatha capsicum field. The infected capsicum samples were detected by DAC-ELISA using serogroup IV orthotospoviruses and the incidence of 10 per cent was observed. Further, detection of species of the orthotospovirus present in the ELISA positive samples using RT-PCR analysis with species specific N-gene primers, followed by cloning and sequencing revealed that, 100% samples were positive for CaCV. Like these finding earlier reported by Krishna Reddy *et. al.,* (2008), Kunklikar *et. al*., (2010), Yogitha Bhora *et. al.,* (2016) and Haokip et. al., (2016).

Typical symptoms of infections of orthotospoviruses observed during the study were chlorotic and necrotic ring spots on leaves and chlorotic ring spots on fruits. Similar types of symptoms have been reported in Karnataka, Tamil Nadu, Himachal Pradesh and Haryana (Krishnareddy*et. al.,* (2008), Kunkalikar *et. al*., 2011, Dar et al 2013; Yogitha Bhora et al. 2016 and Haokip et. al., 2016). In the present study, molecular characterization of CaCV N gene along with 3' UTRs revealed that, the N gene sequence consisted of 828 nucleotides in viral sense encoding 275 amino acids and 3' UTR consisted of 67 nucleotides.

Further, the sequence blast and pair wise analysis ranged between 95- 98.55% with the other available CaCV isolates in the GenBank database.

In the present study, CaCV NP gene was isolated from the Byatha, Karnataka was expressed in the prokaryotic expression system. Prokaryotic expression is valuable and stable nature of expression system for the production of good quality recombinant protein (Yeh *et al*., 1996; Chu *et al*., 2001; Chen *et al*., 2005; Jain *et al*., 2005; Lin *et al*., 2005; Wu *et al*., 2009; Khatabi *et al*., 2012 and Wu *et al*., 2014).Theexpressed recombinantprotein fromCaCV NP gene showed molecular weightof ~32kDa in SDS-PAGE and also proved in western blot using the GBNV polyclonal antibody. However, the GBNVNP antibodies were serologically detecting serogroup IV orthotospoviruses (CaCV, WBNV). Earlier report was conformed the GBNV N gene antibodies serologically detect the WBNV (Jain et al., 2005).

Detection of viruses through molecular assay such as PCR, RT-PCR, IC-RT-PCR and sequencing is costly as well as time-consuming with requirement of equipments. Moreover, DIBA is a simple, sensitive, economical cheaper, less time-consuming without the requirement of any instruments and results can be viewed visually for detection of viruses. The developed recombinant protein fromCaCV NP gene andits detectionwould be useful for the detections of viruses from the field samples.In addition to this, the expressed recombinant protein may be used for further development of polyclonal and monoclonal in the future.

Acknowledgement:

The authors acknowledge the funding from Department of Biotechnology, Government of India, under BioCARe (BT/Bio-CARe/02/290/2011–12) women scientist project.

References:

1. *Anonymous, Horticultural Statistics at a glance. Ministry of Agriculture and Farmers welfare, 2014. http://www.nhb.gov.in.*
2. *Anonymous, Horticultural Statistics at a glance. Ministry of Agriculture and Farmers welfare, GOI, 2018, 514p.*
3. *H. Bayat, A. Hassani-Mehraban, N. Safaei and M. Shams-bakhsh, "Molecular and biological characterization of an isolate of capsicum chlorosis virus from Iran", Jounal of Plant Pathol,. Vol.100(2), (2018), pp. 163-170. https://doi. org/10.1007/s42161-018-0047-5*
4. *M. F. Clark andA. N. Adams, "Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses" Journal of Gen. Virol, vol. 34, (1977), pp. 475– 483.*
5. *T. C. Chen, H. T. Hsu, R. K. Jain, C. W. Huang, C. H. Lin, F. L. Liu, and S. D. Yeh, "Purification and serological analyses of tospoviral nucleocapsid proteins expressed by Zucchini yellow mosaic virus vector in squash", J. Virol. Methods, vol. 129, (2005), pp.113-124.*
6. *K. Chen, Z. Xu, L. Yan and G. Wang, "Characterization of a new strain of Capsicum chlorosis virus from Peanut (Arachis hypogaea L.) in China" Journal of Phytopathology , vol. 155, (2007b), pp. 178-181*

7. *T. C. Chen, J.T. Li , Y. P. Lin, Y. C. Yeh, Y. C. Kang, L. H. Huang and S. D. Yeh, "Genomic characterization of calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses" Journal of Plant Pathol, vol. 61, (2012), pp.183–194.*
8. *Y. Chiaki, K. Kubota, Y. Tomitaka, T. Usugi and T. Sakurai, "Transmission of capsicum chlorosis virus by Thrips palmi (Thysanoptera: Thripidae)", Journal of Appl EntomolZool, (2019),1–5. https://doi.org/10.1007/ s13355-019-00649-7.*
9. *P. Chiemsombat, O. Gajanandana, N. Warin, R. Hongprayoon, A.Bhunchoth, A. and P. Pongsapich,"Biological and molecular characterization of tospoviruses in Thailand",Journal of Arch. Virol., vol. 153, 2008, pp. 571–577.*
10. *M. F. Clark and M. B. Joseph, "Enzyme immunosorbent Assays in Plant Virology", Maramorosch, K. and H. Koprowski (Eds.), Academic Press, New York, USA. Vol.7, (1984), pp. 511-58.*
11. *N. A. Dar, A. K. Handa, M. I. Jeelani, A. Hamid and M. A. Bhat, "Status, symptomatology and partial characterization of virus causing ringspot disease in bell pepper (Capsicum annuum L.) in Himachal Pradesh", Global Journal of Medical Research, vol. 13, (2013), pp. 48-55.*
12. *B. D. Haokip, D. Alice, V.G. Malathi, K. Nagendran, P. Renukadevi and Karthikeyan, "Detection of Capsicum chlorosis virus (CaCV), an emerging virus infecting chilli in Tamil Nadu, India", Journal of Vegetos. Vol. 29, (2016), pp.4.*
13. *V. Hemalatha, G. Pradnya, A. K. Anjali, M. Krishnareddy, H. S. Savithri, "Monoclonal antibodies to the recombinant nucleocapsid protein of a Groundnut bud necrosis virus infecting tomato in Karnataka and their use in profiling the epitopes of Indian tospovirus isolates", Journal of Curr Sci. vol. 95, (2008), pp. 952–957.*
14. *R.K. Jain and A.I. Bhat AI,"Virus resistant transgenic plants for environmentally safe management of viral diseases", Indian Journal of Biotechnology. Vol.1 (2002), pp. 73-86.*
15. *R. K. Jain, N. A. Pandey, M. Krishnareddy and B. Mandal, "Immunodiagnosis of groundnut and watermelon bud necrosis viruses using polyclonal antiserum to recombinant nucleocapsid protein of Groundnut bud necrosis virus", J Virol Methods. 130, (2005), pp.162–164.*
16. *B. Khatabi, B. He, and M. R. Hajimorad "Diagnostic Potential of Polyclonal Antibodies Against Bacterially Expressed Recombinant Coat Protein of Alfalfa mosaic virus", Journal of plant disease. Vol.96 (2012), pp.1352-57.*
17. *M. Krishnareddy, R. Usha Rani , A. Kumar A, K. Madhavireddy K and Pappu HR, "First report of Capsicum chlorosis virus (Genus: Tospovirus) infecting chilli pepper (Capsicum annuum) in India", Journal of Plant Dis. vol. 92, (2008), pp.1469.*
18. *D. Knierim, R. Blawid and E. Maiss,"The complete nucleotide sequence of a Capsicumchlorosis virus isolate from Lycopersicum esculentum in Thailand", Journal of Archives of Virology , vol.151(9), (2006), pp.1761-1782.*
19. *S. Kumar, G. Stecher and K. Tamura, "MEGA7: molecular evolutionary genetics analysis (MEGA) software version 7.0 for bigger datasets", jornal of Mol. Biol. Evol. vol.33, no.7, (2016),pp. 1870–1874.*
20. *S. R. Kunkalikar, P. Sudarsana, P. Rajagopalan, U. B. Zehr,R.A .Naidu and K. S. Ravi, "First report of Capsicum chlorosis virus in tomato in India" Plant Health Prog., 2007. doi:10.1094/PHP-2007-1204-01-BR*

21. *S. R. Kunkalikar, S. Poojari, B. M. Arun, P. A. Rajagopalan, T. C. Chen, S. D. Yeh, R. A. Naidu, U. B. Zehr and K. S. Ravi, "Importance and genetic diversity of vegetable infecting tospoviruses in India", Journal of Phytopathology. Vol. 101, (2011), pp. 367-376.*
22. *S. R. Kunkalikar, P. Sudarsana, P. Rajagopalan, U. B. Zehr and K. S. Ravi, "Biological and molecular characterization of Capsicum chlorosis virus infecting chilli and tomato in India", Journal of Arch Virol. vol. 155, (2010), pp.1047–1057.*
23. *B. Mandal and R. K. Jain, "ELISA kit for tospoviruses detection" ICAR News vol. 16, (2010) , pp.13.*
24. *L. A. McMichael, D. M. Persley and J.E. Thomas, "A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia" Journal of Aust Plant Patho, vol. l 31, (2002), pp. 231–239.*
25. *M. J. Melzer, J. Shimabukuro, M. H. Long, S. C. Nelson, A. M. Alvarez AM et al, "First report of capsicum chlorosis virus infecting waxflower (Hoya calycina Schlecter) in the United States", journal of Plant Dis.vol. 98(4), (2014), pp. 571-571. https://doi.org/10.1094/PDIS-06-13-0588-PDN.*
26. *G. Nolasco, C. de Bias, V. Torres et al, "A method combining immunocapture and PCR amplifi cation in a microtiter plate for the detection of plant viruses and subviral pathogens", J Virol Meth. Vol.45, (1993) , pp. 201–218.*
27. *C.G. Orfanidou, A. Boutsika, G. Tsiolakis, S. Winter, N.I. Katiset al, "Capsicum chlorosis virus: a new viral pathogen of pepper in Greece", journal of Plant Disease,vol.103(2) (2018). https://doi.org/10.1094/PDIS-06-18- 0961-PDN.*
28. *H. R. Pappu, R. A. C. Jones and R. K. Jain, "Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead" Journal of Virus Res. Vol.141,(2009), pp. 219-236.*
29. *A. Plyusnin, B. Beaty, R. Elliott, R. Goldbach, R. Kormelink, A. Lundkvist, C. Schmaljohn and R. Tesh, " Family Bunyaviridae. In: A. M. King, M. J. Adams, E. J. Lefkowitz, E. B. Carstens (eds) Virus taxonomy", Ninth report of the international committee on taxonomy of viruses. Elsevier Academic Press, San Diego, (2012), pp. 725-741.*
30. *P. Renukadevi, K. Nagendran, S. Nakkeeran, G. Karthikeyan, M. Jawaharlal, D. Alice, V. G. Malathi and H. R. Pappu, "First report of Tomato spotted wilt virus infection of chrysanthemum in India" journal of Plant Dis. vol. 99(8), (2015b), pp. 1190.*
31. *J. Sambrook, E. F. Fritschi and T. Maniatis, "Molecular cloning: a laboratory manual", Cold Spring Harbor Laboratory Press, New York. (1989).*
32. *Sambrook, J., Russel, D.W., 2001. Molecular Cloning, a Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York.*
33. *A.Sharma and S. Kulshrestha, "First report of Amaranthus sp. as a natural host of Capsicum chlorosis virus in India", journal of Virus Disease. Vol. 25, (2014), pp.412-413.*
34. *O. P. Singh, N. Anand and A. H. Deshpande, "Improvement of bell pepper. In: Advances in Horticultureed. By KL Chadha and G Kalloo, MPH, New Delhi.,vol.5, (1993), pp. 87-104.*
35. *D. S. Sreedhara, M. G. Kerutagi, H. Vasabaraja, L. B. Kunnal and M. T. Dodamani, "Economics of capsicum production under protected conditions in Northern Karnataka", Karnataka Journal of Agriculture Science.vol.26, (2013), pp.217-219.*

36. *H. Towbin, T. Staehelin andJ. Gordon, "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications", Journal of Proc. Nat. Acad. Sci. U. S. A, vol. 76 (9), (1979), pp. 4350–4354.*
37. *M. Turina, R. Kormelink and R. O. Resende, "Resistance to tospovirusus in vegetable crops: epidemiological and molecular aspects", Journal of Annu Rev Phytopathol. Vol.54, (2016), pp. 347–371.*
38. *G. Vijayalakshmi, B. D. Haokip, G. Karthikeyan, D. Alice, N. Ganapathy, G. Asokan, L. Rajendran, J. S. Kennedy, "First report of field infection of Capsicum chlorosis virus on groundnut(Arachis hypogaea) in India (Tamil Nadu)", Journal of Plant Dis. vol. 100(11), (2016), pp. 2339.*
39. *A. E. Whitfield, D. E. Ullmanand T. L. German, "Tospovirus-Thrips Interactions", journal of Annu Rev Phytopatholvol.43 (2005), pp. 459-489.*
40. *S. Widana Gamage, D. M. Persley, C. M. Higgins andR. G. Dietzgen, "First complete genome sequence of a capsicum chlorosis tospovirus isolate from Australia with an unusually large S RNA intergenic region", Journal of Arch Virol, vol. 160(3), (2015), pp. 869–872. https://doi.org/10.1007/s00705-014- 2324-8*
41. *J. X. Wu, C. Yu, C.Y. Yang, F. L. Deng and X. P. Zhou, "Monoclonal antibodies against the recombinant nucleocapsid protein of tomato spotted wilt virus and its application in virus detection", J. Phytopathol. Vol. 157 (2009.), pp. 344–349.*
42. *S. D. Yeh, I. J. Sun, H. M. Ho and T. F. Chang, "Molecular cloning and sequence analysis of the S RNAof Watermelon silver mottle virus", journal of Acta Hort . vol. 431, (1996), pp. 244–259*
43. *B. Yogita and H. Anil, "Molecular Characterization and Detection of viris Associated with Ring Spot Disease of Bell Pepper (capsicum annum L.)", J Mycol Pl Pathol.vol. 46(4), (2016), pp. 273-286.*
44. *Y. X. Zheng, C. C. Chen and F. J. Jan, "Complete nucleotide sequence of capsicum chlorosis virus isolated from Phalaenopsis orchid and the prediction of the unexplored genetic information of tospoviruses" journal of Arch Virol, vol. 156,* (2011), pp. 421-432.