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Detection and Molecular Characterization of Chilli Veinal Mottle Virus (ChiVMV) in Chili from Karnataka, India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The study aimed to characterize viral infections affecting chili plants in Karnataka, India, addressing the significant agricultural challenge posed by these infections. To tackle this problem, the study utilized multiple diagnostic methods viz., Transmission electron microscopy (TEM), Direct Antigen Coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA and Polymerase Chain Reaction (PCR) using specific. Transmission electron microscopy revealed the presence of flexuous rod-shaped particles, indicative of potyviruses, in symptomatic leaf dip preparations. DAC-ELISA further confirmed the presence of potyvirus across all tested chili samples, with varying absorbance values among locations, highest in Masanige (1.66) and lowest in Dharwad (0.89).

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Positive reactions were consistent across all infected samples, contrasting with healthy and buffer controls. PCR using specific primers amplified a ~1.2 kb fragment from all diseased samples, confirming the presence of Chilli veinal mottle virus (ChiVMV). This comprehensive analysis underscores the prevalence and distribution of potyviruses, particularly ChiVMV, in chili crops in Karnataka, highlighting the importance of continued surveillance and management strategies to mitigate viral impact on agricultural productivity.

Keywords: Chilli; chilli veinal mottle virus; ELISA; potyvirus; PCR; TEM.

1. INTRODUCTION

The popularity of chilli peppers has grown dramatically in recent decades, with hotter varieties leading the surge. An estimated 25% of the world's population now incorporates chillies into their daily meals [1]. Roughly two-thirds of global chilli production comes from Asia [2]. Chilli cultivation offers significant economic advantages for small-scale farmers, boosting their income and improving their social and economic standing [3,4].

Among the various viruses that plague chilli plants, *Chilli Veinal Mottle Virus* (ChiVMV) stands out as the most damaging and widespread, particularly in East Asia and certain African nations. The presence of ChiVMV infection has been documented in numerous countries, including Indonesia [5], Papua New Guinea [6], China [7] and parts of West and East Africa [8]. Classified as a positive-sense single-stranded RNA (+ssRNA) virus, ChiVMV belongs to the Potyvirus genus within the Potyviridae family [9]. Beyond *Capsicum annuum*, ChiVMV is known to infect a range of other plants in the Solanaceae family, including tobacco, tomato, eggplant and Jimson weed [10-14]. The characteristic signs of ChiVMV infection include mottled leaves with a mosaic pattern, distorted or drooping leaves, visible bands along the veins, and stunted fruit development [10,15]. The genetic makeup of ChiVMV is comprised of a single-stranded RNA molecule, about 9.7 kilobases in length excluding the poly(A) tail. This RNA encodes a polyprotein, which is subsequently cleaved by viral proteases into ten functional mature proteins [12,16]. The aphid *Aphis gossypii* has been identified as a non-persistent transmitter of the virus among solanaceous crops [16].

Symptoms of viral diseases include crinkling, browning of leaf tissues, mosaic, necrosis *etc*. However, diagnosing viral diseases based on symptoms is more challenging compared to other pathogens [17]. This difficulty arises because plants also exhibit virus-like symptoms due to various factors such as unfavorable weather, nutritional imbalances, infections by other pathogens or other abiotic agents [18]. In recent decades, a lot of methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods. These methods are rapid and precise and significantly advanced for detection of plant viruses and helpful to farmers for taking management interventions at right time. This study primarily aiming at identifying virus associated typical symptoms observed on chilli crop such as mottled leaves with a mosaic pattern, distorted or drooping leaves, and visible bands along the veins.

2. MATERIALS AND METHODS

Symptomatic chilli plants showing typical symptoms viz., mottled leaves with a mosaic pattern, distorted or drooping leaves, visible bands along the veins (Fig. 1) were collected from major chilli growing districts like Belgaum, Dharwad, Haveri and Gadag of Northern Karnataka, India and used for detection of ChiVMV using DAC-ELISA and PCR.

2.1 Detection of Potyvirus through Electron Microscopy and DAC-ELISA

Fresh symptomatic leaves from the infected chill plants were collected and cut into a small section (about 1 cm^2) from the symptomatic leaf using a clean scalpel or razor blade. The leaf tissue was gently ground the in the drop of Phosphate buffer. This will release the viral particles into the solution. Following which, carbon-coated copper grid, shiny side down, placed onto the drop of the leaf extract and made to sit in the extract for 1-2 minutes to allow the viral particles to adsorb to the grid surface. Immediately after washing with drop of buffer solution to remove any unbound material touch the grid to a drop of 2% (w/v) aqueous uranyl acetate for negative staining. Once the grid was dry, it was kept under transmission electron microscope for particle morphology (Transmission Electron

Microscope facility, Indian Institute of Horticulltural Research, Benguluru, used magnification 100,000x).

A direct antibody coating enzyme linked immuno sorbent assay (DAC-ELISA) technique (Hobbs et al*.,* 1987) was employed for serological detection of the potyvirus associated with diseases field samples of chilli using commercially purchased polyclonal antibodies raised against *Tobacco etch virus* (TEV). The assay was carried out in 96-well polysterene microtitre plate. Positive controls *(Poty virus* Y supplied by manufacturer (Agida) and papaya sample infected with *Papaya ring spot virus)* healthy controls as well as buffer controls were maintained. The mean absorbance values were measured at 405 nm wavelengths. The absorbance values (optical density, OD) are directly proportional to the virus concentrations.

2.2 Detection of Chilli Veinal Mottle Virus through PCR

Total RNA isolation: Qiagen, Germany Kit (ID No.:74534) was used for the isolation of total RNA from the diseased leaf tissue. Virus infected leaf sample was taken and ground to powder in a pestle and mortar using liquid nitrogen. About 50 mg of powder was taken into 450 µl of buffer RLT was added and procedure mentioned in kit was followed for extraction of total RNA. Finally, RNA was eluted by transferring the Rneasy column into a new 1.5 ml eppendorf tube and eluted out using 30-50 µl of Rnase free water by centrifugation for 1 minute at 10000 rpm. The RNA was stored at – 20^0C.

Reverse transcription: Potyviral cDNA was synthesized using Oligo (dT) (5' GCGGGATCC TTTTTTTTTTTTTTTTT-3') as downstream primer. Three microlitres of extracted RNA was used for viral cDNA synthesis following a modified protocol for *Moloney Murine Leukemia Virus* reverse transcriptase (M-MLVRT, Pomega), using 0.375 mM dNTP, 20 U RNase inhibitor, 100 U M-MLV-RT, and incubation at 42^0C for 120 min.

PCR amplification using Degenerate primers for potyvirus (ChiVMV): PCR amplification was performed using CVMV1037Pol (the ChiVMV polymerase primer, 5'-AGCATGGAGAGA GCGACATTAGTC-3') for the coat protein of pepper and tomato potyviruses, as upstream primer and Oligo (dT) (5'-GCGGGATCC TTTTTTTTTTTTTTTTT-3') as the downstream primer. The primer pair CVMV1037 Pol/Oligo (dT) was designed to amplify the 3'-end of ChiVMV genomic c DNA including 3' terminus of the polymerase (Nlb) gene, the CP gene and the 3'-UTR [10]. The components in PCR reaction were standardized and followed as given below in order to amplify a single sharp amplicon. Corbett Palm Cycler (JH Bio) was used to run the PCR programme for amplification. The optimised amplified conditions with 35 cycles of denaturation at 94^0C for 1 min, primer annealing at 58^0C for 1 min and extension at 72^0C for 2 mins and final extension at 72^0C for 5 mins.

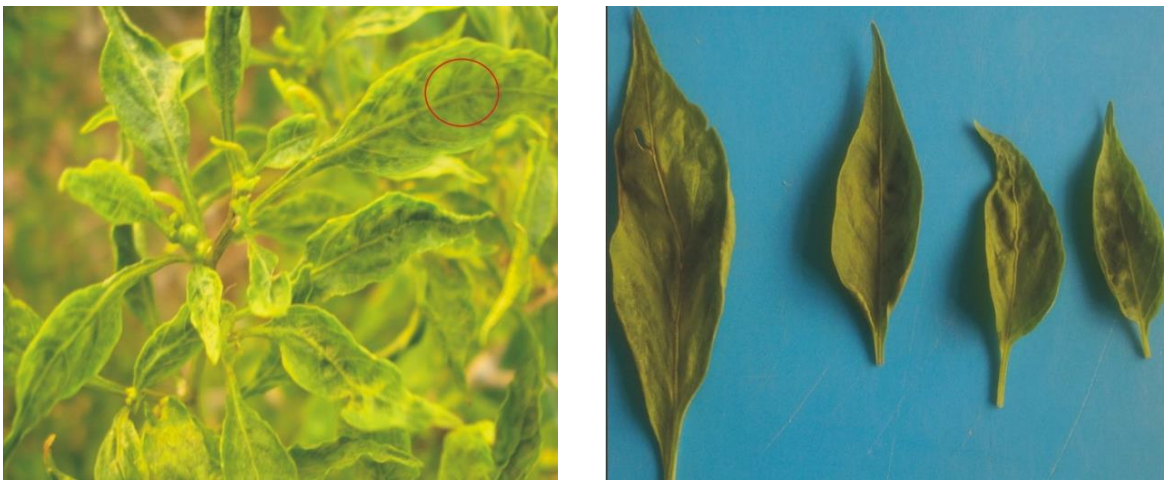


Fig. 1. Chilli leaves showing vein banding, veinal necrosis and green mottling

List 1. PCR amplification using Degenerate primers for potyvirus

Components	Concentrations	Volume (µl)
		Potyvirus
Template cDNA	-	1
Taq assay buffer Awith 15mM MgCl2	10x	2
dNTPs	2.5 mM each	2.5
Forward primer	10 pM	1
Reverse primer	10 pM	1
Taq DNA polymerase	1 U/ µl	1
Nuclease free water to final volume (20 µl)		11.5

3. RESULTS

3.1 Particle Morphology of Virus

Leaf dip preparations of infected symptomatic samples were stained with uranyl acetate and observed under transmission electron microscope. No geminate (icosahedral) particles were observed in partially purified preparations indicating that the absence of begomoviruses. Similarly, quasi-spherical enveloped particles characteristic of tospoviruses were not noticed in dip preparations. Whereas, typical flexuous rod shaped particles measuring about 700 nm were observed in leaf dip preparations which reveals that the presence of potyvirus (Fig. 2).

3.2 DAC-ELISA

The absorbance values revealed that all the infected chilli samples that were brought from field showed positive reaction for TEV antisera. Highest absorbance for potyvirus was recorded in chilli sample brought from Masanige (1.66) of Haveri district followed by Hebbali (1.44) and Shalavaddi (1.34) and least absorbance value was found in sample brought from Dharwad (0.89) (Table 1).

3.3 PCR Detection of Chilli Veinal Mottle Virus

A set of primers CVMV1037Pol/Oligo (dT) designed to amplify the 3'-end of ChiVMV genomic cDNA including the 3' terminus of polymerase (NIb) gene, the CP gene and the 3'-UTR were used at optimized 0.5 pM of final concentration and 58^0C annealing temperature keeping all other components of PCR reaction constant. The predicted ~1.2 kb DNA fragment was amplified in all diseased samples (Fig. 3). The resulted sequences approximately 1200 -1300 nucleotides in length. No PCR product was obtained in uninfected control samples.

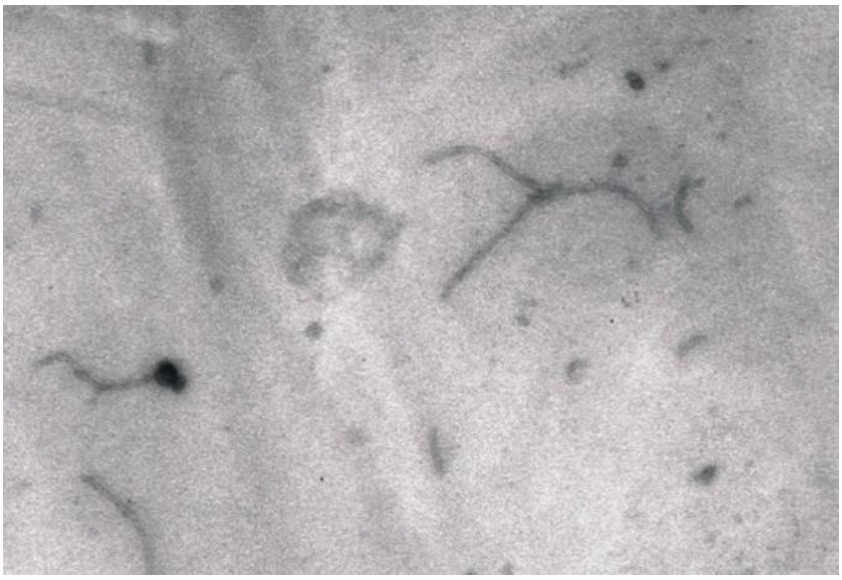
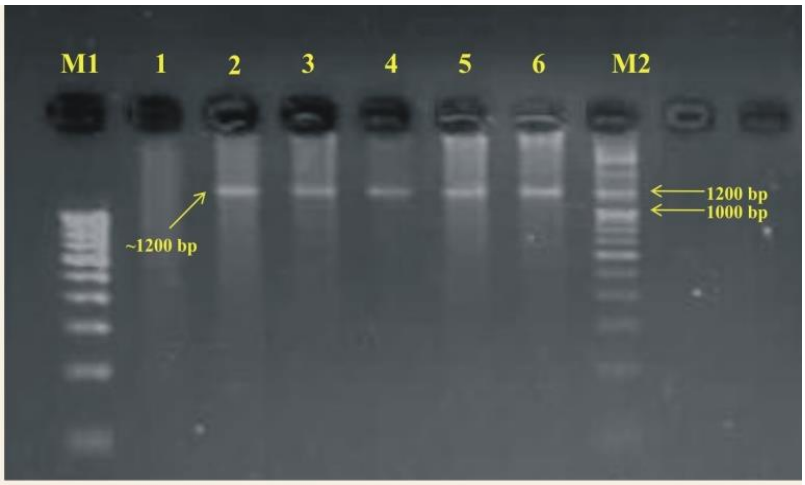


Fig. 2. Electron micrograph showing flexous particles of potyvirus (18000x)

Table 1. DAC-ELISA absorbance values of chilli murda samples for potyvirus (ChiVMV)

Sl. No.	Samples	Location	OD value (405 nm)	Reaction + / -
1	Chilli	Hebbali farm (Dharwad district)	1.44	+
2	Chilli	Masanige (Haveri district)	1.66	+
3	Chilli	Shalavaddi (Gadag district)	1.34	+
4	Chilli	UAS Campus (Dharwad district)	0.89	+
5	Positive control (Potyvirus, Agida)	-	1.21	+
6	Positive control, Papaya (PRSV)	UAS Campus (Dharwad district)	1.45	+
7	Healthy control, Chilli	UAS Campus (Dharwad district)	0.31	-
8	Buffer control (PBS-T)	--	0.23	-

Note: "+"= Positive reaction, "–" = Negative reaction



Lane Index : M1 - 100 bp marker, 1 - Healthy chilli sample,
2, 3, 4 & 5 - Diseased chilli samples,
6 - Dried chilli plants with symptomatic new flush and
M2: DNA medium range ruler

Fig. 3. RT-PCR analysis of chilli samples for potyvirus (ChiVMV)

4. DISCUSSION

Hussain et al. [19] reported Chilli veinal mottle virus (ChiVMV) as one of the prevalent chilli-infecting viruses found throughout chilli growing areas of Pakistan and observed 44.7 per cent relative occurrence of the virus in the country during 2003 and 2004. Individual plants occurring sporadically showing chlorotic, necrotic spots symptoms on leaves with typical apical necrosis were also made by Krishnareddy et al. [20]. The symptomatic plants collected for the present investigation were similar to above mentioned works which are typical for ChiVMV.

Typical flexuous rod shaped particles measuring about 700 nm were detected in all samples through electron microscopy. These observations are in conformity with Ravi et al. [21] who reported for PVBV as flexuous rods with an average length of 900 nm in pepper with mosaic symptoms and Gundannavar [22] and Raju [23] who also stated electron microscopy examination of chilli leaf curl samples revealed flexuous rods (Poty virus). The virion particle morphology of members of potyviridae family consists non-enveloped with a flexuous and filamentous nucleocapsid, 680 to 900 nanometers (nm) long and is 11–20 nm in diameter [24]. Thus, our

study initially identified the major viral group by analyzing particle morphology, which revealed the presence of flexuous rod-shaped particles [25,26].

Potyvirus was detected by DAC-ELISA in all samples with TEV antiserum utilizing crude sap of the samples. However, the value of absorbance was varied from sample to sample. This may be due to variation in the concentration of the virus in the infected samples. This variation in concentration may be due to varietal difference and also due to age of the crop. Previously, Siriwong et al. [27] also observed *chilli vein-banding mottle virus* serologically related to *Chilli veinal mottle potyvirus* from Malaysia and had a distinct serological relationship with *Tobacco etch virus*. Thus our study identified that the PAbs produced against TEV can be also used for detection of ChiVMV.

The most sensitive diagnostic tool polymerase chain reaction (PCR) was performed for detection of the above three referred viruses at genus level. A set of primers CVMV1037Pol/Oligo (dT) designed to amplify the CP gene of *Chilli veinal mottle virus* were used in RT-PCR against all diseased isolates. The predicted ~1.2 kb DNA fragment was amplified in all murda infected samples with no amplification in healthy control. The present findings are in accordance with Tsai et al. [10] who used same set of primers for detection and also studied complete sequence of ChiVMV infecting peppers in India and reported that it shared more than 94.8% nucleotide identity with previously accomplished full length sequence of pepper vein banding virus (PVBV). Ravi et al. [21] compared the N-terminal sequence of PVBV CP with all the potyviral CP sequences and observed the DAG sequence conserved in all aphid-transmitted potyviruses in the N-terminal sequence of the PVBV CP. Similar observations of conserved DAG motif was also made by Tsai et al. [10] in all ChiVMV isolates including Indian isolate. Vector-virus relationship revealed that among the insects, only aphids, Aphis gossypii and Myzus persicae were able to transmit the virus from diseased to healthy chilli seedlings and virus was identified as Potyvirus. Thus, the present investigation revealed that *chilli veinal mottle virus* is the distinct virus of the genus *Potyvirus* associated with symptomatic chilli and may be transmitted by aphid species.

5. CONCLUSION

The combined evidence from transmission electron microscopy (TEM), Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAC-ELISA), and PCR analysis strongly suggests that *chilli veinal mottle virus* which comes under *Potyvirus* genus was the only virus associated with the particular symptomatic chilli plants under study in Karnataka.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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