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Molecular characterization of bell pepper (*Capsicum annuum* L.) infecting *Capsicum chlorosis orthotospovirus*

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Abstract
In 2023, surveys were carried out in key bell pepper cultivation regions of the Andhra Pradesh district to gather virus isolates for analysis. The virus responsible was identified based on its symptoms, how it spreads, and through serological and molecular investigations. Affected plants exhibited stunted growth, dwarfism, and concentric chlorotic ringspot symptoms on their leaves. Infected bell pepper fruits showed deformities with nailhead symptoms, or ripe fruits displayed pale yellow ringspots of various colours. Samples showing characteristic symptoms were gathered and employed to inoculate healthy bell pepper plants. Serological testing using Tospo (I, II, III) and CaCV antisera via DAS-ELISA identified the virus as *Capsicum chlorosis orthotospovirus* (CaCV), as it reacted solely with CaCV antisera. Further molecular analysis, including RT-PCR, confirmed the virus's identity as *Capsicum chlorosis orthotospovirus*.

Keywords: Capsicum, *Capsicum chlorosis virus*, coat protein gene and diversity analysis

Introduction
Capsicum (*Capsicum annuum* L.), originating from Central and South America, holds a crucial position among vegetables within the Solanaceae family. Its worldwide cultivation represents a lucrative sector (Bosland and Votava, 2000) [4]. Nonetheless, bell pepper cultivation faces substantial hurdles globally, attributed to a range of environmental and biological factors, including diverse plant pathogens. Viral infections, in particular, pose significant threats to bell pepper crops, resulting in substantial losses both in experimental settings and actual fields. These infections inflict severe damage annually, leading to considerable financial losses for farmers.

An extensive review of literature concerning the prevalence of various viruses infecting bell peppers in different regions worldwide revealed reports of at least 67 viruses belonging to 25 genera affecting the bell pepper crop (Pernezny *et al.*, 2003) [21]. Out of these, about 20 types of viruses spanning 15 different taxonomic categories have been identified as causing notable damage to the cultivation of bell peppers. Notably, one of the most detrimental virus groups impacting bell peppers is the Orthotospovirus genus, which infects different types of horticultural crops across Tropical, Subtropical, and Temperate regions globally. Diseases induced by *orthotospoviruses* can lead to complete yield loss, often reaching up to 100 percent, with profound economic and social repercussions (Varma, 2007) [27]. *Orthotospoviruses* identified in Asia include *Capsicum chlorosis orthotospovirus* (CaCV), Iris yellow spot virus (IYSV), Groundnut bud necrosis virus (GBNV), Peanut yellow spot virus (PYSV), Melon yellow spot virus (MYSV), Watermelon silver mottle virus (WSMoV), Watermelon bud necrosis virus (WBNV), and several other related species (Chiemsombat *et al.*, 2008) [8].

Capsicum chlorosis orthotospovirus has become a notable threat to capsicum and tomato cultivation in Australia and South east Asia (Widana *et al.*, 2016) [30]. It was initially identified in tomatoes and peppers in Australia (McMichael *et al.*, 2000) [16]. CaCV has been documented in several countries, including Taiwan, China, Thailand, USA, India, and Iran (Bayat *et al.*, 2018) [2], infecting various hosts such as tomatoes, peppers, amaryllis, blood lilies, amaranthus, orchids, calla lilies, peanuts, *Rudbeckia* spp., and wax flowers.

In India, *Capsicum chlorosis orthotospovirus* (CaCV) has been documented infecting tomatoes and bell peppers (Krishnareddy *et al.*, 2008) [14] across various states such as Karnataka, Haryana, and Tamil Nadu, leading to yield losses ranging from 20 to 100 percent. Additionally, reports indicate the virus's presence in Amaranthus and Groundnuts (Sharma and Kulashresta, 2014) [25].

Distinct symptoms have been observed on capsicum plants compared to those typically associated with Tomato spotted wilt virus (TSWV) infection. Bell pepper plants showing symptoms of infection such as yellowing along the edges and between veins on young leaves. These leaves may also exhibit a narrow, curled appearance, reminiscent of a strap. Naturally infected capsicum plants manifest dwarfism, stunted growth, and the development of ring spots on leaves, eventually leading to necrosis and chlorosis. Fruits produced on infected plants may display deformities with nailhead symptoms (Dar *et al.*, 2013) [11].

Materials and Methods

Collection and maintenance of virus isolates

Collected the virus infected samples from diverse capsicum farming regions across the Andhra Pradesh district. After that, samples promptly placed in sealed cold boxes during transportation, and stored at 4 °C until they underwent inoculation and examination using the DAS-ELISA method. Isolates showing characteristic symptoms were chosen and cultured on their original host plants in controlled environments within insect-proof greenhouses. Young plants displaying typical symptoms were delicately removed with soil intact and transplanted directly into earthen pots. Furthermore, they were used for mechanical transmission on healthy capsicum plants. Subsequently, both naturally infected and artificially inoculated plants kept in a greenhouse environment where measures were taken to prevent insect infestation.

Artificial Inoculation

Viral isolates were propagated on healthy young bell pepper plants to ensure a continuous culture throughout the year.

Inoculum Preparation

Initially, the leaves were harvested with care and washed thoroughly with tap water, followed by a rinse with distilled water to eliminate any foreign material adhering to their surfaces. Subsequently, they were dried by interleaving them with layers of blotting paper. After drying, the leaves underwent fine chopping and were crushed in phosphate buffer (0.1 M, pH 7.0) using a sterilized mortar and pestle. The resulting homogenized pulp was filtered through two layers of muslin cloth in a sterilized petri dish. This prepared extract was subsequently used for mechanical inoculations.

Growing and cultivation of test plants

Cultivated healthy bell pepper seedlings in earthen pots containing a blend of sterilized soil and FYM (3:1 ratio v/v). Typically, seedlings aged between fifteen to thirty days and at the 4-5 leaf stage were utilized for both culture maintenance and mechanical inoculation. The potted test plants were maintained in greenhouses protected against insects, with temperatures ranging from 25 to 45 °C during the experiments. The Arka Mohini variety of bell pepper was selected for the experimental investigations.

Mechanical Inoculation

Before going to mechanical inoculation, the mortars and pestles were cooled in a freezer at -20 °C overnight. Mature leaves of test plants were then abraded with 500 mesh carborundum to induce wounds. Bell pepper test plants at the 4-5 leaf stage were then inoculated either by gently rubbing chilled sap onto the leaves with a forefinger or using the cotton swab method, thus exposing plant cells to damage and facilitating virus entry. To ensure virus viability, the inoculum was replenished with freshly ground leaf tissue every 10 minutes. After inoculation, the leaves were immediately rinsed with cold distilled water to eliminate any surplus inoculum and abrasives from the leaf surface. Control plants received a mock inoculation with an inoculation buffer. Special attention was given during the mechanical transmission test to prevent any lethal damage to the leaves from abrasion or hand pressure. All inoculated plants were kept in the greenhouse and regularly checked for the onset of virus symptoms.

Serological detection

Leaf samples showing characteristic symptoms were collected from infected plants in the field and transported to the laboratory in individual labelled polythene bags stored in an icebox to maintain their freshness. These samples were then subjected to serological detection of the causal virus using alkaline phosphatase-based DAS-ELISA, following the procedure outlined below.

Serological detection by DAS-ELISA

Samples collected underwent alkaline phosphatase-based DAS-ELISA initially against Tospo I, II, III antisera, and subsequently against antibodies targeting *Capsicum chlorosis orthotospovirus*, following the protocol outlined by Clark and Adams (1977) [10] with minor adjustments. The assay was conducted using NUNC-immuno Plates Maxisorp F96 polystyrene microplates. It is as follows:

Wells in the microtiter plate were used, except for those in the top and bottom rows at the far left and right sides. Antibodies against CaCV were diluted to 1:500 (i.e., 20µl in 10 ml) in coating buffer. Wells were then coated with 200 µl of diluted anti-CaCV antibodies, and the plate was tightly covered with aluminum foil and incubated in a humid box at 37 °C for 4 hours.

Following the incubation period, the coating antibody suspension was eliminated by thorough shaking over a wash basin. The wells were then filled with 1x PBS-Tween and gently agitated for 2 minutes to empty the plate, after which they were refilled with PBS-Tween. This washing process was repeated three times. Leaf extracts from the test samples, along with positive and negative control wells, were prepared in 1x extraction buffer and filled into wells in 200 µl aliquots.

The plate was again covered with aluminum foil and incubated overnight at 4°C, followed by repeated washing. The specific alkaline phosphatase (ALP)-based conjugated antibody was diluted to 1:500 in conjugate buffer, and 200 µl aliquots were added to each well. The plate was then covered with aluminum foil and placed in a humid box at 37 °C for 5 hours, followed by washing.

The p-nitrophenyl phosphate (pNPP) substrate was dissolved in 1x substrate buffer under dark conditions. Each well received 200 µl aliquots of substrate, and the plates were kept in a humid box under dark conditions at 37 °C

until a yellow color developed in the wells (typically between 30 and 90 minutes). If necessary, the reaction was halted by adding 50 µl of 3M NaOH to each well.

The results were evaluated either by measuring the absorbance value of the hydrolyzed substrate (p-nitrophenyl) at a wavelength of 405nm using a microplate reader (iMarkTM Microplate Reader, BIO-RAD, USA) or through visual screening. For all ELISA serological tests, immune reagents, buffers, positive and negative controls supplied by M/s BIOREBA AG, Switzerland, for Tospo I, II, III, and by GBiosciences (U.S.A) for *Capsicum chlorosis orthotospovirus* were used according to the provided instructions.

The interpretation of ELISA results for detection followed the guidelines outlined by Dijkstra and Jager (1998) [12].

Molecular characterization of *Capsicum chlorosis orthotospovirus* ISOLATE

Total RNA extracted from leaves of symptomatic capsicum plants, where viral infection was confirmed through serological studies, underwent reverse transcription polymerase chain reaction (RT-PCR).

Primers specific to the coat protein region of *Capsicum chlorosis orthotospovirus* were utilized to amplify the desired gene, thereby confirming the results and establishing the precise identity of the virus under investigation. Since CaCV is an RNA virus, the isolation of total RNA from plants is essential for PCR-based diagnostic protocols.

To isolate total RNA from symptomatic capsicum leaves, the 'RNeasy® Plant Mini Kit' (Qiagen, Germany) was employed according to the manufacturer's instructions.

Evaluation of RNA quantity and quality

The concentration and purity of RNA were assessed using a Bio-spectrometer (Eppendorf, Germany) at wavelengths A260/A280nm and A260/A230nm, and calculated using the following formulas:

RNA concentration (g/ml) = A260 x 40 x dilution factor

Total yield (µg) = stock volume x concentration

RNA sample (ml) RNA purity

An A260/280 ratio between 1.8 and 2.0 suggests the RNA purity. Ratios below this range indicate potential polysaccharide contamination, while ratios above it suggest possible phenol contamination.

c-DNA Synthesis by reverse transcription-polymerase chain reaction (RT-PCR)

Once the presence of total RNA was confirmed on an agarose gel, cDNA synthesis was carried out according to the Thermo Scientific cDNA synthesis kit protocol, using an RT primer specifically designed to generate ample amounts of full-length cDNA. Within the cDNA synthesis solution, 2 µl of 10x cDNA synthesis reaction buffers were utilized to enhance reverse transcription across a broad range of templates in the reaction mixture. Subsequently, 1.0 µl of 200 U/µl Reverse Transcriptase was added to facilitate the generation of long cDNA strands. Additionally, 0.8 µl of 10mM dNTP mix and 2.0 µl of 100 µM Random primer were introduced into the medium. A volume of 10 µl of template RNA was incorporated, and sterile nuclease-free water was added to achieve a final volume of 20 µl.

Table 1: Components of the RT-PCR reaction mixture

Components	Reaction volume
10× Reaction buffer	2.0 µl
25× dNTP mix (10 mM)	0.8 µl
10× RT Primer	2.0 µl
Reverse Transcriptase enzyme	1.0 µl
RNase free water	4.2 µl
Template (RNA)	10 µl
Total	20 µl

The complete reaction mixture was set up under chilled conditions to reduce the chance of RNA breakdown and prevent premature c-DNA formation. After vertexing briefly and centrifuging briefly, the mixture was promptly placed into the Pro FlexTM PCR System (applied biosystems, USA) for c-DNA synthesis, following the specified cycles outlined in Table 2.

Table 2: RT-PCR conditions for c-DNA synthesis:

Process	Temperature	Duration	No. of cycles
Stage 1	25 °C	10 minutes	1
Stage 2	37 °C	120 minutes	
Stage 3	85 °C	5 minutes	
Stage 4	4 °C	∞	

After the reaction mixture containing c-DNA was finished, it underwent PCR amplification and was subsequently stored at -20 °C with appropriate labelling for future use.

Amplification of cDNA via PCR

The polymerase chain reaction (PCR) amplifies a single DNA molecule or a fragment across a wide range of levels, producing multiple copies of the DNA fragment. The c-DNA, which was generated during the reverse transcription step, was amplified using the Pro FlexTM PCR System (Applied Biosystems, USA).

Table 3: Primer used for coat protein gene amplification of *Capsicum chlorosis orthotospovirus*

S. No.	Code	Sequence	Product size (bp)
1.	MhMVL-F MhMVL-R	ATGTCTAMCGTYARGCAACTTAC CCCTYACAMTTCHAKAGAWGDRCTAG	834bp

Table 4: Components of PCR reaction mixture for amplification of CP region of *Capsicum chlorosis orthotospovirus*

Components (Stock concentration)	Reaction Volume
c-DNA	1.0 µl
Forward Primer (MhMVl-F)	1.0 µl
Reverse Primer (MhMVL-R)	1.0 µl
10X Reaction Buffer	2.0 µl
dNTP mix (10mM)	2.0 µl
Taq DNA Polymerase (3U/µl)	0.3 µl
Nuclease free water	17.2 µl
Total	25.0µl

The reaction mixture consisted of various components: Taq DNA polymerase (3U/µl), a 2.5mM dNTP mix (originally 10mM), and a 10X reaction buffer containing Tris with 15 mM MgCl2. The final volume was adjusted to 25 µl using DEPC treated water. After thorough mixing, PCR amplification was performed under the following conditions:

Table 5: PCR cycle set up for amplification of coat protein gene of the *Capsicum chlorosis orthotospovirus*

Steps	Temperature	Duration	No. of cycles
Initial Denaturation	94 °C	5.00 minutes	35
Denaturation	94 °C	1.00 minute	
Annealing	60 °C	1.00 minute	
Extension	72 °C	1.30 min	
Final Extension	72 °C	10.00 minutes	

After completion of the PCR reaction, 10 µl of the reaction mixture from each tube was mixed with loading dye and then placed onto a 1% agarose gel. A 100 bp DNA ladder served as the molecular marker. Electrophoresis was conducted in 1x TAE buffer (pH-8.0) at 80V. The DNA bands on the gel were visualized using the Gel Documentation System (BIO-RAD Gel DocTM XR+ Imaging System), and the primer pair displaying the anticipated size was chosen.

Agarose Gel Electrophoresis of the PCR product:

Gel electrophoresis was performed using a submerged horizontal agarose gel system, adhering to the methodology described by Sambrook and Russel (2001).

A 1% agarose gel was prepared for the electrophoresis of the PCR product. To make a 100 ml solution, 2 ml of 50x TAE buffer was added to 98 ml of distilled water, and then 1 gram of agarose was dissolved in the solution. The mixture was heated in a microwave oven for 2 minutes, allowed to cool, and then 3 µl of ethidium bromide was added. The lukewarm gel was poured into a casting tray with combs fixed in place. After solidification, the casting tray with the gel was placed in the electrophoresis tank containing 1x TAE buffer at pH 8.0.

Afterward, 5 µl of PCR product mixed with 1 µl of 6x bromophenol blue gel loading dye for each sample was deposited into the wells. The size of the bands on the gel was determined by running a 100 bp marker ladder (StepUpTM, GeNeiTM) concurrently with the PCR product. Electrophoresis was conducted at 80V. Following electrophoresis, the gel was analyzed using a Gel Documentation System (BIO-RAD Gel DocTM XR+ Imaging System) and photographed.

Results and Discussion

Symptomatology

Naturally infected bell pepper plants exhibited dwarfism, showed inhibited growth, and presented typical chlorotic ringspot symptoms on their infected leaves, with concentric rings of varying sizes appearing. As the infection progressed, these ringspots merged, covering the entire leaf surface. Plants infected at very early stages remained stunted and produced few to no fruits. The fruits produced on infected plants displayed either deformities with nailhead symptoms or developed pale yellow ringspots of different colours (see Plate 1). Dar *et al.* (2013) [11] also noted that infected bell pepper plants exhibited dwarfism compared to healthy ones and showed chlorotic ringspot symptoms on their leaves. Similar symptoms were observed by Bohra *et al.* (2016) [3], who observed concentric rings of varying sizes on the leaf surface, with the entire leaf surface eventually covered by merged ringspots. Many other researchers, including McMichael *et al.* (2002) [17], Kunkalikar *et al.* (2007) [15], and Cheimsombat *et al.* (2010) [9], also reported similar symptoms on bell pepper as well as on other host plants.

Collection and maintenance of virus isolates

Three virus isolates displaying chlorotic ringspots were gathered from distinct bell pepper cultivation areas within West Godavari district, identified based on the symptoms exhibited by the bell pepper plants. Plants showing symptoms collected from open fields were labeled as C-1, those from polyhouses as C-2, and those from vegetable farms as C-3. Inoculum was prepared from infected leaves of these plants and artificially inoculated onto healthy bell pepper plants to sustain the cultures for further examination. These artificially inoculated plants were maintained in insect-proof greenhouses, where the temperature ranged from 25 to 40°C. Subsequently, all the test plants developed ringspot symptoms upon artificial inoculation.

Serological Detection

The infected samples underwent serological detection using Tospo (serogroup I, II, III) antisera developed against the genus Orthotospovirus and CaCV antisera specific to *Capsicum chlorosis orthotospovirus*, which falls under subgroup IV of the genus Orthotospovirus. Initially, the infected samples were tested against Tospo (serogroup I, II, III) antisera using Double Antibody Sandwich (DAS) form of ELISA. However, none of the samples yielded positive results, indicating that the causal virus did not belong to serogroup I, II, III of the genus Orthotospovirus. Following this confirmation, the infected samples were then examined using CaCV antiserum, revealing positive reactions in six samples during DAS-ELISA. Notably, virus isolate C6 exhibited a particularly strong reaction with an O.D. value of 2.049 in ELISA compared to other isolates (see Plate 2).

The optical density (O.D.) data, representing absorbance values at 405 nm (A405), are presented in Tables 6 and 7. Given that isolate C6, obtained from a vegetable farm, displayed typical ringspot symptoms characteristic of *Capsicum chlorosis orthotospovirus* and showed a robust reaction in DAS-ELISA, it was chosen for further molecular studies.

ELISA stands out as one of the foremost serological tests for detecting plant viruses due to its simplicity, versatility, and sensitivity. The current research concludes that DAS-ELISA effectively detects Orthotospovirus isolates in infected bell pepper plants. Previous studies have demonstrated that ELISA employing polyclonal antisera against virus isolates results in positive reactions in symptomatic plants with GBNV and WSMoV antisera. Given that *Capsicum chlorosis orthotospovirus* falls under the WSMoV subgroup, i.e., subgroup IV of the genus Orthotospovirus, polyclonal antisera against GBNV and WSMoV can be utilized for CaCV detection. Several reports have documented the utilization of both polyclonal and monoclonal antibodies against *Capsicum chlorosis orthotospovirus* in ELISA. For instance, Chen *et al.* (2012) [7] conducted serological detection on infected calla lily samples using antiserum against CaCV and monoclonal antibodies against WSMoV, with both yielding positive reactions.

Table 6: Optical density values of tested isolates against Tospo I, II, III antisera using DAS-ELISA.

Isolates	Optical density values at A_{405}
Open field: C1	0.515
C2	0.444
Polyhouse: C3	0.552
C4	0.557
C5	0.502
Vegetable Farm: C6	0.514
C7	0.538
C8	0.476
COH Farm: C9	0.545
C10	0.509
Positive Control	0.678(+)
Negative Control	0.286 (-)

Plate 1: Necrotic and chlorotic symptoms of Capsicum chlorosis virus on leaves and fruits of capsicum

Manyam *et al.* (2014) conducted analogous investigations, examining infected chili samples with polyclonal antibodies tailored for GBNV, resulting in positive reactions. Similar findings were reported by other researchers, including Sharma *et al.* (2016) [26], Basvaraj *et al.* (2020) [1], and Chen *et al.* (2022) [6].

Table 7: Optical density readings of examined isolates with *Capsicum chlorosis orthotospovirus* antisera using DAS-ELISA.

Isolates	Optical density values at A_{405}
Open field: C1	0.939(+) 1.099(+)
C2	
Polyhouse: C3	0.681
C4	0.988(+)
C5	0.637
Vegetable Farm: C6	2.049(+)
C7	0.802(+)
C8	1.036(+)
COH Farm: C9	0.636
C10	0.624
Positive Control	1.048 (+)
Negative Control	0.394 (-)

Molecular Characterization

Nucleic acid-based detection methods were employed to molecularly characterize the isolate, aiming for precise identification of the virus under investigation. The subsequent paragraphs outline the various steps involved in molecular characterization.

RNA Isolation

Initially, infected samples underwent testing in DAS-ELISA, followed by the isolation of total plant RNA from seropositive samples using the 'RNeasy® Plant Mini Kit' (Qiagen, Germany). The concentration and purity of the RNA were assessed using a Bio spectrometer (Eppendorf, Germany) at A260/A280nm and A260/A230nm. Additionally, 3µl of the isolated RNA was analyzed on a 1.2 percent denatured agarose gel to confirm the presence and quality of total RNA, while the remaining RNA was promptly stored at -80 °C for future use. On the gel, two bands of RNA, corresponding to 28s and 18s, were observed.

cDNA Synthesis

The RNA acquired was employed for cDNA synthesis through Reverse Transcription Polymerase Chain Reaction (RT-PCR), utilizing reverse transcriptase enzyme as per the guidelines outlined in the Thermo Scientific c-DNA synthesis kit protocol and RT-PCR conditions provided in Table 2. The resulting cDNA was then utilized for amplification, employing a CaCV-specific degenerate primer pair.

Amplification of the complementary DNA (CDNA) strand

cDNA obtained from RNA extracted from diseased bell pepper leaves served as the basis for PCR amplification. This process utilized a pair of degenerate primers specific to the Watermelon silver mottle virus (WSMoV) group, targeting sequences within the nucleocapsid gene:

Forward primer (MhMVL-F):
5' ATGTCTAMCGTYARGCAACTTAC

Reverse primer (MhMVL-R):
5' CCCTYACAMTTCHAKAGAWGDRCTAG

The reaction mixture contained the components outlined in Table 4, and the PCR cycle setup for amplification followed the instructions provided in Table 5. Following completion of the amplification process in the thermocycler, the PCR product underwent assessment on 1% agarose gel through electrophoresis. The gel was then examined using a Gel Documentation System and photographed. Clear bands were observed at a 1µl concentration of both forward and reverse primers with 1µl of cDNA template, without the formation of primer dimers. A distinct band of approximately 834 bp was observed in lane C1 compared to the 100 bp marker ladder on the gel (see Plate 3). The results indicated that the infected samples subjected to nucleic acid-based detection were infected with *Capsicum chlorosis orthotospovirus*.

The utilization of RT-PCR technique for molecular detection of CaCV has been documented by numerous researchers from various regions worldwide. Kunkalikar *et al.* (2007) [15] employed a similar technique for the amplification of the nucleocapsid gene of virus isolates and observed an amplicon of approximately 828 bp. Krishnareddy *et al.* (2008) [14] also conducted RT-PCR using GBNV and CaCV specific primers, noting no amplification with GBNV specific primers, while an amplicon of 850 bp was observed with CaCV specific primers. Many researchers have conducted similar studies across different crops using primers specific to CaCV, and their findings align with the results of the present study (Cheimsombat *et al.*, 2008; Bohra *et al.*, 2016; Sharma *et al.*, 2016; Orfanidou *et al.*, 2019; Bayat *et al.*, 2018; Rajmanickam *et al.*, 2020; Haokip *et al.*, 2021; Vinodhini *et al.*, 2021) [8, 3, 26, 20, 2, 13, 29].

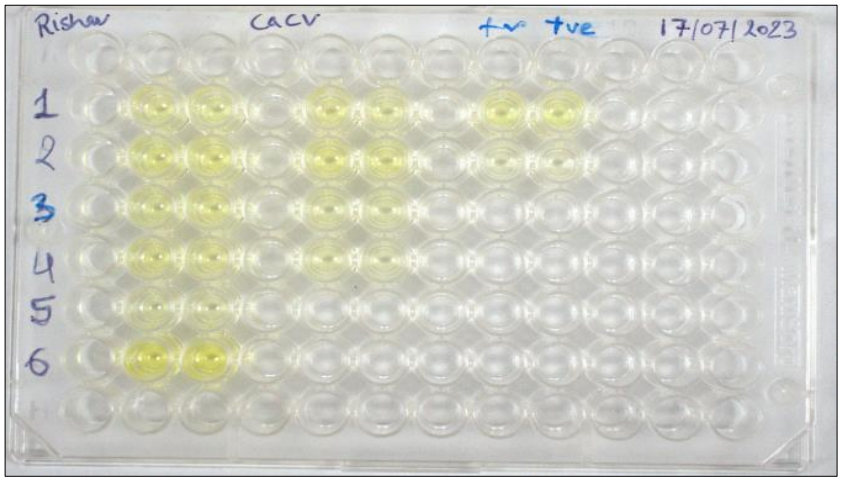

Plate 2: Serological Detection of CaCV on ELISA plate

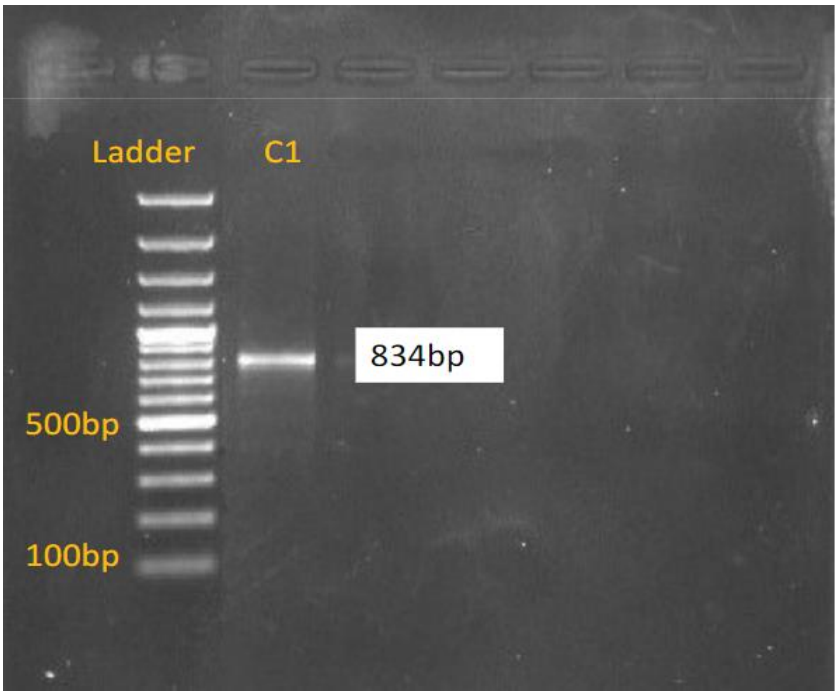

Plate 3: Amplicon of PCR product on 1% agarose gel

Conclusion

The disease under examination prominently manifests through chlorotic ringspots of varying sizes on leaves, which converge during later infection stages, covering the entire leaf surface, along with symptoms like stunted growth and deformed fruits. Ripe bell peppers display ringspots of assorted colours, diminishing fruit quality and rendering them unsuitable for market. Three isolates demonstrating typical *Capsicum chlorosis orthotospovirus* symptoms were gathered from distinct locations and labelled as C1, C2, and C3. These isolates were artificially introduced into healthy bell pepper plants via sap inoculation and cultivated under controlled conditions in insect-proof glasshouses for further analysis. Notably, C1 isolate, collected from open fields, induced the most prominent symptoms upon sap inoculation. Serological testing using Tospo (I, II, III) and CaCV antisera through DAS-ELISA revealed that out of 10 samples examined, none reacted positively with Tospo (I, II, III) antisera, while six samples showed positive reactions against CaCV antisera, with C6 exhibiting the highest O.D. value of 2.049. Given its significant symptomatology in the field, the C6 isolate was selected for subsequent molecular investigations. Efforts were made to characterize the virus molecularly by isolating total RNA from seropositive bell pepper samples. Subsequently, cDNA was synthesized from the RNA template using reverse transcriptase enzyme, and polymerase chain reaction was performed using degenerate primers specific to the nucleocapsid (N) gene of Watermelon silver mottle virus subgroup of the genus Orthotospovirus. Electrophoresis of the PCR product on a 1% agarose gel yielded desired amplicons of approximately 830 bp, confirming the presence of CaCV in the infected samples.

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