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RESEARCH ARTICLE

Serological and molecular characterization of Cucumber Mosaic Virus (CMV) infecting cucumber (*Cucumis sativus* L.) in southern Tamil Nadu

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Abstract

Cucumber Mosaic Virus (CMV) is a significant threat to cucumber (*Cucumis sativus* L.) cultivation, causing mosaic patterns, leaf crinkling, fruit distortion and stunting. This study investigates the serological and molecular characteristics of a CMV- Anantha nambi Nambi kuruchi isolate (CMV-ANK) collected from cucumber plants in major growing areas of Tamil Nadu. Host range studies revealed that CMV-ANK caused systemic infection in multiple crops and chlorotic lesions in okra, chenopodium and wild cucumber. The virus was confirmed using Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS-ELISA) and Reverse transcription polymerase chain reaction (RT-PCR) with Coat Protein (CP) gene-specific primers, producing a 1200 bp amplicon. Nucleotide sequencing demonstrated that 99.85 % identity with a CMV isolate from tomato (MZ298672), indicating close genetic similarity. Phylogenetic analysis showed that CMV-ANK clusters with other CMV isolates affecting various crops. Physical property analysis revealed a dilution endpoint at 10^{-5}, thermal inactivation at 65 °C and longevity *in vitro* up to three days at room temperature and five days under refrigeration. Seed transmission studies confirmed an 83.33 % transmission rate through seeds from infected plants, reinforcing the risk of seed-borne spread. These findings underscore the adaptability and resilience of CMV-ANK, highlighting the need for effective disease management strategies, including the development of resistant cucumber varieties and integrated pest management. The insights gained from this study contribute to understanding CMV epidemiology and offer a foundation for future research aimed at controlling its impact on cucumber production.

Keywords: cucumber; CMV (Cucumber Mosaic Virus); TAS-ELISA; RT-PCR

Introduction

Cucumber (*Cucumis sativus* L.) is commonly known as Khira or Sasha which is one of the widely cultivated commercial vegetable crops grown in large greenhouses as well as small farms. This is the summer vegetable crop that belongs to the Cucurbitaceae family grown in tropical and subtropical areas of the country. Overall Cucurbitaceae family has 118 genera and 825 species among which 36 genera and 100 species are found in India (1). Cucumber is considered to be one of the oldest vegetable crops grown in India for over 3000 years (2). In India, cucurbitaceous crops contribute about 5.6 % of the total vegetable production (3). Tamil Nadu is one of the most important state for the production of various cucurbits. The sap, seed and aphid transmission of CMV was reported and found that 80 species of aphids in 33 genera were able to transmit CMV in non-persistent manner (4). The different aphid species transmitting CMV were *Myzus persicae, Aphis gossypii, Macrosiphum euphorbide, Myzocallis asclepiadis, A. craccivora ,A. nerri, Acyrthisyphon pisum, Dactynotus carthami, A. glycines, Therioaphis trifolli, A. spiraecola, Rhapalosiphum maidis, A. fabae, Nearctaphis bakeri, Lipaphis erysimi* (5-8).

Materials and Methods

Isolation of virus and maintenance of inoculum

A survey was conducted for the occurrence of CMV incidence in the major cucumber growing areas of Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari districts. Based on the severity of symptoms, the cucumber leaf samples were collected from Anantha nambi kuruchi village of Thoothukudi

district was inoculated in cucumber and cowpea plants and maintained. CMV infected cucumber samples collected from Anantha nambi kuruchi village was used for the virus isolation. This isolate was named as CMV- Anantha Nambi Kuruchi (CMV-ANK) and which was used in further studies. From the infected leaf, the virus extraction was done by using 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 % Mercaptoethanol and inoculated on seven days old healthy cucumber plants (Vallanadu local). Virus inoculation was done by dusting 600-mesh carborundum powder on the leaves which act as an abrasive to make minute injuries on the plant leaf surface. After inoculation, plants were observed periodically and recorded the symptoms. Through sap transmission CMV-ANK isolate was multiplied in cucumber plants (Vallanadu local) and maintained under insect proof glass house (9).

Physical properties of virus isolate

The physical properties of the virus such as Dilution End Point (DEP), Thermal Inactivation Point (TIP) and Longevity *in vitro* (LIV) were determined in the extract collected from CMV infected cucumber plant with sodium phosphate buffer (0.1 M, pH 7.0). For DEP, CMV inoculated cucumber plant showed systemic mosaic symptoms were homogenized in sodium phosphate buffer (1:2 W/V) and serial dilution was done from 10^{-1} to 10^{-7}. Undiluted virus leaf extract was served as control. 10^{-1} dilution was prepared by diluting one mL of undiluted sap in 9 mL of buffer, like wise dilutions were made up to 10^{-7}. The cucumber plant in two leaf stage were inoculated separately with each dilution and the development of symptom was recorded. Finally, the symptoms produced under each dilution were compared. To test the TIP, The CMV extract was prepared by macerating infected cucumber leaves with 0.1M sodium phosphate buffer and filtered through cheese cloth and 2 mL of CMV extract was taken in each of the eight screw capped test tubes. Each test tube was kept in water bath for 10 minutes under different temperatures *viz*., 20, 30, 40, 50, 60, 70, 80 and 90 °C. Thereafter, the crude sap was cooled by keeping the test tubes in ice cold water and subsequently inoculated on test plants to observe the symptoms expression for determining the thermal inactivation point of CMV. The number of plants infected at different temperature was recorded and compared. The LIV was performed where the CMV extract was stored at room temperature (28 - 30 °C) and refrigerated temperature (6 - 8 °C) for different periods *viz*., 0, 2, 4, 6, 8, 12 hr and 1, 2, 3, 4, 5 days to determine the longevity *in vitro* of CMV. The different temperature virus extract samples were inoculated separately on cucumber plants maintained in insect proof glasshouse. The symptoms produced in each treatment were recorded and compared (8).

Host range studies

The virus isolate was inoculated on *Luffa acutangula, Trichosanthes cucumerina, Cyamopsis tetragonoloba, Vigna unguiculate, Cucurbita pepo, Momordica charantia, Cucumis anguria, Abelmoschus esculentus* and *Chenopodium album* in insect proof glass house condition at two leaf stage. Infected cucumber leaf samples were grounded by using Phosphate buffer (pH 7.0) containing 0.1 % mercaptoethanol was added and macerated well. For abrasiveness, 600 mesh carborundum was dusted on the leaf surface to facilitate the virus entry (10). The inoculated plants were kept in insect proof glass house condition for symptom expression. Three replications were performed for each of the selected host plants. The virus present in the host plants were confirmed by TAS-ELISA.

Seed transmission studies

Cucumber fruit was collected from the CMV infected cucumber plants maintained under glass house. From the fruit, seeds were extracted and dried. For transmission studies 25 seeds were randomly taken and sown individually in each pot containing mixture of soil, sand, Farmyard Manure (FYM) in the ratio of 2:1:1. Similarly, seeds were extracted from fruit of healthy cucumber plant maintained in insect proof cage. From this, 25 seeds were randomly selected and sown individually in pots for the purpose of healthy control. The plants were observed periodically and recorded the symptoms. (11). The seeds from the infected and healthy cucumber plants were subjected to TAS-ELISA.

TAS-ELISA

Triple Antibody Sandwich ELISA (TAS-ELISA) was performed according to the methodology of (12). 100 mg of sample from sap transmitted cucumber plants, crop and weed species expressed symptoms under host range studies, seeds from CMV infected plants maintained in seed transmission study were ground separately in 1 mL of carbonate buffer centrifuged at 8000 rpm for 10 min. CMV polyclonal antibody, monoclonal antibody and Alkaline Phosphatase (ALP) conjugated rabbit antimouse IgG (Source: NRCB, Trichy) were diluted with coating buffer at 1:1000, 1:1000 and 1:2000 respectively. The CMV polyclonal antibody @ 100 µL was added in each well in polystyrene plates and incubated at 37 °C for 1 hr. The ELISA plates were washed three times with Phosphate Buffered Saline (PBS) Tween for three times at three minutes interval after each step. 100 µL of antigen prepared from the samples were added separately in each well and incubated at 4 °C for overnight. CMV monoclonal antibody @ 100 µL was added in each well and incubated at 37 °C for 1 hr. The 100 µL of ALP conjugated rabbit anti mouse IgG was added in each well and incubated at 37 °C for 1 hr. Then 100µl of freshly prepared substrate buffer was added in each well and incubated at 37 °C for 30 - 60 min. After which 50 µL of 3M NaOH was added to each well to stop the enzyme substrate reaction. The absorbance was read at 405 nm in ELISA reader (BioTek Gen5). The samples were positive when the A405 values were twice greater than negative samples.

RNA extraction and RT-PCR

Total RNA was extracted from the cucumber plants mechanically transmitted with CMV-ANK isolate through Trizol method (13). The RNA extracted was quantified in nano drop spectrometer and uniform dilution was made (3000 ng µL-1). The RNA was subjected to cDNA synthesis by following the instruction given in Revert aid First Strand complementary DNA synthesis kit. The reaction mixture (5 µL - total RNA; 1 µL - Random primer; 7 µL - Nuclease free water; 4 µL - 5X Reaction buffer; 1 µL - 2 mM dNTPs; 1 µL - reverse transcriptase; 1 µL- RNase inhibitor) was prepared and incubated at 42 °C for 1 hr and 70 °C for 5 min each in one cycle (14).

Bioinformatic analysis of CMV genome

The phylogenetic analysis was done using MEGA version 7.0 for the sequenced CMV genome (Accession number: OL624845) and the neighbour joining tree was made by using related sequences from NCBI database via nucleotide BLAST search. The CMV genome specific parameters were analysed using Softberry software (FGENESV) for further characterization. The ORF finder was used to identify the potential protein encoding segments of the CMV-ANK isolate. The pairwise nucleotide identity was calculated using Sequence Demarcation Tool (SDT1.2).

Results and Discussion

Physical properties of the virus isolate

The infectivity of CMV was evaluated under different physical conditions to determine its dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV). The results of DEP revealed that the percent transmission decreased progressively with increasing dilution of the CMV inoculum. The highest transmission was observed in the undiluted control (100.30 ± 4.82 %), followed by 10^{-1} (93.12 ± 8.98 %) and 10^{-2} (86.75 ± 3.52 %). A notable decline was seen at 10^{-3} (72.81 ± 6.08 %) and 10^{-4} (35.31 ± 2.27 %), while no transmission was recorded from 10^{-5} onwards. These results indicate that CMV lost infectivity at the 10^{-5} dilution (Table 1). This is consistent with previous scientific reports in cucumber (8) and in banana (11). The results of TIP revealed that CMV infectivity declined with increasing temperatures. While the unheated control recorded the highest transmission (100.30 ± 4.82 %), no significant reduction was seen up to 40 °C (95.48 ± 3.87 %). A gradual decline was noted at 45 °C (83.30 ± 6.96 %) and 50 °C (63.44 ± 4.08 %), with further reduction at 55 °C (43.79 ± 4.18 %) and 60 °C (25.48 ± 1.30 %). No infectivity was observed at 65 °C and 70 °C, suggesting the thermal inactivation point lies between 60 °C and 65 °C (Table 2). These findings agree with those of previous scientific investigations which reported similar TIP values for CMV (8). For LIV, CMV retained high infectivity up to 4 hr at room temperature (25-28 °C), after which a gradual reduction was observed, with complete inactivation by the third day. Under refrigerated conditions (6 -8 °C), infectivity was better preserved, with significant transmission (80.84 ± 7.72 %) at 12 hrs and detectable infectivity (26.41 ± 2.67 %) up to four days. By the fifth day, no infectivity was observed under either condition. These results indicate that CMV remains viable for a longer period when stored at lower temperatures (Table 3). Similar trends were reported and confirmed the role of cool storage in prolonging CMV stability (8). Overall, these findings suggest that CMV infectivity is sensitive to dilution and heat, but relatively stable under refrigerated conditions, emphasizing the importance of careful handling and storage for accurate biological assays.

Table 1. Determination of Dilution End Point (DEP) of CMV

Sl. No.	Dilution	Percent transmission *
1.	Undiluted (control)	100.30 ± 4.82^{a}
2.	1:10 (10^{-1})	93.12 ± 8.98^{ab}
3.	1:100 (10^{-2})	86.75 ± 3.52^{b}
4.	1:1000 (10^{-3})	72.81 ± 6.08^{c}
5.	1:10,000 (10^{-4})	35.31 ± 2.27^{d}
6.	1:1,00,000 (10^{-5})	0.00 ± 0.00^{e}
7.	1:10,00,000 (10^{-6})	0.00 ± 0.00^{e}
8.	1:10,000,000 (10^{-7})	0.00 ± 0.00^{e}
	SE(m)	2.57
	CD @5 %	7.7

* Mean of three replications

Table 2. Determination of Thermal Inactivation Point (TIP) of CMV

Sl. No.	Thermal inactivation point	
	Temperature (° C)	Percent transmission*
1.	Unheated (control)	100.30 ± 4.82^{a}
2.	28	98.65 ± 9.52^{a}
3.	40	95.48 ± 3.87^{a}
4.	45	83.30 ± 6.96^{b}
5.	50	63.44 ± 4.08^{c}
6.	55	43.79 ± 4.18^{d}
7.	60	25.48 ± 1.30^{e}
8.	65	0.00 ± 0.00^{f}
9.	70	0.00 ± 0.00^{f}
	SE(m)	2.81
	CD @ 5 %	8.35

* Mean of three replications

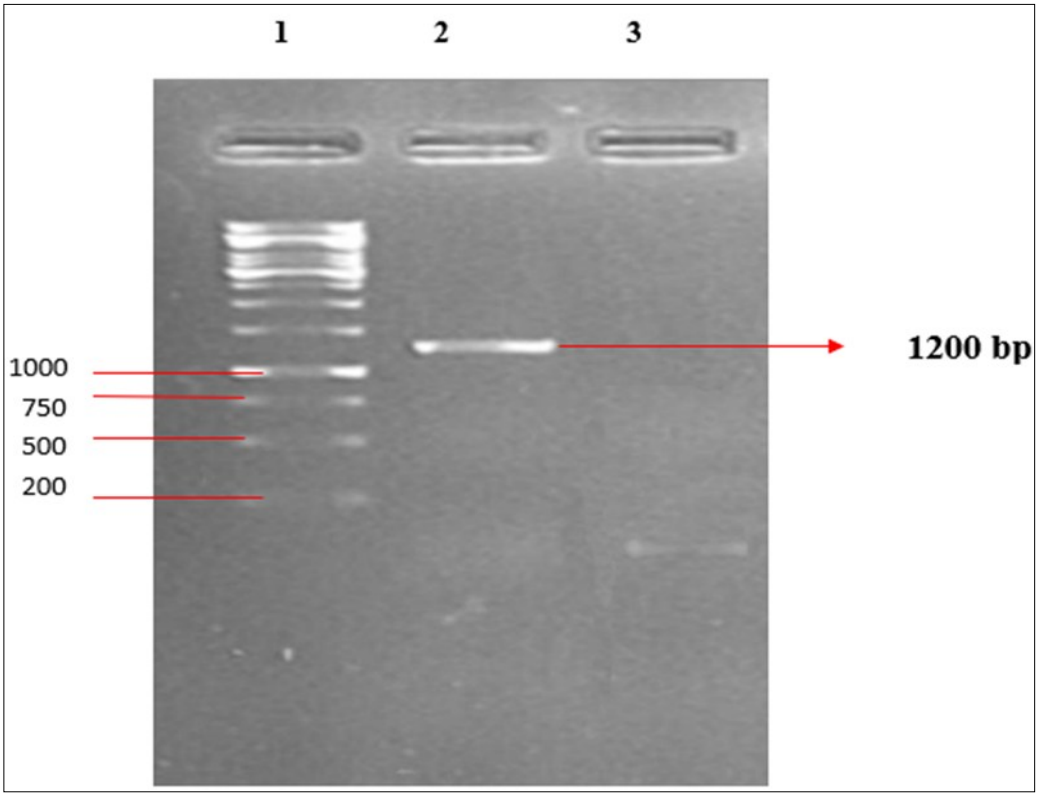
Table 3. Determination of Longevity *in vitro* (LIV) of CMV

Sl. No.	Storage period	Percent transmission *	
		Room temperature (25-28 °C)	Refrigerated temperature (6-8 °C)
1.	Control	100.30 ± 4.82^{a}	100.30 ± 4.82^{a}
2.	2 h	97.14 ± 9.37^{a}	98.65 ± 9.52^{ab}
3.	4 h	94.97 ± 3.85^{a}	96.71 ± 3.92^{ab}
4.	6 h	78.20 ± 6.53^{b}	90.16 ± 7.53^{bc}
5.	8 h	67.12 ± 4.32^{c}	82.45 ± 5.31^{cd}
6.	12 h	65.23 ± 6.23^{c}	80.84 ± 7.72^{cd}
7.	1 day	61.30 ± 3.14^{c}	76.75 ± 3.93^{d}
8.	2 days	51.43 ± 4.75^{d}	44.94 ± 4.15^{e}
9.	3 days	0.00 ± 0.00^{e}	36.38 ± 2.23^{e}
10.	4 days	0.00 ± 0.00^{e}	26.41 ± 2.67^{f}
11.	5 days	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}
	SE (m)	2.80	3.11
	CD @ 5 %	6.81	9.11

*Mean of three replications

Detection of CMV in sap transmitted cucumber plants

Total RNA was extracted from both infected and healthy tissues of sap-inoculated and uninoculated cucumber plants. RT-PCR was performed using coat protein (CP) gene-specific primers. A distinct amplicon of approximately 1200 bp was amplified from the infected tissues, while no amplification was observed in the healthy samples. The 1200 bp amplicon (Fig. 1) was subsequently sequenced (16). Tissue samples from virus-inoculated cucumber plants exhibiting mosaic symptoms and from healthy controls were further subjected to TAS-ELISA using both monoclonal and polyclonal antibodies specific to CMV (Fig. 2). The infected samples showed strong positive reactions, recording an A_{405} value of 1.057 ± 0.02, whereas healthy samples showed negative reactions with an A_{405} value of 0.258 ± 0.01. In addition, host range studies confirmed CMV



Lane 1 - 1 Kb DNA ladder

Lane 2 - CMV-ANK isolate inoculated cucumber sample

Lane 3 - Healthy cucumber sample

Fig.1. Detection of CMV inoculated cucumber sample by RT-PCR using CMV specific coat protein primer.

Fig. 2. Development of mosaic symptom on sap transmitted cucumber plant.

infection in several cucurbit crops, including melon (mosaic), bitter gourd (mosaic and vein banding), angled luffa (malformation, mosaic and vein banding), chayote (mosaic) and pumpkin (mosaic), all of which tested positive in TAS-ELISA with both antisera. Notably, significant serological responses were recorded in seven cucurbit species-cucumber, melon, watermelon, pumpkin, chayote, angled luffa and bitter melon-further confirming CMV infection (17).

Symptoms of CMV in test plants

Mechanical inoculation of CMV on various host species resulted in differential symptom expression and incubation periods, indicating varied host responses to the virus. Mosaic symptoms were observed in *Luffa acutangula* (24 days), *Trichosanthes cucumerina* (14 days), *Cyamopsis tetragonoloba* (16 days) and *Vigna unguiculata* (23 days), indicating systemic infection. *Momordica charantia* developed vein banding symptoms with the shortest incubation period of 11 days, suggesting rapid symptom onset and high susceptibility. In *Cucurbita pepo*, dark green spots appeared after 19 days. These findings align with those of other research findings (16, 18, 19), expressing the chlorotic symptoms. The symptom of vein banding, small dark green spots and small chlorotic lesion were observed in the host plants of *Momordica charantia, Chenopodium album* and *Cucurbita pepo* from the previous research reports (20-22), expressing the similar symptoms of vein banding, small dark green spots and small chlorotic lesion from the CMV infected host plants (Fig. 3, Table 4).

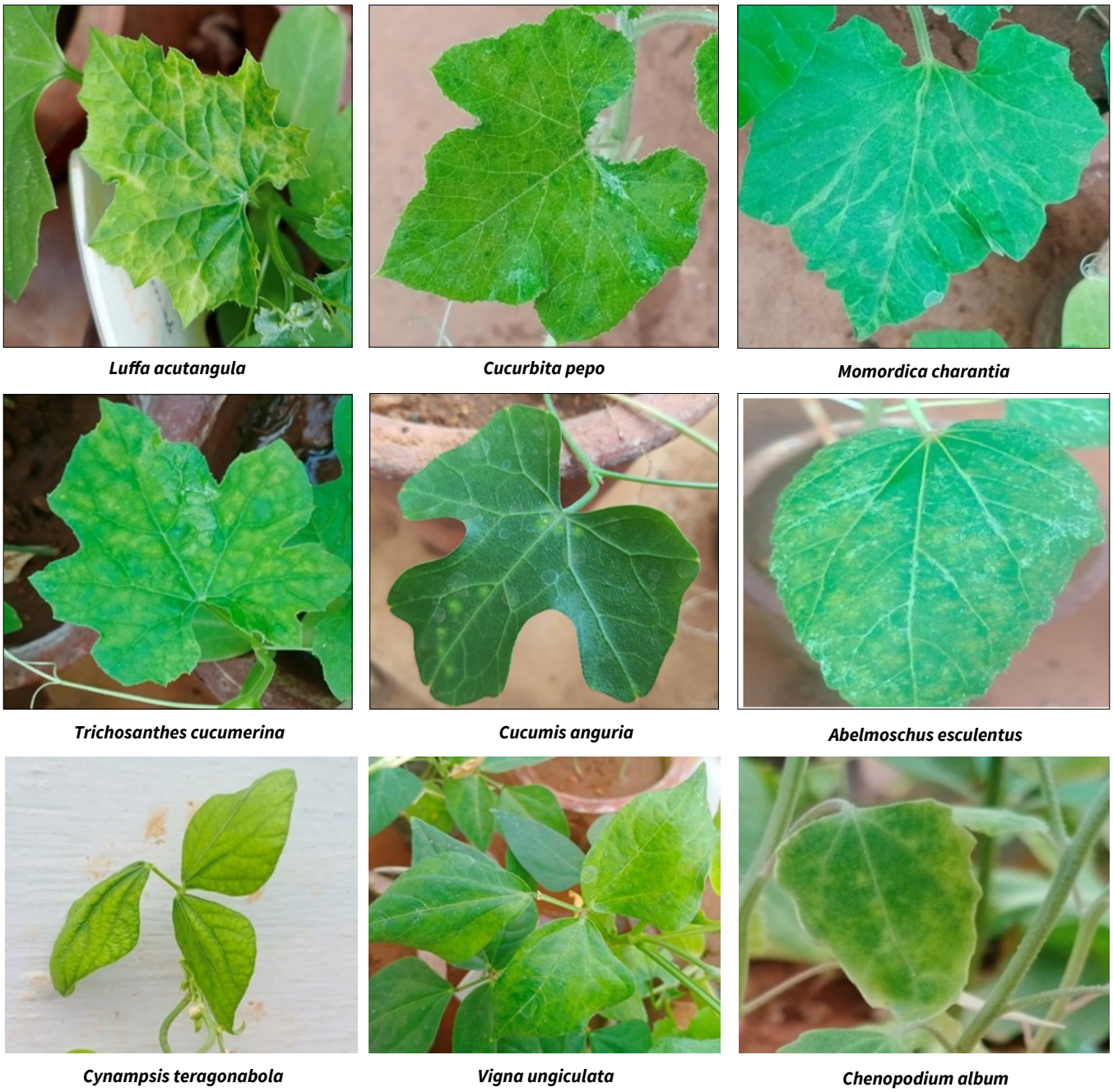


Fig. 3. Expression of different symptoms of CMV-ANK isolate on different host.

Table 4. Expression of different symptoms on inoculation of CMV in different host

Sl. No.	Common Name	Scientific name	Incubation period (days)	Types of Symptoms
1.	Ridge gourd	*Luffa acutangula*	24	Mosaic
2.	Pumpkin	*Cucurbita pepo*	19	Dark Green spots
3.	Bitter gourd	*Momordica charantia*	11	Vein banding
4.	Snake gourd	*Trichosanthes cucumerina*	14	Mosaic
5.	Gherkin	*Cucumis anguria*	28	Chlorotic lesion
6.	Bhendi	*Abelmoschus esculentus*	21	Chlorotic lesion
7.	Cluster bean	*Cyamopsis tetragonoloba*	16	Mosaic
8.	Cowpea	*Vigna unguiculata*	23	Mosaic
9.	Chenopodium	*Chenopodium album*	22	Chlorotic lesions

Detection of CMV in different host plants using TAS-ELISA

TAS-ELISA was employed to detect Cucumber Mosaic Virus (CMV) in various host plants, with absorbance values measured at 405 nm in both infected and healthy tissues. A clear distinction in absorbance was observed between infected and healthy samples, indicating the effectiveness of TAS-ELISA for CMV detection. Among the infected samples, *Luffa acutangula* recorded the highest absorbance value (0.915 ± 0.04), indicating a strong positive reaction. This was followed by *Trichosanthes cucumerina* (0.534 ± 0.04), *Cucurbita pepo* (0.495 ± 0.05), *Cyamopsis tetragonoloba* (0.492 ± 0.03) and *Abelmoschus esculentus* (0.460 ± 0.04), all of which exhibited significantly higher absorbance values than their respective healthy controls, confirming CMV infection. Moderate absorbance values were recorded in *Cucumis anguria* (0.407 ± 0.03), *Chenopodium album* (0.441 ± 0.03) and *Momordica charantia* (0.359 ± 0.01), while the lowest positive reaction was observed in *Vigna unguiculata* (0.332 ± 0.03) (Table 5). The significant positive results for the presence of CMV were detected in TAS-ELISA (23) from virus infected natural weed hosts.

Detection of CMV in the cucumber seeds

Among the 25 seeds taken from infected and healthy cucumber plants, 18 and 20 seeds were germinated respectively. Among the 18 plants grown 15 plants were infected with CMV and expressed the mosaic symptom whereas, no symptoms were observed from the plants grown from healthy seeds. This study revealed that CMV is transmitted through seed at 83.33 %. The TAS-ELISA results indicated that seeds from infected plants showed positive reaction to CMV polyclonal and monoclonal antiserum. The A405 value of infected seeds ranged from 0.416 to 0.562 whereas the A405 value with the range of 0.199 to 0.293 was recorded in healthy seeds (Table 6). Similarly, the seeds extracted from different capsicum accessions show the presence of CMV through antigen coated plate ELISA (11).

Sequencing and phylogenetic analysis

The nucleotide sequence obtained from the 1200 bp size of amplicon from the CMV-ANK isolate was submitted in NCBI GenBank database (Accession Number: OL624845). Similar size of CMV amplicon was obtained from CMV infected snake gourd samples (16). The sequence was analysed through blast and CLUSTAL-W. The phylogenetic analysis revealed that the nucleotide sequence of CMV-ANK isolate was found to 98.95, 98.34, 98.95 and 97.48 % with CMV infected legume, lilies and tomatoes respectively. Cluster dendrogram was constructed based on CLUSTAL-W analysis of nucleotide sequence of CP gene of CMV-ANK isolate (Accession Number: OL624845) with the other CMV CP gene sequences already available in NCBI database. The results revealed that CMV-ANK isolate was closely related to multiple CMV isolates from different hosts and forming one cluster (Fig. 4). These results align with the other research findings (23), where the phylogenetic tree for CMV tomato isolate (EF15734) with the other members of genus *Cucumovirus*. The analysis clearly divided strains into three clusters of subgroup IA, IB and II. The CMV tomato isolate clade with the subgroup IB which showed closest relationships with tomato (Y16926) and tobacco (D28780) strains of CMV Indian isolates

Table 6. Detection of CMV in cucumber seeds through TAS-ELISA

Seed No.	Absorbance value at 405 nm	
	Infected	Healthy
1.	0.463	0.203
2.	0.491	0.211
3.	0.571	0.290
4.	0.251	0.291
5.	0.434	0.209
6.	0.562	0.215
7.	0.445	0.199
8.	0.495	0.268
9.	0.544	0.277
10.	0.236	0.255
11.	0.443	0.293
12.	0.482	0.244
13.	0.441	0.257
14.	0.224	0.284
15.	0.489	0.239
16.	0.542	0.246
17.	0.432	0.211
18.	0.416	0.251

Table 5. Detection of CMV in different hosts through TAS-ELISA

Sl. No.	Details of sample	Absorbance value at 405 nm	
		Infected samples	Healthy samples
1.	*Luffa acutangular*	0.915 ± 0.04^{a}	0.362 ± 0.02^{a}
2.	*Cucurbita pepo*	0.495 ± 0.05^{bc}	0.195 ± 0.02^{b}
3.	*Momordica charantia*	0.359 ± 0.01^{ef}	0.179 ± 0.01^{bc}
4.	*Trichosanthes cucumerina*	0.534 ± 0.04^{b}	0.119 ± 0.01^{f}
5.	*Cucumis anguria*	0.407 ± 0.03^{de}	0.146 ± 0.01^{de}
6.	*Abelmoschus esculentus*	0.460 ± 0.04^{cd}	0.135 ± 0.01^{ef}
7.	*Cyamopsis tetragonoloba*	0.492 ± 0.03^{bc}	0.131 ± 0.01^{ef}
8.	*Vigna unguiculata*	0.332 ± 0.03^{f}	0.162 ± 0.01^{cd}
9.	*Chenopodium album*	0.441 ± 0.03^{cd}	0.119 ± 0.01^{f}
	SE(m)	0.02	0.01
	CD @5 %	0.06	0.02

* Mean of three replications

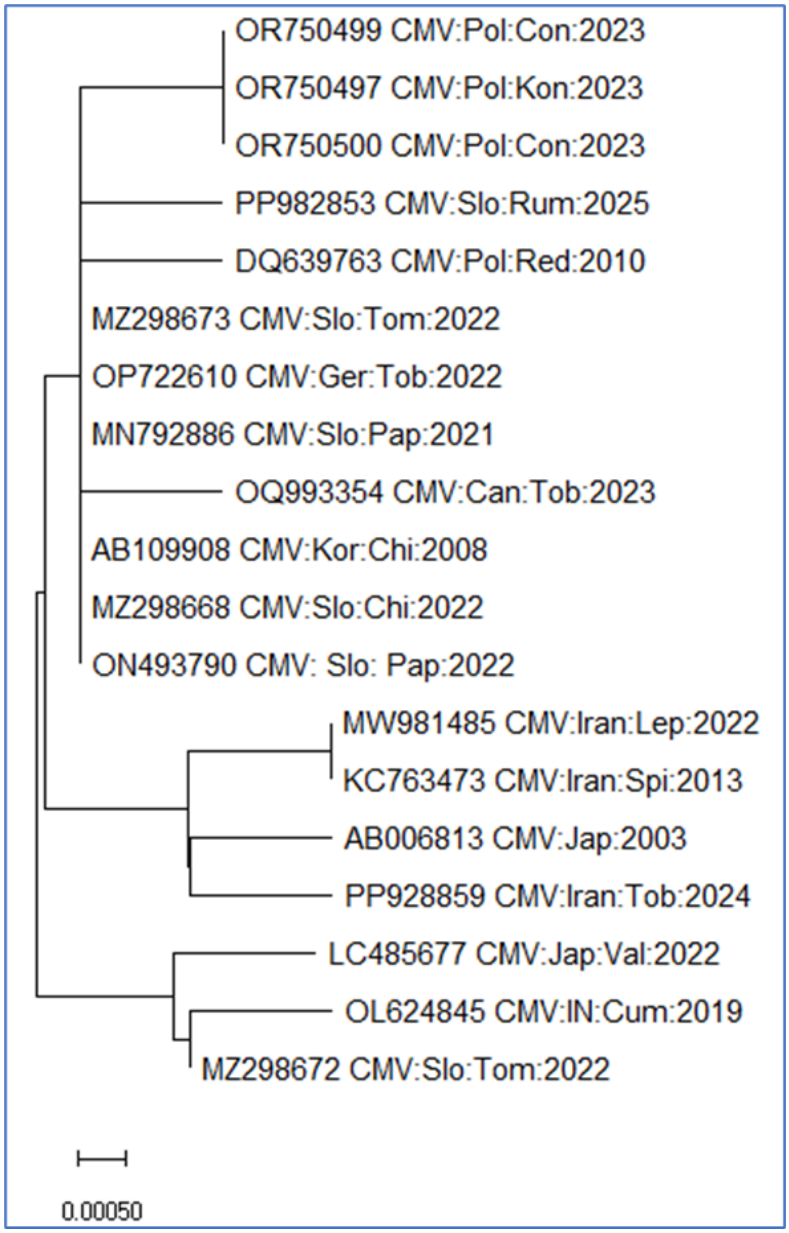


Fig. 4. Phylogenetic relationship of coat protein of CMV-ANK isolate with other CMV isolates from NCBI database.

Analysis of CMV-ANK isolate

The results of further analysis of sequenced CMV-ANK isolate using FGENESV of softberry software revealed that there is one predicted potential gene in the CMV ANK isolate. The Open Reading Frame (ORF) finder analysis revealed that there were four open reading frames, ORF 1 is in positive strand with 657 nucleotide length followed by ORF 3 in positive strand with 288 nucleotide length, ORF 2 in positive strand with 84 nucleotide length and ORF 4 in negative strand with 96 nucleotide length. Previous studies have proved that the CP gene encode 657 bp region (25, 26). The results of the pairwise nucleotide identity analysis, that CMV-ANK (OL624845) has nucleotide similarity of 99.5 % to 100 % with other CMV isolates retrieved from NCBI database. The nucleotide percentage similarities were visualized as color matrix (Fig. 5). Similarly, previous experiments showed that the coat protein of CMV isolate from passion fruit had 98 % nucleotide similarity with CMV isolate infecting yam retrieved from NCBI database (25).

Conclusion

This study successfully characterized the Cucumber mosaic virus (CMV) isolate (CMV-ANK) affecting cucumber in southern Tamil Nadu. The virus was confirmed through host range studies, TAS-ELISA and RT-PCR. Nucleotide sequencing showed 99.85 % identity with CMV isolates infecting tomato, highlighting close genetic similarity. Physical properties analysis revealed a dilution end point at 10^{-5}, thermal inactivation at 65 °C and longevity of up to three days at room temperature. Host range studies confirmed CMV's infectivity in multiple crops and weeds. The detection of CMV in seeds emphasizes the risk of seed transmission. These findings provide insights into CMV epidemiology and suggest the need for resistant varieties and integrated disease management strategies.

Acknowledgements

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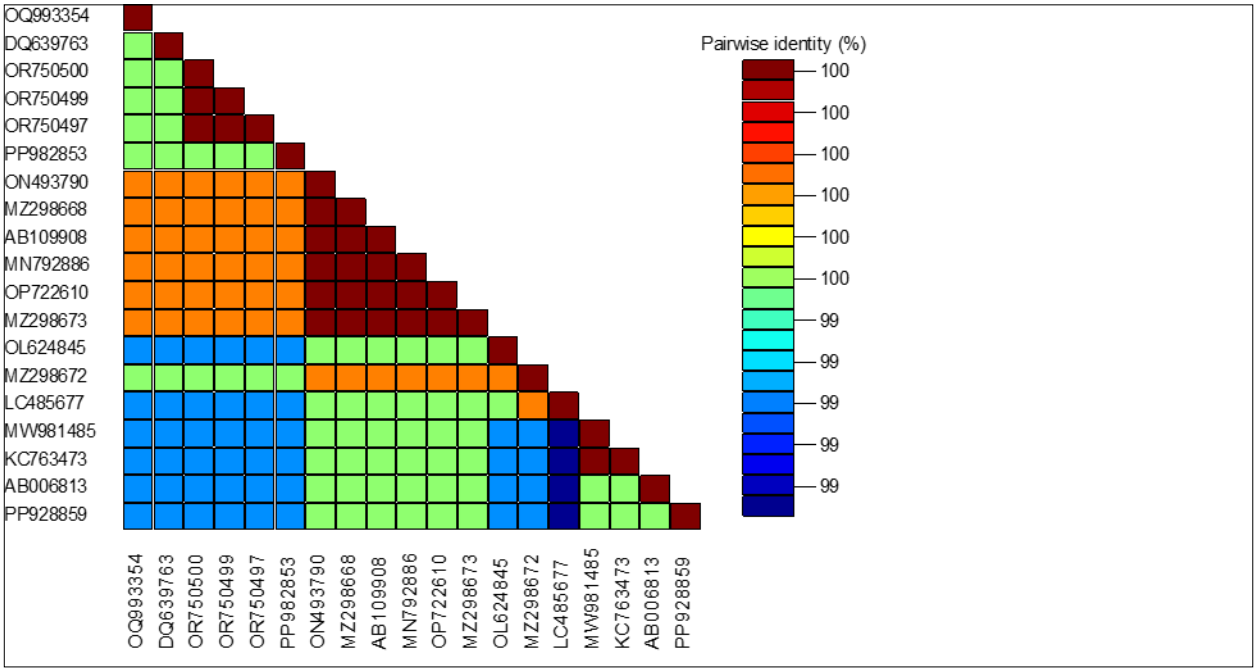

Fig. 5. Pairwise nucleotide identity of CMV-ANK isolates with other CMV isolates.

Authors' contributions

SE collected the articles and wrote the first draft. SR, KR, GD, SM, SD and EK edited the manuscript. RN assisted in correcting the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The author(s) declared that they have no competing interests.

Ethical issues: None

References

1. Chakravarthy HL. Fasicles of flora of India. Calcutta: Botanical Survey of India; 1982.
2. Agindotan B, Winter S, Lesemann D, Uwaifo A, Mignouna J, Hughes J, et al. Diversity of banana streak-inducing viruses in Nigeria and Ghana: Twice as many sources detected by immunoelectron microscopy (IEM) than by TAS-ELISA or IC-PCR. African Journal of Biotechnology. 2006;5(12):1194-203.
3. Rai M, Pandey S, Kumar S, Pitrat M. Cucurbit research in India: A retrospect. Varanasi: Indian Institute of Vegetable Research; 2008. p. 285-94.
4. Palukaitis P, Garcia-Arenal F. Cucumoviruses. Advances in Virus Research. 2003; 62:241-323. https://doi.org/10.1016/S0065-3527(03)62005-1
5. McClanahan RJ, Guyer GE. The role of insects in the spread of cucumber mosaic virus. Canadian Journal of Plant Science. 1964;44(1):1-6. https://doi.org/10.4141/cjps64-001
6. Jones RAC, Proudlove W. Further studies on cucumber mosaic virus infection of narrow‐leafed lupin (*Lupinus angustifolius*): seed‐borne infection, aphid transmission, spread and effects on grain yield. Annals of Applied Biology. 1991;118(2):319-29. https://doi.org/10.1111/j.1744-7348.1991.tb05631.x
7. Gildow FE, Shah DA, Sackett WM, Butzler TM, Nault BA, Fleischer SJ. Transmission efficiency of cucumber mosaic virus by aphids associated with virus epidemics in snap bean. Phytopathology. 2008;98(11):1233-41. https://doi.org/10.1094/PHYTO-98-11-1233.
8. Chandankar VD, Mondhe MK, Bhoyar PR, Ninawe BN, Jadesha G. Biophysical characterization, host range and transmission studies of cucumber mosaic virus. The Bioscan. 2013;8(2):437-41.
9. Subramanian KS, Narayanasamy P. Mechanical transmission of white fly-borne yellow mosaic virus of lablab niger medikus (*Dolichos lablab* L.). Current Sciences. 1970.
10. Kavyashri VV, Pappachan A, Padmaja AS, Nagaraju N, Rangaswamy KT. Biological and molecular characterization of cucumber mosaic virus isolate causing severe mosaic in Gherkin (*Cucumis anguria* L.) in India. Journal of Pure and Applied Microbiology. 2016;10(3):2089-99.
11. Arogundade O, Balogun OS, Kumar PL. Seed transmissibility of Cucumber mosaic virus in *Capsicum* species. International Journal of Vegetable Science. 2019;25(2):146-53. https://doi.org/10.1080/19315260.2018.1487498
12. Agindotan B, Winter S, Lesemann D, Uwaifo A, Mignouna J, Hughes J, Thottappilly G. Diversity of banana streak-inducing viruses in Nigeria and Ghana: Twice as many sources detected by immunoelectron microscopy (IEM) than by TAS-ELISA or IC-PCR. African Journal of Biotechnology. 2006;5(12).
13. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Annals of Biochemistry. 1987;162(1):156-9. https://doi.org/10.1016/0003-2697(87)90021-2.
14. Ramesh B, Sreenivasulu P. Detection of cucumber mosaic virus in brinjal and chilli by RT-PCR. International Journal of Pharmacy and Biological Sciences. 2018;8(3):128-31.
15. Ahamedemujtaba V, Cherian AK, Namitha PM, Louis V, Beena S. Detection and biophysical characterization studies of cucumber mosaic virus causing infectious chlorosis disease of banana. Journal of Pharmacognsy and Phytochemistry. 2019;8(1):2606-11.
16. Nagendran K, Priyanka R, Aravintharaj R, Balaji CG, Prashant S, Basavaraj B, et al. Characterization of cucumber mosaic virus infecting snake gourd and bottle gourd in India. Physiology and Molecular Plant Pathology. 2018;103:102-6. https://doi.org/10.1016/j.pmpp.2018.05.001
17. Supyani SA, Chandra F, Rochman DN, Septariani N, Widadi S. Occurrence and distribution of cucumber mosaic virus in cucurbits in Karanganyar, Central Java, Indonesia. African Journal of Agricultural Research. 2017;12(18):1593-601. https://doi.org/10.5897/AJAR2017.12247

18. Rani A, Jansirani P, Rabindran R. Screening and identification of ridge gourd [*Luffa acutangula* (L.) Roxb] genotypes against Cucumber mosaic virus (CMV) tolerance. International Journal of Current Microbiology and Applied Sciences. 2017;6(3):119-27. https://doi.org/10.20546/ijcmas.2017.603.014.

19. El-Aziz MHA, Younes HA. Detection of Cucumber mosaic cucumovirus in infected cowpea plants (*Vigna unguiculata* L.) from northern Egypt. Novel Research in Microbiology Journal. 2019;3:326-40.

20. Daryono BS, Natsuaki KT. Survey on the occurrence of viruses infecting cucurbits in Yogyakarta and Central Java. Jurnal Perlindungan Tanaman Indonesia. 2009;15(2):83-9. https://doi.org/10.22146/jpti.11769

21. Adhab MA, Al-Ani RA. Amaryllis and shrimp plant are secondary hosts of cucumber mosaic cucumovirus (CMV) in Iraq. Agriculture and Biology Journal of North America. 2011;2(5):872-5. https://doi.org/10.5251/abjna.2011.2.5.872.875

22. Salaudeen MT, Oluwatosin O, Gana AS. Reactions of commercial cultivars of okra, pepper and tomato to cucumber mosaic virus disease. Agro-Science. 2018;17(2):27-36. https://doi.org/10.4314/as. v17i2.5

23. Akhtar KP, Anwer M, Saleem MY, Yousaf S, Ullah N, Cheema HMN. Identification of natural weed hosts of Cucumber mosaic virus subgroup-I and the absence of seed transmission in weed hosts in Pakistan. Journal of Horticultural Science and Biotechnology. 2019;94(4):468-74. https://doi.org/10.1080/14620316.2019.1565947

24. Pratap D, Kumar S, Snehi SK, Raj SK. Biological and molecular characterization of cucumber mosaic virus isolate causing shoestring disease of tomato in India which has closer affinity to European or East Asian isolates of CMV. Indian Journal of Virology. 2012;23(1):57-63. https://doi.org/10.1007/s13337-012-0074-0

25. Srivastava A, Chandra G, Raj SK. Molecular characterization of a strain of cucumber mosaic virus based on coat protein and movement protein genes. Acta Virologica. 2004;48(4):229-39. https://doi.org/10.4149/av_2004_04_229

26. Liu YY, Yu SL, Lan YF, Zhang CL, Hou SS, Li XD, et al. Molecular variability of five cucumber mosaic virus isolates from China. Acta Virologica. 2009;53(2):89. https://doi.org/10:4149/av_2009_02_89

27. Arogundade O, Atanda H Y, Ebre V O, Aliyu T H, Banji A. Detection and characterization of cucumber mosaic virus (CMV) infecting passion fruit in Nigeria. Indian Phytopathology. 2023;76:879-87. https://doi.org/10.1007/s42360-023-00649-2

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