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RESEARCH ARTICLE

Etiology and immuno-molecular detection of snake gourd (*Trichosanthes anguina* L.) mosaic disease in Kerala, India

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Abstract

In August 2022, snake gourd plants (cv. Kaumudi; n = 90) were observed with mosaic, mottling, blistering, vein banding and deformed fruits at Vellayani (N 8°25'59.6", E 76°59'09.5"), Kakkamoola (N 8°25'26.0", E 77°00'21.9") and Manamboor villages (N 8°42'58.0", E 76°46'36.0") of Thiruvananthapuram district of Kerala, India. Disease incidence ranged between 28.5 and 100%. The viruses were sap transmissible and maintained in snake gourd and in local lesion hosts *viz*., *Chenopodium amaranticolor* and *Nicotiana tabacum* var Samsun. The viruses were mechanically transmissible to cucurbitaceous crops and *N. glutinosa*. No symptoms were produced in tomato, chili, brinjal and papaya. The symptomatic snake gourd samples reacted positively with *Papaya ringspot virus* (PRSV) polyclonal antiserum (DSMZ, Germany) in double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and *Cucumber mosaic virus* (CMV) polyclonal antiserum (ICAR-NRC, Tamil Nadu) in Direct antigen coating ELISA. Reverse transcriptase polymerase chain reaction gave amplicons of size 1200 and 400 bp with primers specific to the coat protein gene of PRSV and the 2a protein gene of CMV, respectively. Comparative nucleotide sequence alignment of isolates revealed 86.5 and 93.8% homology with PRSV and CMV isolates of snake gourd from Tamil Nadu. Phylogenetic analysis identified PRSV isolates as type W. The results of the current study revealed for the first time the etiology of the snake gourd mosaic disease complex in Kerala, which can pave the way for devising management strategies.

Keywords: Snake gourd, PRSV, CMV, ELISA, RT-PCR.

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Introduction

Snake gourd (*Trichosanthes anguina* L.) of Family Cucurbitaceae is an important vegetable crop widely cultivated in humid lowland tropics of the world (Pradheep *et al*., 2015). The crop originated from Southeast Asia and some regions of Africa/Australia in wild form and later got domesticated. Even though the economic potential and nutritional benefits of snake gourd are very high, it is grown as a minor vegetable in the majority of countries due to its lowest global production, limited processing technologies, marketing facilities and consumption (Idowu *et al*., 2019). Snake gourd is a rich source of proteins, fat, fiber, carbohydrates, vitamins, phenolics, flavonoids and minerals. It also has several medicinal properties viz., antidiabetic, hepatoprotective, anti-inflammatory and antibiotic properties (Kritikar & Basu, 1999; Kumar *et al*., 2009). Dried seeds of snake gourd are used as an alternative medicine to treat diarrhea anthelmintics and also exhibit antibacterial properties (Yusuf *et al*., 2007). It is also an important summer vegetable crop grown in Kerala, India, during January-March and August-December for its tender fruits. In Kerala, it is cultivated in an area of 745 hectares with an annual production of 8023 tonnes (Department of Economics and Statistics, 2023).

Viruses are reported to infect snake gourd at different stages of the crop, causing yield losses. Wide host range, lack of efficient insect control options and susceptibility of host genotypes are the reasons for the occurrence of such damaging virus epidemics. Viruses coming under the genera *viz*., *Potyvirus*, *Cucumovirus* and *Tobamovirus* are reported to be associated with the mosaic disease in snake gourd (Ariyaratne *et al*., 2005; Jadao *et al*., 2010; Kumar *et al*., 2014). Ashwini *et al*. (2016) reported the mixed infection of *Papaya ring spot virus* (PRSV) and *Cucumber mosaic virus* (CMV) in bottle gourd from Kerala. PRSV, *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV) are the reported prevalent viruses coming under the *Potyvirus* genera infecting cucurbits (cucumber, bitter gourd, watermelon, etc). The flexuous, non-enveloped rod-shaped particles of the *Potyvirus* genus are 680 to 900 nm long and 11 to 13 nm in diameter. Potyviruses have monopartite, positive sense RNA genome which encodes a single polyprotein that is further cleaved into nine or ten mature proteins (Khanal & Ali, 2018). The genus is sap and aphid transmissible in a non-persistent manner. The combined host range of the numerous Potyviruses is quite wide; however individual Potyvirus like PRSV have a limited host range (Coutts *et al*., 2013). The genus *Cucumovirus* includes CMV, the economically devastating plant virus as a type member with an icosahedral particle of size 28 to 30 nm in diameter, infecting cucurbitaceous crops. Its genome is a single-stranded positive-sense tripartite RNA composed of three genomic RNAs, *viz*., RNA1, RNA2, RNA3 and two subgenomic RNAs *viz*., RNA4 and RNA4A (Sivakumaran *et al*., 2002). CMV have a wide host range and can infect over 1200 plant species in 100 plant families, with an average yield loss of 10 to 20% under field conditions (Liu *et al*., 2009). CMV is naturally transmitted through more than 85 aphid species in a non-persistent way as well as sap transmissible and sometimes seed-borne (Palukaitis & Arenal, 2003).

In August 2022, mosaic, mosaic mottling, vein banding, blistering, distortion, puckering and deformed fruits were observed in snake gourd plants (cv. Kaumudi; n = 90) at Vellayani (N 8°25'59.6", E 76°59'09.5"), Kakkamoola (N 8°25'26.0", E 77°00'21.9") and Manamboor villages (N 8°42'58.0", E 76°46'36.0") from Thiruvananthapuram district of Kerala, India. Continuous vigilance and detection of these emerging viruses are very significant to protect the snake gourd crop from the snake gourd mosaic disease by reducing its incidence and severity. The wide and overlapping host range of these viruses, close relationships with polyphagous insect vectors and difficulties in predicting their outbreaks pose challenges to the development and implementation of effective management programs. So, investigations are required to determine their distribution and their economic impact on snake gourd production in the country. Potyviruses (family Potyviridae and genus *Potyvirus*) and Cucumoviruses (family Bromoviridae and genus *Cucumovirus*) are widespread in cucurbitaceous crops of Kerala; hence, they were assumed to be associated with the mosaic disease in snake gourd. In this context, the study was carried out at the College of Agriculture, Vellayani, in 2022 with the objective of symptomatology, maintenance of the virus in systemic and local lesion hosts, host range studies, immunological diagnosis and molecular diagnosis of the viruses causing mosaic disease in snake gourd (*Trichosanthes anguina* L.).

Materials and Methods

Survey and sample collection

In August 2022, mosaic, mosaic mottling, vein banding, blistering, distortion, puckering and deformed fruits were observed in snake gourd plants of cv. Kaumudi (n = 90) from Vellayani, Kakkamoola and Manamboor villages of Thiruvananthapuram district, Kerala. Mosaic diseased snake gourd samples were collected from these areas and symptomatology was studied. The percent disease incidence (PDI) of snake gourd mosaic disease was calculated from all the locations. Disease incidence at different growth stages of the snake gourd plants, *viz*., one month after transplanting (MAT) to three MAT, were recorded. Days taken for symptom development and the nature of symptoms developed on snake gourd were observed. The percent disease incidence was calculated using the formula.

$$\text{Disease incidence (D. I.)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Maintenance of virus through mechanical transmission

The diseased samples collected from fields were mechanically transmitted to a systemic host *i.e*., snake gourd cv. Kaumudi at the three-leaf stage and the local lesion hosts *viz*., *Chenopodium amaranticolor* (at 8 leaf stage) specific for *Potyvirus* and *Nicotiana tabacum* var. Samsun (at 10 days after transplanting) specific for *Cucumovirus* under insect proof glasshouse conditions. Sap was prepared from infected snake gourd leaves by grinding with 0.01 M potassium phosphate buffer (pH – 7.2) containing 0.1% 2-mercaptoethanol @ 1.5 ml/g of leaf, using pre-chilled mortar and pestle. The tissue was strained through non-absorbent muslin cloth, the homogenate was taken in an ice box and immediately used for inoculation. The sap was rub-inoculated on to the test seedlings, previously dusted with carborundum. After 5 min, the excess sap was washed off with distilled water. The development of symptoms on these plants was recorded up to 60 days after inoculation (DAI).

Host range studies

Host range studies of the virus with host plants belonging to the family Cucurbitaceae *viz.* pumpkin var. Saras, bitter

gourd var. Preethi, cucumber var. Vishal, ridge gourd var. local, bottle gourd var. local and water melon var. Sugar Baby; and family Solanaceae *viz*., tomato var. Anagha, chili var. Athulya, brinjal var. Surya and *Nicotiana glutinosa;* and papaya of family Caricaceae was carried out. Mechanical transmission of viruses were carried out on the seedlings of Cucurbitaceae at three-leaf stage, Solanaceae at ten days after transplanting, two-month-old seedlings of Caricaceae and were maintained in the insect proof glass house. The sap was extracted from snake gourd leaves showing typical mosaic symptoms and transmission was conducted with 0.01 M Potassium phosphate buffer (pH 7.2) at the rate of 1 g per 1.5 ml of the buffer using pre-chilled mortar and pestle. The development of symptoms on these host plants was recorded up to 60 days after inoculation (DAI).

Immunological diagnosis

Symptomatic snake gourd samples from surveyed locations and mechanical transmission studies were analyzed by DAC-ELISA and DAS-ELISA, using the polyclonal antisera specific to coat protein gene of CMV and PRSV respectively, in order to investigate the etiology of snake gourd mosaic disease.

Direct antigen coating-Enzyme linked immunosorbent assay (DAC- ELISA)

DAC-ELISA was performed for the detection of CMV following the manufacturer's instruction (National Research Centre (NRC) for Banana, Tamil Nadu). Infected young snake gourd leaf (1 g) was homogenized in 5 ml of coating buffer (Carbonate buffer) containing 2% (w/v) PVP under chilled condition. Healthy plant extract was prepared by using leaves of healthy plants and the homogenate was centrifuged at 5000 rpm for 10 min at 4°C. Sample was dispensed at the rate of 200 ml into Nunc immunological plates. The treatment was replicated thrice. After 1 h of incubation at 37 °C, the wells were washed with PBS-T (three times each for a duration of 3 min) using an ELISA plate washer (PW-40, BIORAD). Blocking was done with 100 ml of 5% spray dried milk (SDM) for 2 h at 37 °C. After incubation, plate was washed with PBS-T as before. Then plate was treated with 100 ml of polyclonal antibody, diluted in PBS-T Polyvinyl pyrrolidone ovalbumin (PBS-TPO). Three replications were maintained for each treatment and was incubated overnight at 4°C. The plate was washed again with PBS-T, then treated with 100 ml of antirabbit immunoglobulin (SIGMA-Aldrich) diluted in PBS-TPO and incubated for 2 h at 37°C. Wells were washed with PBS-T as before, the substrate, PNPP in diethanolamine buffer (1 mg per ml) was added to each well (100 ml per well) and incubated at 37 °C for 1 h. Reaction was stopped by adding 50 ml of 4% Sodium hydroxide. The absorbance was read at 405 nm in an ELISA reader (Microplate Reader 680, BIORAD).

Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS- ELISA)

DAS-ELISA was performed for detection of PRSV following the manufacturer's instruction (DSMZ Gmbh, Braunschweig, Germany). The primary antibody was diluted in coating buffer (1:1000) and 200µl was added to each well of the microtiter plate. The plate was covered and incubated at 37°C for 2 h. The plate was washed with Phosphate Buffer Saline – Tween (PBS-T) for 3 times. Infected leaf sample was extracted @ 1:20 (w/v) in extraction buffer and 200 µl aliquots of the sample was added in the wells (in three replications). Plate was covered and incubated overnight at 4°C. Plate was washed three times and 200 µl of enzyme conjugate in conjugate buffer (1:1000) was added. Plate was covered and incubated at 37°C for 3 hours. Plate was washed three times and 200 µl of freshly prepared substrate (para- nitrophenyl-phosphate (PNPP) in substrate buffer @ 1 mg/ml) was added to each well. The plate was covered and incubated at 37°C for 30 min to obtain clear reactions. Enzyme-mediated colour reaction was assessed by checking absorbance at 415 nm in an ELISA reader (BIORAD, Model 680 Microplate reader). Sample was recorded as positive if the absorbance value exceeds the healthy sample value by 2 or 3 times.

Molecular diagnosis

Molecular diagnosis *via* Reverse Transcription-Polymerase chain reaction (RT-PCR) was carried out with primers specific to the coat protein of PRSV and 2a protein of CMV (Table 1) for the detection of viruses infecting snake gourd. Total RNA from infected snake gourd samples (100 mg) were extracted using TRIzol (Thermo Fisher Scientific) method. The quantity and quality of the isolated RNA was analyzed in Nanodrop. The quality of RNA isolated was checked using 1% agarose gel electrophoresis. The RNA isolated from leaf samples were reverse transcribed to cDNA using Two Step AMV RT-PCR kit (GeNei) as per the manufacturer's instructions. The PCR amplification reactions were carried out in a 50 µl reaction mixture. The reaction mix was gently mixed and template RNA (2 µg / reaction) was added to the individual PCR tubes. For the amplification of PRSV, reaction mixture was run in a thermocycler (96 well Thermal cycler, Applied

Table 1: Gene specific primers used for the detection of snake gourd mosaic viruses

Primers	*Sequences (5′-3′)*	*Target region*	*Product size (bp)*	*Annealing temperature*	*References*
PRSV-F1 PRSV-F2	ATGATAGAGTCATGGGG GTTGCGCATACCCAGGAGAG	Coat protein	1200	50°C	Jain *et al*. (1998)
CMV 1 CMV 2	GATCATCGCCTGAGAATA TTCCAGAGATGCCTTCG	2a	400	57°C	Kumari *et al*. (2021)

Biosystems) under the conditions standardized for PRSV primers with initial denaturation of 3 min at 94 °C followed by 34 cycles of denaturation at 94 °C for 20 s, annealing and extension temperature being specific for particular amplicon and finally an extension time of 10 min at 72°C. For the amplification of CMV, the reaction conditions standardised were an initial denaturation of 2 minutes at 94°C, followed by 30 cycles of denaturation at 94°C for 20 seconds, annealing and extension temperature being specific for particular amplicon and finally an extension time of 10 min at 72°C.

Sequencing of PCR products and phylogenetic analysis

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. Purification of PCR products were done using sephadex G-50 followed by run in ABI 3730 XL DNA Analyzer 3 (Applied Biosystems). The sequence quality was checked using Sequencing Analysis Software v5.2 (Applied Biosystems). The PCR products were sequenced and deposited in NCBI Genbank using Bankit software and accession numbers were obtained. The characterisation of PRSV and CMV isolates from snake gourd was done by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and the sequences were matched with existing database from NCBI. The nucleotide sequences based on the coat protein region of the PRSV isolate and other related PRSV isolates were retrieved from NCBI Genbank data base and compared. Similarly, the nucleotide sequences based on the 2a protein region of the CMV isolate and other related CMV isolates were also retrieved and compared. Multiple nucleotide sequence alignment and phylogenetic analysis was done using Molecular Evolutionary Genetics Analysis 11 software (MEGA 11) (Kumar *et al*., 2018). The estimation of genetic diversity and structure was done through alignment using the CLUSTALW algorithm (Higgins, 1994), which is integrated in the MEGA software. The p-distance method was used over nucleotide substitution models and the genetic structure of the isolates was depicted through phylogenetic trees, constructed using Minimum Evolution (ME) method (1000 bootstrap replicates).

Results and Discussion

Survey and sample collection

Mosaic, mosaic mottling, vein banding, blistering, distortion, puckering on leaves and deformed fruits were the symptoms of snake gourd mosaic disease observed from the surveyed fields of Vellayani, Kakkamoola and Manamboor (Figure 1).

Figure 1: Symptoms of snake gourd mosaic disease observed from surveyed locations

Symptom of mosaic is described as light green, dark green or yellow areas forming a variegated pattern. Mosaic mottling is described as discoloured light and dark green patches including specks, dots, spots etc. with respect to size, shape, distinctness of boundary and number of patches. Vein banding is described as tissues along the veins turning to dark green compared to interveinal areas (Bos & Bos, 1970). The initial symptom of snake gourd mosaic disease observed during the survey was slight mottling on younger leaves followed by chlorotic spots / mosaic on leaves, sometimes localized or covering the entire leaf area along with vein banding, formation of blisters, puckering or raised surfaces and distortion of leaf. Several workers have described similar type of symptoms for snake gourd mosaic disease. Joseph & Menon (1979) from Kerala reported that natural infection of *Cucumis virus* 1 on snake gourd produced mosaic, dark green blisters and malformations on the leaf lamina. Mosaic mottling, blistering and distortion on leaves were the symptoms on snake gourd infected by PRSV-W strain (Kumar *et al*., 2014). Symptoms of mosaic, thickened leaf, rosetting accompanied by downward curling of leaf were documented in snake gourd plants infected by CMV (Rajapaksha *et al*., 2019; Barkavi *et al*., 2021). The mosaic disease occurred throughout the growing seasons of snake gourd in all surveyed locations with the highest disease incidence of 94-100% observed from fields of Vellayani followed by Kakkamoola (64.5%) and Manamboor (28.5-64%) at 3 MAT. The disease incidence in majority of the fields was found to be over 25.0%. Monocropping, crop rotation with related crop hosts, presence of weeds, polyphagous nature of insect vectors, crop canopy and seasonal variation were the reasons for high disease incidence and severity of snake gourd mosaic disease.

Maintenance of virus through mechanical transmission

Mechanical transmission of snake gourd mosaic viruses into snake gourd cv. Kaumudi took 14 days for development of mosaic symptoms, with a% transmission of 100. Local lesion assay using *Chenopodium amaranticolor* specific for *Potyvirus* and *N. tabaccum* var. Samsun specific for *Cucumovirus*, produced chlorotic local lesions at 21 and 15 days after transmission with percent transmission of 73.3 and 80.0 respectively (Figure 2). However, the mean number of lesions produced were less in *Chenopodium* (7) compared to *N. tabacum* (10). This may be due to less virus titre in *Chenopodium* compared to *N. tabacum*. Thus, the local lesion assay indicated the association of *Potyviruses* and *Cucumoviruses* with snake gourd mosaic disease. Concurrent with the findings of the present study, Kumar et al. (2014) reported that *C. amaranticolor* produced chlorotic spots in response to the inoculation with PRSV-W from snake gourd. Barbosa *et al*. (2016) recorded the development of chorotic local lesion on *C. amaranticolor* leaves after mechanical inoculation with PRSV-W.

Uninoculated Inoculated Uninoculated Inoculated

Figure 2: Chlorotic local lesions on leaves of *Chenopodium amaranticolor* and *Nicotiana tabacum* var. Samsun in response to virus inoculation

Host range studies

Experimental findings showed that the sap inoculation successfully transmitted the viruses to plants belonging to the family Cucurbitaceae. Symptoms observed on cucurbitaceous hosts *viz*., pumpkin, bitter gourd, cucumber, ridge gourd, bottle gourd and watermelon ranged from mosaic, chlorosis, vein banding, leaf blisters and leaf distortion. Pumpkin, bitter gourd and cucumber took 12, 15 and 13 days for the development of systemic symptoms with 100% transmission. Ridge gourd, bottle gourd and watermelon took 17, 10 and 18 days for the development of systemic symptoms with 90, 100 and 86.6% transmission. Tomato, chili, brinjal and papaya did not develop any symptoms after mechanical transmission with snake gourd mosaic viruses. *Nicotiana glutinosa* took 18 days for the development of mosaic mottling symptoms with a percent transmission of 70. Similarly, Kumar *et al*. (2014) reported that cucurbitaceous crops *viz*., snake gourd, bottle gourd, ridge gourd, cucumber, pumpkin and melon took 15-17 days for symptom expression in response to mechanical inoculation with PRSV-W. Nagendran *et al*. (2018) recorded the development of necrotic lesions with a yellow halo on inoculated leaves of *N. glutinosa* within 5 to 7 days of post-inoculation with snake gourd CMV isolate, followed by the appearance of systemic mosaic mottling on newer leaves. They also recorded the development of systemic symptoms *viz*., mosaic mottling, vein clearing, stunted growth and chlorotic spots in bottle gourd, pumpkin, ridge gourd, watermelon, cucumber and ash gourd.

According to the host range specificity, PRSV is classified into two biotypes: (i) PRSV-W, formerly *water mosaic virus* 1, which naturally infects Cucurbitaceae crops but is unable to infect papaya, and (ii) PRSV-P, which naturally infects papaya (*Carica papaya*) and can be transmitted experimentally to cucurbits. PRSV-P has a narrow host range, including 15 species in three families (Caricaceae, Chenopodiaceae and Cucurbitaceae) (Roy *et al*., 1999). PRSV-W has a wide host range, including 40 plant species, 38 of which are

Table 2: Reaction of symptomatic snake gourd samples to polyclonal antibodies of PRSV and CMV in ELISA

Snake gourd samples	*PRSV in DAS-ELISA*				*CMV in DAC-ELISA*			
	Absorbance at 415 nm (Values are mean of three replications)							
	Diseased	*Healthy*	*Ratio of absorbance*	*Remarks*	*Diseased*	*Healthy*	*Ratio of absorbance*	*Remarks*
Mosaic	0.022	0.008	2.7	Positive	0.075	0.037	2.0	Positive
Vein banding	0.021	0.008	2.6	Positive	0.077	0.037	2.1	Positive
Leaf blister	0.019	0.008	2.3	Positive	0.031	0.037	0.8	Negative
Puckering	0.010	0.008	1.3	Negative	0.085	0.037	2.2	Positive
Leaf distortion	0.026	0.008	3.2	Positive	0.055	0.037	1.5	Negative

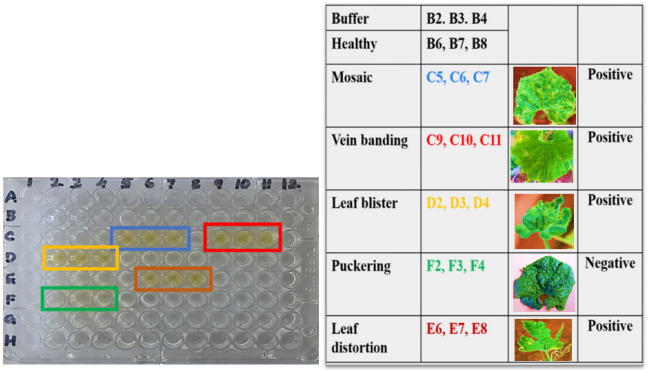

Figure 3: Reaction of symptomatic snake gourd samples to polyclonal antibody of PRSV in DAS-ELISA

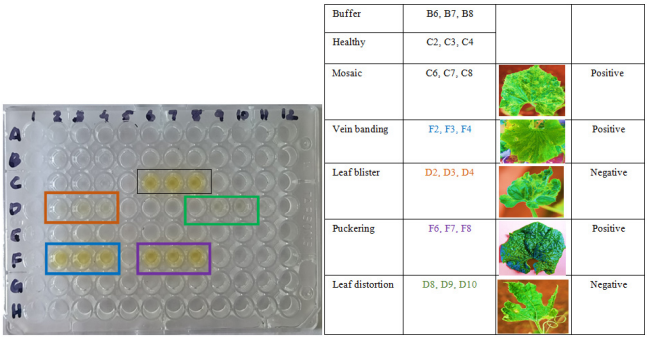

Figure 4: Reaction of symptomatic snake gourd samples to polyclonal antibody of CMV in DAC-ELISA

in the family Cucurbitaceae and two of which are in the family Chenopodiaceae (Lecoq & Desbiez, 2012). CMV has a wide host range and is found to infect over 1200 species belonging to 100 families of monocots and dicots, including many vegetable crops (Zitter & Murphy, 2009). Based on this literature information and results of the current host range studies, snake gourd mosaic viruses were assumed as a complex of PRSV Type-W and CMV.

Immunological diagnosis

ELISA was preliminarily used to detect PRSV and CMV from infected samples showing the characteristic symptoms. The samples of mosaic, vein banding, leaf blister and leaf distortion reacted positively to a polyclonal antibody specific for coat protein gene of PRSV with 2.7, 2.6, 2.3 and 3.2-fold increases in titer values over healthy samples in DAS-ELISA (Figure 3). The samples of mosaic, vein banding and puckering reacted positively to a polyclonal antibody specific for the coat protein gene of CMV with 2.0, 2.1 and 2.2 folds increase in titer values over healthy samples in DAC-ELISA (Table 2) (Figure 4). ELISA results revealed that PRSV produced symptoms such as blistering and leaf distortion, whereas CMV produced symptoms such as puckering on leaves. Both PRSV and CMV induced the symptoms of mosaic and vein banding. ELISA results confirmed that the combined infection of PRSV and CMV causes the snake gourd mosaic disease. Similarly, Abdelkhalek *et al.* (2022) reported that symptomatic leaf samples of squash reacted positively with polyclonal antiserum of CMV in DAS-ELISA with a maximum ELISA absorbance value (0.781) recorded at 12 days post-inoculation.

Molecular diagnosis

Molecular diagnosis with RT-PCR is the most reliable technique to detect plant RNA viruses. Even though immunological techniques are easy to perform, low virus titer in a sample can limit its sensitivity, producing false negatives when the virus concentration is below the detection threshold. From symptomatic leaves, total RNA of good quality (1.9–2.0) was extracted, followed by RT-PCR using specific primers for the coat protein gene of PRSV and CMV, which gave amplicons of sizes 1200 and 400 bp, respectively (Figures 5 and 6). Thus, RT-PCR detected PRSV and CMV from infected snake gourd samples.

Sequencing of PCR products and phylogenetic analysis

The PCR product obtained using primer specific to the coat protein of PRSV was subjected to comparative nucleotide sequence alignment using BLAST software, which showed 86.5% homology with PRSV isolate of snake gourd from Tamil Nadu (KP161501.1). Similarly, the sequence of the PCR product obtained using a primer specific to 2a protein of CMV showed 93.8% homology with CMV isolate of snake

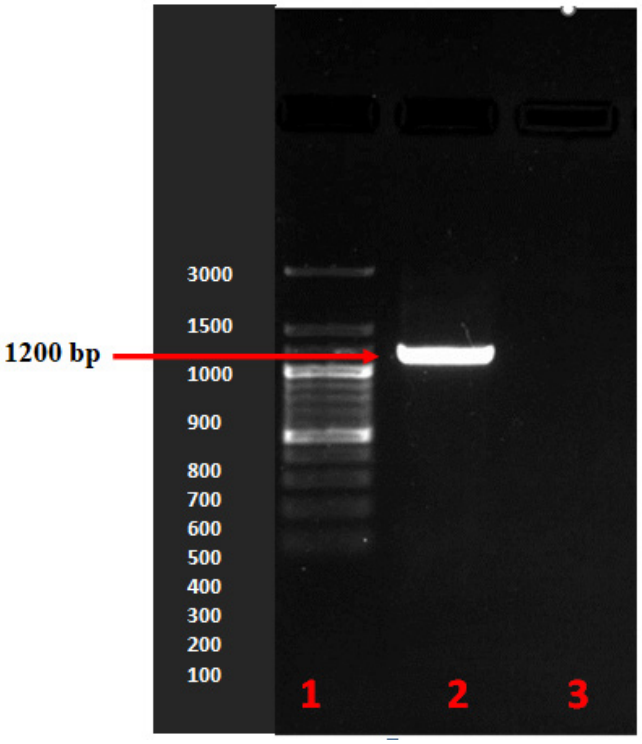

Figure 5: Electrophoresis gel image of amplified PRSV- RNA using primer PRSV-F1 and PRSV-F2. Lane 1) 100 bp DNA ladder 2) Infected snake gourd 3) Healthy (control)

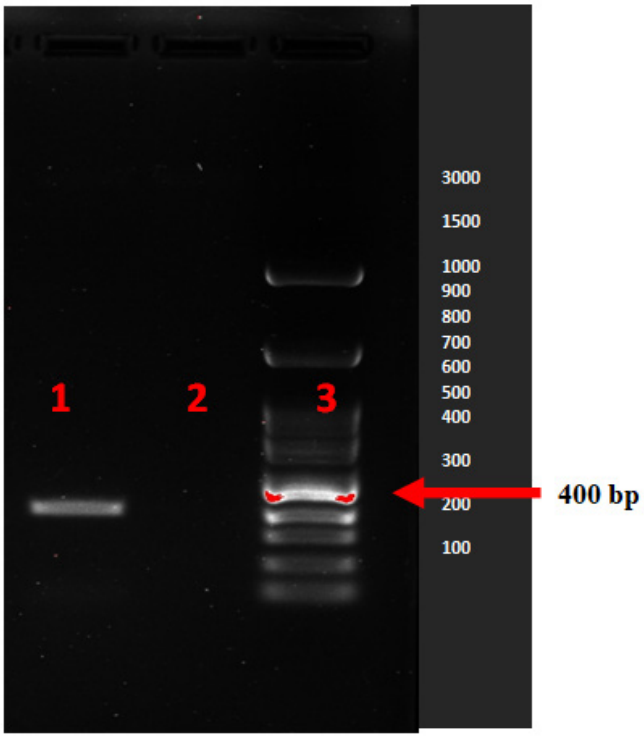

Figure 6: Electrophoresis gel image of amplified CMV- RNA using CMV 1 & CMV 2 primers Lane 1) Infected snake gourd 2) Healthy (control) 3) 100 bp DNA ladder

gourd from Tamil Nadu (KJ778898.1). The sequences were deposited in NCBI Genbank using Bankit software and the accession numbers *viz*., OR601008 and OR601007 were obtained for PRSV and CMV, respectively. The genetic structure of PRSV and CMV was depicted through phylogenetic trees, constructed using MEGA 11 software by the minimum evolution (ME) method and nucleotide diversity was estimated. Nucleotide diversity represents the average distance, indicating the proportion of nucleotide differences between pairs of sequences. It serves as a metric for assessing the genetic variation within a virus population (Rubio *et al*., 2020). The PRSV isolate pertaining to this study was grouped in the same clade along with other reported PRSV type-W isolates. Thus the isolate was identified as PRSV type-W (Figure 7). Similarly, CMV isolates from the snake gourd, grouped in the same clade along with other related CMV isolates from the snake gourd and *Musa* sp. These results were in confirmation of the findings of several workers. Kumar *et al*. (2014) detected PRSV-W from mosaic-infected snake gourd samples and obtained an amplicon of 650 bp using RT-PCR with potyvirus-specific primers HRP 1, HRP 2, HRP 3 and HRP 4. Nagendran *et al*. (2018) carried out RT-PCR analysis of snake gourd mosaic diseased samples with primers specific to 1a, 2a, 2b, 3a and 3b protein of CMV and obtained amplicons of the size ~1200 bp. They carried out the sequence analysis which unveiled

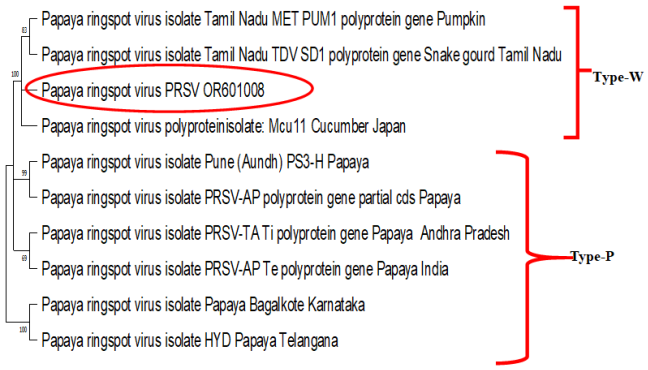

Figure 7: Dendrogram showing the relationship of PRSV isolate with reported PRSV isolates

that their isolate exhibited a 97% nucleotide identity with the CMV strain identified from India. Kumari *et al*. (2021) revealed that the RT-PCR using 2a protein-specific primers successfully diagnosed natural CMV infection in cucurbits *viz*., snake gourd, bitter gourd, sponge gourd and ridge gourd from Varanasi. They developed a phylogenetic tree, which showed that CMV isolates affecting cucurbits had a common lineage with CMV strains identified from tomatoes, snake gourd, bottle gourd, pepper and banana from India.

Conclusion

In the surveyed locations of Thiruvananthapuram district, Kerala *viz*., Vellayani, Kakkamoola and Manamboor villages,

snake gourd mosaic disease is caused by the combined infection of PRSV-W and CMV. Mosaic, mosaic mottling, blistering, vein banding, leaf distortion and deformed fruits were the recorded typical symptoms of snake gourd mosaic disease. ELISA and RT-PCR identified the snake gourd mosaic viruses as PRSV and CMV. Host range study identified the associated PRSV as Type W since it produced systemic symptoms on all the tested cucurbitaceous hosts, whereas no symptoms were produced in solanaceous hosts and papaya. Phylogenetic tree analysis further confirmed the PRSV isolates from snake gourd as type-W.

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सारांश

अगस्त 2022 में, वेल्लयानी, कक्कामूला और मनमबूर गांवों में चिचिंडा के पौधों में मोज़ेक, धब्बेदार, फफोलेदार, नस बैंडिंग और विकृत फल देखे गए। रोग की घटना 28.5 से 100% के बीच थी। विषाणु रस संचरित होते थे और चिचिंडा और स्थानीय घाव मेजबानों, चेनोपोडियम अमारेटिकोलर और निकोटियाना टैबैकम में बने रहते थे। वायरस यांत्रिक रूप से अन्य कुकुर्बिटेशियस फसलों और सोलेनेसियस मेजबान, जैसे- एन. ग्लूटिनोसा में संचारित होते थे। टमाटर, मिर्च, बैंगन एवं पपीता में कोई लक्षण उत्पन्न नहीं हुए। डायरेक्ट एंटीजन कोटिंग एलीसा में, रोगसूचक चिचिंडा के नमूनों ने डबल-एंटीबॉडी सैंडविच एंजाइम लिंक्ड इम्युनोसॉरबेट परख (डीएएस-एलिसा) खीरा मोज़ेक वायरस (सीएमवी) पॉलीक्लोनल एंटीसेरम (आईसीएआर-एनआरसी, तमिल) और पपीता रिगस्पॉट वायरस (पीआरएसवी) पॉलीक्लोनल एंटीसेरम (डीएसएमजेड, जर्मनी) के साथ सकारात्मक प्रतिक्रिया व्यक्त की। रिवर्स ट्रांसक्रिपटेस पोलीमरेज़ चेन रिएक्शन ने क्रमशः पीआरएसवी के कोट प्रोटीन जीन और सीएमवी के 2 ए प्रोटीन जीन के लिए विशिष्ट प्राइमरों के साथ 1200 बीपी और 400 बीपी आकार के एम्प्लिकॉन दिए। आइसोलेट्स के तुलनात्मक न्यूक्लियोटाइड अनुक्रम संरेखण का तमिलनाडु के चिचिंडा के पीआरएसवी और सीएमवी आइसोलेट्स के साथ 86.5 और 93.8% समरूपता का पता चला। फ़ाइलोजेनेटिक विश्लेषण से पता चला कि पीआरएसवी को डब्ल्यू प्रकार के रूप में अलग किया गया है। वर्तमान अध्ययन के परिणामों से पहली बार केरल में चिचिंडा मोज़ेक रोग परिसर के एटियोलॉजी का पता चला है, जो प्रबंधन रणनीतियों को तैयार करने का मार्ग प्रशस्त कर सकता है।