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Elucidation of *Cucumber mosaic virus* incidence on Chilli in Bhagalpur district of Bihar and its Biological characterization

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Abstract
Chilli (*Capsicum annuum* L.) is one of the most important vegetable crops grown throughout the globe. It is prone to viral infections under field conditions. Among the viruses; Cucumber mosaic virus is severely affecting the production and productivity of Chilli. Cucumber mosaic virus is most devastating plant pathogen infecting more than 1200 species across 100 families of monocot and dicot plants. In the current investigation we have conducted field survey for the identification of the CMV. During the field survey in the Bhagalpur district of Bihar, we were collected 95 chilli leaves samples showing characteristics symptom of CMV, such as mild mosaic, mottling and leaf distortion. DAC-ELISA was performed for detection and screening of the CMV. The observation that the chilli samples exhibited CMV symptoms from Sabour and Kahalgaon block, accounting for 25.71% and 16.66% of the ELISA findings against CMV antiserum, was surprising. The highest incidence of CMV was recorded in sabour block i.e. 25.71% and lowest incidence was recorded in Kahalgaon block i.e. 16.66%. After confirmation with the DAC-ELISA, single lesion assay was performed for the purification of the CMV virus from other contaminants. Identified CMV strain was biologically characterized by mechanically transmissible to the host plant. Seven days after the inoculation, we saw chlorotic local lesions on *Chenopodium amaranticolar*. It is comparable to several CMV research published by different scientific groups

Keywords: Chilli, DIBA, DAC-ELISA, *Cucumber mosaic virus* and Chenopodium

Introduction
Chilli is one of the most important economic crops grown in India. It is grown almost everywhere in the country. There are about 400 different types of chiles in the world. Bell pepper, sweet pepper, cayenne pepper, and hot pepper are some other names for it. It is known by the scientific name "*Capsicum annuum*". The Assamese hills close to the Indian village of Tezpur are home to the "Naga Jolokia", the spiciest chilli in the world. Many varieties are grown for pickles, spices, condiments, sauces, and veggies.

The Indian states with the quickest rates of growth are Andhra Pradesh, Orissa, Madhya Pradesh, Karnataka, Tamil Nadu, West Bengal, Maharashtra, Gujarat, and Bihar. Tomato production and productivity are affected by a wide range of biotic and abiotic stresses. One significant component is viruses. Among biotic stresses; viruses are a major factor reducing tomato productivity. Tomato leaf curl virus (TLCV), tomato mosaic virus (TMV), and cucumber mosaic virus (CMV) are the three primary viruses that infect tomatoes.

In this study, we have only focused at the Cucumber mosaic virus. To provide a brief, the CMV belongs to the genus Cucumovirus and family Bromoviridae. Cucumber mosaic virus (CMV) was the first viral disease in plants discovered by Doolittle and Jagger in 1916 [2]. It is one of the dangerous plant viruses to eradicate because of its broad host range. Owing to its significance, it affected about 1200 species from 100 families of monocots and dicot. Because of this it placed fourth among the top ten scientific/economic viruses in recent survey reports.

It is primarily associated with temperate and tropical regions, causing a wide range of symptoms in ornamental plants, woody and semi-woody plants, crops, and vegetables. It is merely spread through mechanical immunization.

Additionally, more than 80 different species of aphids spread it non-persistently. The host species affects how CMV spreads through seeds. In various host species, including weed species, it ranges from 0% to 100%. With 60-65% of their nucleotide similarities, the viruses known as Peanut Stunt Virus (PSV) and Tomato Aspermy Virus (TAV) belong to the same genus as the Cucumber Mosaic Virus. With a diameter of 29 nm, its CMV virion particles are isometric in form. Genetic material of CMV is RNBA. Whole genome of the CMV is divided into three part that why its genome is known as tripartite genome, in nature, RNA is a single stranded, positive-sense molecule (Palukaitis *et al*., 1992) [10]. Five ORFs in total are encoded by the whole genome. The 111-kDa protein 1a is encoded by RNA1 (about 3.3 kb long) the proteins 2a and 2b are encoded by RNA2 (about 3.0 kb long). 2a protein has a size of 98 kDa, whereas 2b protein has a size range of 13 to 15 kD.

The subgenomic RNA RNA4A is the source of the 2b. RNA3 is bi-cistronic in nature and has a size of about 2.2 kb. RNA codes for both coat protein (CP) and movement protein (MP). The size of the CP is 25 kDa, whereas the MP and CP are 30 kDa. Canto and Associates (2005) [1]. On the basis of their molecular, physical, biological, and serological traits, CMV strains were split into two groups, I and II. Strains from further grouping I were divided into subgroups IA and IB based on nucleotide variation in the 5' non-coding region of RNA3 (Roossinck *et al* 1999) [12]. Subgroups II and IA are distributed globally, but subgroup IB is concentrated in Asia. A field survey for the current study was conducted at a chilli field in the Sabour and Kahalgaon block of Bhagalpur.

Materials and Methods

Culture of viruses

Based on the viral symptoms, samples of likely chilli leaves were collected from the Bhagalpur district of Bihar. The number of samples collected from the sabour and Kahalgaon is shown in Table 1. Viral diagnoses were made using the direct antigen coated enzyme linked immunosorbent assay test (DAC-ELISA). Additionally, Chenopodium quinoa was maintained on Nicotiana rustica through mechanical inoculations of sap and employed in the single lesion assay for the purification of the Cucumber mosaic virus.

DAC-ELISA

After making a few slight modifications, the virus-infected chilli samples were screened using DAC-ELISA in accordance with the recommendations given by Hobbs *et al*., 1987 [4] & Mowat and Dawson, 1987 [8]. Using a mortar and pestle and 1000 µl of 0.05 M carbonate buffer (pH 9.6), 200 mg of infected tomato test samples, a healthy sample, a positive control, and a negative control were separately ground into powder to minimize cross-contamination. Ground samples were placed in eppendorf tubes and centrifuged for one minute at 8,000 rpm. An aliquot of 150 µl of supernatant was applied to each well on the ELISA plate. Following an hour of incubation at room temperature, the plate was cleaned three times with PBS-T (0.15 M NaCl in 0.1 M) at five-minute intervals.The antisera was diluted using PBS-TPO buffer (0.15 M NaCl, 0.1 M phosphate buffer, 0.05% Tween 20, 2% polyvinyl pyrrolidine, 0.2% ovalbumin). After applying 200 µl of CMV antisera (1: 20,000 dilution) to the well, it was allowed to incubate at room temperature for two hours. To halt the reaction, 50 µl of a 3 M NaOH solution was added to each well. The absorbance at 405 nm wavelength or visually (yellow color intensity) were used to screen the positive samples.

Biological characterization

Plant viruses are unable to enter plant cells on their own because they are devoid of the enzymes required to dissolve cell walls. As a result, unlike bacteria or fungi, they enter plant cells either by wounds caused by mechanical trauma or naturally occurring apertures like leaf stomata or stem lenticels. In a laboratory environment, celite and caborundam 500 mesh were used to produce a mechanical injury. Once the samples passed the ELISA and DIBA tests, they were used for further investigation. 200 mg of CMV-confirmed samples were crushed in a cold mortar and pestle with inoculation solution (50 mM phosphate buffer, pH 7.0). [Di-potassium hydrogen phosphate (K2HPO4): 5.40 g; mercaptoethanol: 1.56 ml, distilled water: 1000 ml; potassium di-hydrogen phosphate (KH2PO4): 2.40 g].

An inoculum-wetted muslin cloth was used to gently rub on the upper surface of the leaves of a healthy chenopodium plant after it had been dusted with carborundum 500 mesh abrasively. Using the palm of the left hand, the leaves were supported from below during inoculation to avoid any injury and to provide uniform pressure and inoculum dispersion. After being inoculated, infected leaves were washed with distilled water using a wash bottle to remove any remaining inoculum and carborundum powder. The innoculated plants were kept in an insect-free glasshouse or net house for two weeks in order to monitor symptoms.

Results and Discussion

Virus Culture

About 95 field samples from chilies suspected of being infected with a virus were collected by Mr. Abhishek Kumar and Dr. Mahesh Kumar in 2022-2023 while on a field trip to the Sabour and Kahalgaon Block of Bhagalpur. The quantity of samples collected from Sabour and Kahalgaon is listed in Table 1. On infected samples taken from the field, DAC-ELISA was carried out using CMV antisera.

Table 1: Virus infected samples collected from Bhagalpur District of Bihar

S. No.	Area	No of samples collected
01	Sabour	35
02	Kahalgaon	60
Total		95

Diagnosis of the Sample through DAC-ELISA

In a field situation, it is exceedingly challenging and impossible to identify the viruses based alone on their symptoms. The majority of the time, collecting a field sample on the presumption of a certain viral infection led to erratic laboratory findings. Ninety-five samples of likely CMV-containing chili leaves were collected in the field for the current study. In Direct Antigen Coating-ELISA (DAC-ELISA) serodiagnosis, cucumber mosaic virus (CMV) antiserum was employed. Visual scoring on ELISA plates was used to determine the results (data not shown). The observation that the chilli samples exhibited CMV symptoms from Sabour and Kahalgaon block, accounting for 25.71% and 16.66% of the ELISA findings against CMV antiserum, was surprising. The highest incidence of CMV

was recorded in sabour block i.e. 25.71% and lowest incidence was recorded in Kahalgaon block i.e. 16.66%. Details of the CMV infestation are mentioned in Table 2.

Table 2: Virus infected samples collected from Bhagalpur District of Bihar

S. No.	Area of Bhagalpur	No of samples collected	No of ELISA positive	% of Positive
01	Sabour	35	09	25.71
02	Kahalgaon	60	10	16.66
Total		95	19	20.00

To authenticate, further ELISA positive samples were confirmed through DIBA assay.

Conclusion

This study highlights the significant incidence of Cucumber mosaic virus (CMV) affecting chilli crops in Bhagalpur district, Bihar. Field surveys and DAC-ELISA diagnostics revealed a notable infection rate, with the highest incidence in Sabour block (25.71%) and the lowest in Kahalgaon block (16.66%). The biological characterization confirmed the mechanical transmission of CMV to host plants. These findings emphasize the need for effective CMV management strategies to enhance chilli production. Future research should focus on developing resistant chilli varieties and exploring advanced diagnostic techniques for early detection and control of CMV.

Biological Characterization

By inoculating the experimental and indicator host plants with sap of the inoculums, it was observed that the identified CMV strain was mechanically transmissible to the host plant. Seven days after the inoculation, we saw chlorotic local lesions on *C. amaranticolar*. It is comparable to several CMV research published by different scientific groups (Jorda *et al*., 1982; Madhubala *et al*., 2005, Kumar *et al*., 2020 and Kumar *et al*., 2017) [5, 9, 6, 7]. Given that these two viruses are the most common infections in the field, it will be important to do application-oriented research to determine the yield losses and commercial consequences caused by them.

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