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Detection and biological characterization of *Cucumber mosaic virus* on chilli (*Capsicum annuum*) in Pothia block of Kishanganj

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Abstract
Within the family Bromoviridae, the genus Cucumovirus includes the rapidly spreading plant pathogen known as *Cucumber mosaic virus* (CMV), which affects over 1200 species across 100 families of monocot and dicot plants. Chilli is highly susceptible to CMV, which can result in a variety of symptoms as mottling and yellowing of the leaves, and ultimately the distortion of the chilli leaf and fruit, which can cause significant financial losses. In the current investigation, we have gathered the chilli leaves exhibiting the typical CMV symptoms from the Pothia block of Kishanganj, specifically from Kharkhari, Motihara, Ganyabari, Lodhabari, and Arrabari. The CMV was identified using the Dot immunobinding assay and the direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA of 165 suspicious samples collected, 47 were found to be positive for the CMV antisera. Remaining 118 field samples tested negative for CMV antisera, which may be affected with other biotic and abiotic stresses. Incidence of *Cucumber mosaic virus* was varies in between 20% to 33% against CMV antiserum in and around of the Pothia block of Kishanganj.

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

Introduction
One of India's most significant commercial crops is chili. It is grown practically all around the nation. The globe over is home to approximately 400 distinct varieties of chiles. Other names for it include bell pepper, sweet pepper, cayenne pepper, and hot pepper. The scientific name for it is "*Capsicum annuum*". The world's spiciest chilli, known as "Naga Jolokia," is grown in the Assamese hills near the village of Tezpur, India. For vegetables, spices, condiments, sauces, and pickles, several types are cultivated.

Andhra Pradesh, Orissa, Madhya Pradesh, Karnataka, Tamil Nadu, West Bengal, Maharashtra, Gujarat, and Bihar are the states in India with the fastest rates of growth.

Numerous biotic and abiotic stressors have an impact on tomato productivity and production. Viruses are a prominent factor limiting tomato yield among biotic stress. The three main viruses that infect tomatoes are tomato leaf curl virus (TLCV), tomato mosaic virus (TMV), and *Cucumber mosaic virus* (CMV).

We have focused solely on the *Cucumber mosaic virus* in this investigation. To give a basic overview of the CMV, it is a member of the Bromoviridae family and genus Cucumovirus. Doolittle and Jagger (1916) [2] identified the first viral disease in plants as the *Cucumber mosaic virus* (CMV). Due to its wide host range, it is one of the most difficult plant viruses to eradicate. Due to its importance, it was ranked fourth among the top ten scientific/economic viruses in recent survey reports, affecting almost 1200 species from 100 families of monocots and dicots. 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%. The viruses known as Peanut Stunt Virus (PSV) and Tomato Aspermy Virus (TAV) are members of the same genus as the *Cucumber mosaic virus*, sharing 60-65% of their nucleotide similarities.

Its virion particles of the CMV are isometric in nature having a diameter of 29 nm. CMV genetic material is RNA. Its whole genome is divided into three part i.e. tripartite genome. RNA is positive-sense single stranded in nature (Palukaitis *et al.* 1992) [10]. The entire genome encodes five ORFs in total. RNA1 is approximately 3.3 kb long and encodes the 111-kDa protein 1a; RNA2 is approximately 3.0 kb long and encodes the proteins 2a and 2b. The size of 2a protein is 98 kDa, while the size of 2b protein ranges from 13 to 15 kDa. The 2b, which is produced from RNA4A, a subgenomic RNA. RNA3 is roughly 2.2 kb in size and is likewise bi-cistronic in nature. Movement protein (MP) and coat protein (CP) are encoded by RNA. The MP and CP have a size of 30 kDa, while the CP is 25 kDa. Canto and others, 2005). CMV strains were divided into two categories, I and II, according to their molecular, physical, biological, and serological characteristics. Based on nucleotide variation in the 5' non-coding region of RNA3, strains from further subgroup I were separated into subgroups IA and IB (Roossinck *et al.* 1999) [12]. Subgroups II and IA are dispersed around the globe, whereas subgroup IB is primarily found in Asia. A field survey for the current study was conducted at a chilli field in the pothia block of Kishanganj.

Materials and Methods

Culture of Viruses

Samples of probable chilli leaves were gathered from the Pothia block of Kishanganj based on the virus symptoms. Table 1 lists the number of samples gathered from the Pothia Block belonging. The direct antigen coated enzyme linked immunosorbent assay test (DAC-ELISA) was used to diagnose viruses. Furthermore, Chenopodium quinoa used to purify the *Cucumber mosaic virus* using the single lesion assay and maintained on Nicotiana rustica by sap mechanical inoculations

DAC-ELISA

With a few minor modifications, DAC-ELISA was utilized to screen the virus-infected chilli samples that had been obtained, following the guidelines provided by Hobbs *et al.*, 1987 & Mowat and Dawson, 1987 [4, 8]. To prevent cross-contamination, 200 mg of suspected tomato test samples, a healthy sample, a positive control, and a negative control were pulverized separately using a mortar and pestle and 1000 µl of 0.05 M carbonate buffer (pH 9.6). Samples that had been ground were centrifuged for one minute at 8,000 rpm in eppendorf tubes. To every well of the ELISA plate, 150 µl of supernatant was added as an aliquot. After one hour of room temperature incubation, the plate was cleaned three times at five-minute intervals using PBS-T (0.15 M NaCl in 0.1 M phosphate buffer, 0.05% Tween 20). PBS-TPO buffer (0.15 M NaCl, 0.1 M phosphate buffer, 0.05% Tween 20, 2% polyvinyl pyrrolidine, 0.2% ovalbumin) was used to dilution the antisera. 200 µl of CMV antisera (1: 20,000 dilution) was applied to the well, and it was incubated for two hours at room temperature. 50 µl of a 3 M NaOH solution was added to each well to stop the reaction. The positive samples were screened by measuring the absorbance at 405 nm wavelength or visually (yellow color intensity).

DIBA, or Dot Immunobinding Assay

Total soluble proteins from infected and healthy leaf samples were separated for the DIBA assay. After macerating 500 mg of leaf samples for 2 ml at 4°C in TB Buffer (50 mM Tris Acetate pH7.4, 10 mM MgCl2, 250 mM KCl, and 20% glycerol), 1 mM PMSF and 1 mM DTT were added, and the mixture was centrifuged for 10 minutes at 15,000 rpm. After being collected, the supernatant was put to use in more research. The PVDF membrane was trimmed to the required size and then immersed in methanol. On the PVDF membrane, a dot representing the healthy control, positive control, and 5µl of total soluble proteins from the ELISA-confirmed infected chili sample was put. Following the drying of the dots, the membrane was incubated for 30 minutes at room temperature on a rocker in blocking buffer (5% milk Nestle daily, 0.02 M Tris, 0.5 M NaCl, 0.05% tween-20, pH 7.5). Following incubation, 1:10,000 dilutions of CMV antisera were added to the plate, and it was left to sit at room temperature on a rocker for two hours. After decanting the solution, the membrane was three times, every five minutes, cleaned with TBS-T. For a further incubation period of, it was incubated in the antibody buffer containing the secondary antibody-enzyme conjugate (0.02 M Tris, 0.5 M NaCl, 0.05% tween-20, pH 7.5, and 5% milk). The membrane was incubated for the development of color using 300 µl of BCIP/NBT substrate. Distil water was added to halt the reaction, and the membrane was then dried on tissue paper.

Inoculation and sap transmission

Since plant viruses lack the enzymes necessary to break down cell walls, they are unable to infiltrate plant cells on their own. Therefore, unlike bacteria or fungus, they penetrate plant cells either naturally through openings like leaf stomata or stem lenticels, or by wounds brought on by mechanical injury. Celite and caborundam 500 mesh were utilized in a lab setting to create a mechanical injury. The samples were utilized for additional research after passing both the ELISA and DIBA tests.

In a cold mortar and pestle, 200 mg of CMV-confirmed samples were crushed using inoculation buffer, or 50 mM phosphate buffer with a pH of 7.0. [Potassium di-hydrogen phosphate (KH_2PO_4)-2.40 g, di-potassium hydrogen phosphate (K_2HPO_4)-5.40 g, mercaptoethanol-1.56 ml and distilled water1000 ml]. A healthy Chenopodium plant was given an abrasive dusting with carborundum 500 mesh, and the upper surface of the leaves were gently rubbed with muslin cloth that had been moistened with inoculums. To prevent any damage and provide even pressure and inoculums dissemination during inoculation, the leaves were supported from below using the palm of the left hand. Using a wash bottle, infected leaves were rinsed with distilled water following inoculation to get rid of extra inoculum and carborundum powder. To track symptoms, the inoculated plants were housed in a glasshouse or net house free of insects for two weeks.

Results and Discussion

Virus Culture

During a field trip to the Pothia Block in Kishanganj in 2019, Mr. Abhishek Kumar and Dr. Mahesh Kumar gathered about 165 field samples from chillies that were suspected of being infected with a virus. Table 1 lists the number of samples that were taken from the Pothia block settlement and its environs. These are the specific collection areas: Kharkhari, Motihara, Kharkhari, Arrabari, Lodhabari, and Ganyabari. Using CMV antisera, DAC-ELISA was performed on infected samples that were obtained from the field.

Table 1: Virus infected samples collected from Pothia block region mainly Kharkhari, Motihara, Ganyabari, Lodhabari and Arrabari

S. No.	Pothia block	No of samples collected
01	Kharkhari	30
02	Motihara	34
03	Ganyabari	25
04	Lodhabari	41
05	Arrabari	35
Total		165

Diagnosis of the Sample through DAC-ELISA

It is very difficult and undetectable to identify the viruses solely on their symptoms in a field setting. In most cases, gathering a field sample under the assumption of a specific virus infection produced inconsistent laboratory results. In the current investigation, 210 samples of probable CMV-containing chili leaves were gathered in the field. *Cucumber mosaic virus* (CMV) antiserum was used in Direct Antigen Coating–ELISA (DAC-ELISA) serodiagnosis. As seen in fig. 1, results were determined by visual scoring on ELISA plates. It was unexpected to see that chillies samples were showing symptom of the CMV from five villages; Kharkhari, Motihara, Ganyabari, Lodhabari, and Arrabari are accounted for 33%, 29.41%, 32%, 31.7% and 20% respectively for the ELISA results against CMV antiserum. The maximum incidence of the CMV was recorded for Kharkhari i.e. 33% and Minimum for the Arrabari i.e. 20%. Details of the CMV infestation are mentioned in Table 2. To authenticate, further ELISA positive samples were confirmed through DIBA assay.

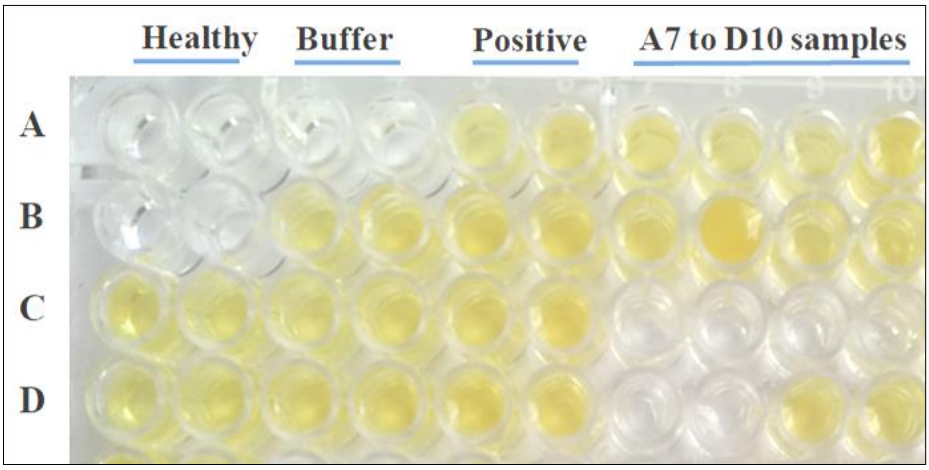

Fig 1: Representative DAC-ELISA plate for screening of the chilli infected samples

A1-A2, A3-A4 and A5-A6 wells of the ELISA plate represent as healthy control, buffer control and positive control. A7 to D10 well of the Plate used for the Chill infected field samples. Each samples were loaded as duplicate. Yellow colour showed Positive against CMV antisera.

Table 2: ELISA results of field collected samples from Pothia region mainly Motihara, Ganyabari, Lodhabari, and Arrabari

S. No.	Villages of Pothia block	No of samples collected	No of ELISA positive	% of Positive
01	Kharkhari	30	09	33.00
02	Motihara	34	10	29.41
03	Ganyabari	25	08	32.00
04	Lodhabari	41	13	31.70
05	Arrabari	35	07	20.00
Total		165	47	28.48

Dot Immuno Binding Assay (DIBA)

Ten symptomatic field samples in total were chosen at random to validate the ELISA results. As stated in the material and methods, total soluble proteins were isolated and spotted onto PVDF membrane. Ten samples were randomly examined using TSV antisera every sample had a positive result that coincided with the ELISA result.

Inoculation and sap transmission

The identified CMV strain was found to be mechanically transmissible by sap inoculation to the experimental as well as indicator plants host plant. We observed chlorotic local lesions on C. *amaranticolar* after seven days post inoculation. It is similar with various investigations for CMV reported by various group of scientist (Jorda *et al.*, 1982; Madhubala *et al.* (2005) [5, 9]. It will be noteworthy to perform an application orientated research to check the yield losses and commercial implications due to these two viruses as these are predominant infections in the field.

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