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MOLECULAR CHARACTERIZATION OF *CUCUMBER MOSAIC VIRUS* ASSOCIATED WITH CHILLI IN KOSHI REGION OF BIHAR INDIA

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ABSTRACT

One of the most common vegetables and spices grown in tropical and subtropical regions of the world is the chilli (*Capsicum annuum*). Viral infections, which result in low fruit quality and quantity, pose a serious danger to chilli output. There are around 65 known viral diseases that may affect chillies worldwide. Cucumber mosaic virus is one of notorious virus; severely infecting the chilli worldwide; resulting into severe economic losses. In the present studies we have collected chilli samples showing mosaic disease from Koshi region (mainly Kasba, Jalalgarh and Dagarua) of Bihar during the period 2021–2022. Leaves and fruits with typical mosaic disease, chlorotic spots followed by mild mosaic and leaf distortion symptoms were observed under field conditions. Infected leaves were first screened by DAC-ELISA. Sap inoculation was carried on host plant from DAC-ELISA positive sample. Total RNAs were isolated from the host plant showing characteristic symptom of CMV after the post inoculation. Reverse transcription -polymerase chain reaction (RT-PCR) for coat protein (CP) gene of CMV with CP specific primers resulted into 657 bp amplification from the samples were collected from Kasba, Jalalgarh and Dagarua.

Keywords **:** Chilli, DAC-ELISA, and cucumber mosaic virus, RT-PCR and Coat protein.

Introduction

Chilli (*Capsicum annuum*) is one of the major vegetables and spices that is cultivated worldwide in tropical and subtropical climates. In addition, it is grown as a decorative plant and a medicinal herb. India, China, Pakistan, Indonesia, Mexico, Nigeria, and South Korea are the primary countries that cultivate chillies, with a combined acreage of 20.20 million and a production of 37.62 million tonnes. Almost 87% of the world's chilli production comes from China and India [FAOSTAT, 2017]. Eight lakh tonnes of dry chili are produced annually from 9.3 lakh hectares of chilli cultivation in India. Five of the 25 identified species in the genus Capsicum ; *C. annuum, C. frutescens, C. chinense, C. baccatum, and C. pubescens*can be identified as domesticated species based on the fruits morphology.

While *C. frutescens* and other cultivated species are thought to have originated in South America, *C. annuum* is thought to have originated in Mexico. Vitamins A, C, and E are abundant in chilli fruit and play a major role in preventing secondary radiation damage [Verghese J, 1999]. The production of chilies is greatly threatened by viral infections, which lead to

low fruit quality and quantity. Worldwide, chilli has been documented to be infected by more than 65 viral infections [Devi & Devi, 2020]. Of these, thirteen viruses have been identified as coming from India; these include the following: *Capsicum chlorosis virus (CaCV)*, *Chilli leaf curl virus* (ChiLCV), *Chilli leaf curl India virus* (ChiLCINV), *Chilli leaf curl Vellanad virus* (ChiLCVV), *Cucumber mosaic virus* (CMV), *Tobacco ring spot virus* (TRSV), *Potato virus X* (PVX), *Chilli leaf curl virus* (ChiLCV), *Tobacco leaf curl virus* (TbLCuV), *Potato virus Y* (PVY), *Pepper vein bending virus* (PeVBV), *Tomato leaf curl Joydebpur virus* (ToLCJV), and *Tomato leaf curl New Delhi virus* (ToLCNDV), [Vinoth Kumar et al., 2015 Wahyuni *et al*., 1992]. The most dangerous of them, according to reports, are CMV, ChiVMV, and ChiLCV, which can cause marketable fruits to lose up to 100% of their yield and occasionally force farmers to abandon their fields before they are ready for harvest.

The most common and economically significant virus in all of the world's regions that grow bell peppers and chilies is CMV. In 1916, cucumber was the source of the first known case of CMV. More than 1200 plant species from 100 families have reportedly been reported to be infected by the virus since then [30]. One of the most significant viruses that seriously harms India's annual crop of chili peppers is CMV [FAOSTAT, 2017]. Cucumber, black pepper, amaranthus, datura, devil pepper, Barbados nut, Egyptian henbane, gladiolus, tomato, geranium, banana, lily, tulasi, betelvine, brinjal, bottle gourd, snake gourd, carrot, castor, chilli, chrysanthemum, gerbera, geranium, jatropha, ornithogalum, and petunia are among the host plants in India that the virus is known to infect [Pavithra *et al*., 2019].

In natural environments there is 80 different species of aphids have been reported to transmit CMV.It is a member of the genus Cucumovirus, family Bromoviridae. The tripartite, positive sense ssRNA genome of CMV is named RNA1, RNA2, and RNA3 for each segment, respectively, based on decreasing molecular weight. Every RNA segment is enclosed within a distinct particle. Proteins 1a and 2a, which are important in the assembly of the replicase complex for viral replication, are encoded by RNA1 and RNA2, respectively [Palukaitis *et al*., 1992]. Additionally, RNA2 encodes protein 2b, which is involved in viral motility and serves as a pathogenicity determinant and suppressor of gene silencing. Two proteins, 3a and 3b, which are encoded by RNA3, are involved in cell-to-cell mobility and virus transmission, respectively.

CMV infected chilli plants exhibit various symptoms including mosaic, mottling, leaf deformation, yellow discoloration, vein clearing, shoestring, stunted growth and whitish streaks on the fruits [ArliSokmen *et al*., 2005].

Materials and Methods

Survey and collection of isolates

CMV infected leaves& fruits were collected from farmers field of chilli in and around of Koshi region of Bihar. In each location 4 to 6 fields were surveyed. The disease incidence was recorded in each field by visual examination of symptomatic and non-symptomatic plants in five randomly selected micro-plots in each field. Symptom's variability and variety grown in each location were recorded. From each field randomly symptomatic and asymptomatic samples were collected, labeled and sealed in polythene bag and transported to the Molecular Biology & Genetic Engineering laboratory, Dr Kalam Agricultural College, Kishanganj Bihar Agricultural University, Sabour, Bhagalpur, Bihar for further analysis.

Detection of virus by DAC-ELISA

Field collected samples were subjected to direct antigen coating assay (DAC-ELISA) against CMV antisera as described by (Hobbs *et al*., 1987 &Mowat and Dawson, 1987) with slight modification as mentioned below. Approximately, 200 mg of infected and healthy plant leaf were macerated with 500 µl of 0.05 M carbonate buffer (pH 9.6) using pestle. 200 µl was added to each well of ELISA plate. Plate was incubated for 2 h at room temperature (RT) and this was washed with PBS-T 5-minute interval. The CMV antisera were diluted to required volume in PBS-TPO buffer (0.15 M NaCl, 0.1 M phosphate buffer, 0.05% Tween-20, 2% polyvinyl pyrrolidine, 0.2% ovalbumin). Then 200µl of respective antisera (1: 10,000 dilution) was added into the well and incubated for 2 h at room temperature. After incubation, the solution was decanted and washing steps were repeated as above to remove the unbound antibody. After that 200 µl of secondary antibody (1:10,000 dilution)-alkaline phosphatase conjugate was added to the wells, incubated and washing steps repeated with PBS-T thrice at 5 min interval. The reaction was developed by adding 200 µl of the substrate solution (9.7% diethanolamine, 50 mg p-nitrophenyl phosphate pH 9.8) and kept for incubation at room temperature for 10 min in the dark. Reaction is considered as positive, if the absorbance values of the test samples are double the value of the healthy samples.

Mechanical transmission

Sap transfer into the host plant was carried out using a DAC-ELISA positive sample. After adding 0.05 M potassium phosphate buffer (pH 7.0, supplemented with 0.02%, 2-beta mercaptoethanol before to use) at a rate of 1 ml/g of leaf tissue, infected leaves were macerated using a pestle and mortar. After filtering, 0.025 g/ml of celite powder was combined with the macerated sap. On the immature leaves of *Chenopodium quinoa* and *Phaseolus vulgaris*; the inoculum was lightly applied and rubbed in a single direction. Extra amount of inoculums were removed by rinsing with distilled water after ten to fifteen minutes of inoculation. To express symptoms, the plants were kept in an insect-proof glasshouse.

Designing of primers

Complete nucleotide sequences of CMV were retrieved from the NCBI data bank and aligned by the Bio-Edit software. Conserved regions were selected for designing of primers. The forward primer was designed manually; taking 18-24 bases from the conserved region of the sequence. Reverse primer was designed by taking 3′ conserved nucleotide (18-24 bases) sequences of target gene. The selected sequence was placed in complementary software from just bio. Both primers were checked for melting temperature using IDT oligocal online tool.

Reverse transcription and 1st strand cDNA synthesis or CMV RNAs

Total RNA isolated from CMV virus was used as a template for 1st strand cDNA synthesis by using MMLV Biotechnologies Kit. The cDNA was synthesized; according to manufacturer's protocol. Briefly, 2 µl (100 ng) of RNA was mixed with 8.5 µl nuclease free water and 10.0 µM gene specific reverse primer and it was incubated at 65ºC for 2 min in a water bath and it was cooled down to RT. To this mixture, 7.5 µl cocktail (2.0 µl 10X reaction buffer, 2.0 µl of 100 mM DTT, 2.0 µl of 5 mMdNTPs, 0.5 µl of ScriptGuardRNAse inhibitor and 1.0 µl MMLV Reverse Transcriptase) was added and incubated at 37ºC for 90 min. After incubation, the reaction was terminated by heating at 85ºC for 5 min. This mixture was kept on ice for 1 min and used directly to amplify the gene by PCR with the specific sets of primers.

Polymerase Chain Reaction (PCR)

PCR is performed as described by standardized protocol; Template was added to the cocktail of 25 µl reaction containing 2.5 µl of 10X buffer (50 mMTris-HCL pH 8.8 and 50 mM KCL, 1.5 mM $MgCl_2$)50 µMdNTP's, 0.5 µM of each specific forward and reverse primers and 1unit of *Taq* DNA polymerase. Initial denaturation step was carried out at 94°C for 4 min, then 30 cycles of denaturation at 94°C for 50 sec, different annealing temperature and extension time 1 min+1 min/ 1 kb of template @ 55°C followed by final extension at 55°C for 10 min. The amplified PCR product is subjected to electrophoresis on 1% agarose gel and was documented by UV-platinum gel documentation instrument.

Results

DAC-ELISA for detection of viruses associated with chilli

Survey for the incidence of CMV was conducted in Koshi region of Bihar based on the visual symptoms in 10chilli fields and subsequent detection of CMV by DAC-ELISA in 70 symptomatic chilli samples collected from fields revealed the ubiquitous prevalence of CMV in all these locations. Further, incidence ranged from 35 to 50% based on visual symptoms in the fields (data not shown). Further, presence of CMV was confirmed by DAC-ELISA in 22infected samples out of 70 samples collected from Koshi region of Bihar mainly; Kasba, Jalalgarh and Dagarua. Percentage of infected samples confirmed with CMV presence by DAC-ELISA ranged from 40 to 54.45% in different region of Koshi area (Data not shown). Infected chilli plants exhibited various symptoms including mosaic, mottling, and their combination, leaf deformation and narrowing, stunted growth, reduced fruit setting and light green mottling on fruits (Fig. 1). Slight difference was observed in viral disease incidence between the fields surveyed, it might be due to the difference in chilli varieties grown across the three area (Kasba, Jalalgarh and Dagarua) region of Koshi region.

Molecular characterization

The genomic RNA isolated from CMV infected leaf was used as a template for cDNA synthesis. PCR was performed with coat protein specific primer [(forward primer: 5' GGGATCCCAATGGCTTTC CAAGGT ACCAGT 3') and reverse primer: AAGAC CGTTAACCACCTGCGG)], 10X buffer, dNTP's, and *Taq* DNA polymerase as mentioned in material and methods section. Thermo profile was optimized with the help of gradient PCR. The best annealing temperature for amplification of the coat protein of CMV was found @ 55ºC for 30 sec. Amplified PCR product was loaded on 1% agarose gel along with 1.0 kb ladder. The PCR product showed sharp band near the 657 bp (fig 2) in all the infected leaves collected from of Kasba, Jalalgarh and Dagarua area of the Purnea district of Bihar.

Discussion

Many disorders caused by CMV subgroups IA and II are prevalent throughout the world, but subgroup IB is limited to Asia. Reports of illnesses brought on by CMV subgroups I and II isolates During the study, infected chilli plants displaying symptoms similar to those previously described severe mosaic, mottling, leaf curl, and stunted growth were found in the majority of the fields. These plants may not always exhibit symptoms that are connected to CMV. As a result, serological identification of the virus in randomly selected infected samples verified the incidence evaluation based on visual symptoms in order to provide an accurate estimate of the illness incidence caused by CMV. According on visual assessment, the results indicate a prevalence of CMV-caused illness of up to 43%; however, this may not be accurate, as other viral diseases may present symptoms that are similar to those of CMV. DAC-ELISA testing amply demonstrated this, revealing that only 54.45% of infected samples tested positive for CMV, despite the fact that all samples were taken from plants exhibiting essentially identical symptoms. There are previous reports from Bihar regarding Incidence of cucumber mosaic virus in tomato (*Lycopersicon esculentum*) in Koshi region of the Bihar (Kumar et al., 2019 and Kumar et al., 2020). Therefore, visual evaluation backed by further confirmatory tests like ELISA or PCR for random samples is crucial for accurate estimation of the viral illness incidence.

A B

Fig. 1 : Chilli plants showing **A :** mosaic**, B-** mosaic and bushy appearance symptoms under natural conditions

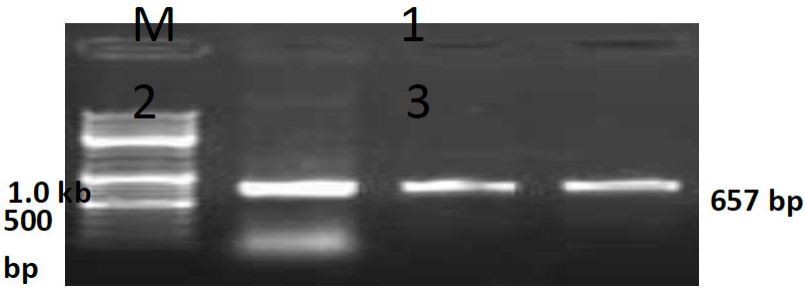

Fig 2: Reverse transcription -polymerase chain reaction (RT-PCR) for coat protein gene of CMV in 1% agarose gel. Lane 1, 2 and 3; PCR amplified product for coat protein gene of CMV from Kasba, Jalalgarh and Dagarua respectively. M; 1 kb ladder, right side of the gel indicate 657 bp for PCR amplified product.

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References

ArliSokmen, M., Mennan, H., Sevik, M.A., Ecevit, O. (2005). Occurrence of viruses in field grown pepper crops and some of their reservoir weed hosts in Samsun, Turkey. *Phytoparasitica,* **33**: 347–58.

Hobbs, H.A., Reddy, D.V.R., Rajeshwari, R., Reddy, A.S. (1987). Use of antigen coating method and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Disease,* **71**: 747-749.

FAOSTAT. Food and agriculture organization of the United Nations. 2017. http://faostat.fao.org.

Khan, S.M., Raj, S.K., Bano, T., Garg, V.K. (2006). Incidence and management of mosaic and leaf curl diseases in cultivars chilli (*Capsicum annum* L). *J Food Agric Environ*. **4**: 171–4.

Kumar, M., Kumar, A., Shamim, M., Kumar, S., Jha, V. (2020). Incidence of cucumber mosaic virus in tomato (*Lycopersicon esculentum*) in Koshi region of the Bihar. *J Crop Sci Biotechnol*., **9**: 64–67.

Kumar, M., Erayya, Shamim, M., Hari, O. (2017). Survey and identification of cucumber mosaic virus on tomato in Kishanganj district of Bihar. *Progressive Research -An International Journal,* **12** (Special-V) : 2908-2910.

Mowat, W., Dawson, S. (1987). Detection and identification of plant viruses by ELISA using crude sap extracts and unfractionated antisera. *Journal of Virological Methods*, **15**: 233-347.

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B. (1992). Cucumber mosaic virus. *Adv Virus Res*., **41**: 281–348.

Pavithra, B.S., Govin, K., Renuka, H.M., Krishnareddy, M., Jalali, S., Samuel, D.K., Himabindu, K. (2019). Characterization of Cucumber mosaic virus infecting coleus (*Plectranthus barbatus*) in Karnataka. VirusDis.. https://doi.org/10.1007/s13337-019-00536-3.

Verghese, J. (1999). Scanning paprika oleoresin and chillicolour specifications/quality parameters. Indian, *Spices*, 36: 7–11.

Vinoth, K.R., Singh, A.K., Singh, A.K., Yadav, T., Basu, S., Kushwaha, N., Chattopadhyay, B., Chakraborty, S. (2015). Complexity of begomovirus and betasatellite populations associated with chilli leaf curl disease in India. *J Gen Virol*., **96**: 3143–58.

Wahyuni, W.S., Dietzgen, R.G., Hanada, K., Francki, R.I.B. (1992). Serological and biological variation between and within subgroup I and II strains of Cucumber mosaic virus. *Plant Pathol*, **41**: 282–97.