Molecular identification of *Cucumber mosaic virus* isolates of subgroup IB associated with mosaic disease of eggplant in India

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Abstract Association of *Cucumber mosaic virus* (CMV) with severe mosaic disease of eggplant (*Solanum melongena* L.) collected from Lucknow and Kanpur, India was initially detected by host reaction and serological assay and confirmed by RT-PCR employing coat protein gene specific primers. Further, molecular identification of the virus isolates was done by cloning and sequence analysis of the complete RNA3 genome. Based on 97–99 % identities and phylogenetic relationships, the virus isolates infecting eggplant were identified as members of CMV subgroup IB.

Keywords Eggplant · Mosaic disease · Sequence analyses · Phylogenetic relationships

Cucumber mosaic virus (CMV), type member of genus *Cucumovirus* of family *Bromoviridae*, has the largest host range among the known RNA viruses of plant kingdom. It harbour plus sense tripartite genome of single-stranded linear RNAs designated as RNA1, 2 and 3, packaged in 29 nm spherical virus particles with a central core [6]. CMV has been divided into IA, IB and II subgroups and Asian strains including Indian have been grouped under subgroup IB [8]. Eggplant (*Solanum melongena* L., family *Solanaceae*) is a popular kitchen garden and vegetable crop plant grown in Asia. India is the second largest producer of

eggplants and therefore has high agricultural and socio-economic impact for the country and growers. Unfortunately, the commercial production of eggplant has setback of several plant viruses causing significant losses in fruit yield and CMV is one among them [4, 5].

During the year 2010, eggplants were observed exhibiting severe mosaic symptoms in the fields of Lucknow and Kanpur, India. The naturally infected plants exhibited mosaic, blistering, leaf distortion (Fig. 1a) flower color breaking and stunting symptoms as compared to healthy plants. The leaf sap from infected samples collected from both the locations were subjected for virus transmission, electron microscopy (EM), Western blot immunoassay and reverse transcription-polymerase chain reaction (RT-PCR) for initial detection of the associated virus. Successful sap transmission on seedlings of eggplant and *Cucumis sativus*, an indicator host of CMV, presence of ~29 nm spherical particles resembling CMV [3] in leaf dip preparations, positive reaction with antibody of CMV (PVAS 242a, ATCC, USA) resulting in ~26 and ~52 kDa (dimer) protein bands (Fig. 1b) indicated CMV infection in eggplant samples. For molecular detection of the virus isolates, total RNA was isolated from two representative symptomatic eggplant samples (KKG-MP and KKG-K collected from Lucknow and Kanpur, respectively) using TRIzol (Sigma-Aldrich, India) and RT-PCR was performed using CMV-CP specific primers. RT-PCR resulted in ~650 bp band in naturally as well as inoculated eggplants (Fig. 1c) which confirmed the association of CMV with the mosaic disease of eggplant collected from both the locations. Further, molecular identification of virus was done by cloning and sequence analysis of the complete RNA3 genomes from these two representative samples.

For molecular identification, the complete RNA3 genome was amplified using CMV-RNA3 specific primers

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Fig. 1 **a** Naturally infected eggplant leaf showing severe mosaic symptoms. **b** Western blot immunoassay showing ~26 and ~52 kDa (dimer) protein bands with antibody of CMV (PVAS 242a, ATCC, USA) in infected eggplant samples collected from Lucknow (L), Kanpur (K) as compared to CMV-Banana taken as positive control (PC, [12]) but not in a healthy (H) sample. **c** Agarose gel electrophoresis of RT-PCR amplicons with CMV-CP specific primers showing ~650 bp amplification from infected eggplant samples of Lucknow (L), Kanpur (K) and sap inoculated samples (L-In and K-In), similar as in CMV-Banana (PC [12]) taken as positive control, but not in a healthy eggplant (H). Lane M λ -DNA *EcoRI/HindIII* double digested (Genei Pvt. Ltd., India) taken as DNA marker

[12] and cloned into pGEM-T vector (Promega, Corporation, Madison, USA) following manufacturer's instruction. The positive clones were deposited in GenBank database under the accessions HQ343232 (KKG-MP isolate) and

GU906293 (KKG-K isolate). The RNA3 genome of both the isolates were found to be 2,219 nucleotides consisted of two ORFs: movement protein (MP) and coat protein (CP). The MP ORF was of 840 nucleotides (ATG and TAG at 123 and 962 positions, respectively) translating 229 amino acid residues, while CP was of 657 nucleotides (ATG and TAG at 1,261 and 1,917 position, respectively) translating 218 amino acid residues. The MP and CP ORFs were separated by 300 nucleotide long intergenic region (IR) and flanked by 5'untranslated region (UTR) and 3'UTR of 122 and 302 nucleotides, respectively.

BLASTn analysis of the sequence data of HQ343232 (KKG-MP) and GU906293 (KKG-K) isolates showed 98 % identity together. Independently, KKG-MP isolate showed highest 97 % sequence identities with CMV strains of banana (EF178298) reported from India and CLW2 (JN054635) from Malaysia of subgroup IB, whereas KKG-K isolate showed highest 99 % identity with the only CMV strain of banana (EF178298). The sequence identities were also scored by Genomatix DiAlign analysis using selected strains of CMV of subgroup IA, IB and II. During analysis both KKG-MP and KKG-K isolates shared 97 % identity with each other and 91–96 % identities with other CMV strains of subgroup IB. The identities were <86 % with CMV strains of subgroup IA and least (60–62 %) with

Fig. 2 Phylogenetic tree showing relationships of CMV-KKG-MP and -KKG-K isolates of eggplant with CMV subgroup IB strains as compared to subgroup IA and II members. The tree was generated using complete RNA3 genome sequences of strains listed in Table S1

at 1,000 bootstrap values and rooted using TAV and PSV sequences as out group reference members. *CMV* Cucumber mosaic virus, *PSV* Peanut stunt virus, *TAV* Tomato aspermy virus

subgroup II strains. When ORFs of RNA3 genome were analyzed independently the MP ORF shared 86–98 and 83–97 % identities at nucleotide and amino acid level, respectively with CMV strains of subgroup IB. While CP ORF shared 89–98 and 94–98 % identities at nucleotide and amino acid level, respectively. The 5'UTR, IR and 3'UTR shared 93–99, 80–99 and 91–99 % identities, respectively with CMV strains of subgroup IB (Table S1).

The phylogenetic tree generated using RNA3 genome sequences of KKG-MP and KKG-K virus isolates along with selected CMV strains of subgroup IA, IB and II clearly grouped all the CMV strains into three separate clusters. The Indian CMV strains of subgroup IB clad into one cluster except a strain from Malaysia (CLW2) while other CMV strains from abroad clad in a separate cluster. KKG-MP and KKG-K isolates showed closest relationships with CMV strains infecting banana, Gerbera, Petunia and CLW2 belonging to subgroup IB (Fig. 2). Based on high 97–99 % identities and closest phylogenetic relationships with CMV strains of Ban, Ger, Pet and CLW2 belonging to subgroup IB, the virus isolates infecting eggplant were identified as new members of CMV subgroup IB.

The natural occurrence of *Alfalfa mosaic virus*, *Cucumber mosaic virus* [4], *Eggplant blister mottled virus* [10], *Eggplant mottled crinkle virus* [9], *Eggplant mottle dwarf virus* [2], *Eggplant severe mottle virus*, *Eggplant mosaic potyvirus* and *Tomato mosaic virus* [1] has already been reported on eggplant. The limited reports of CMV on eggplant also exist from India [5, 11], which were based on biological and serological studies only but molecular identification and proper sub grouping of these CMV isolates has not been done. Therefore, the present study aimed on sequence analysis of RNA3 genome of the two isolates of CMV infecting eggplant for their proper identification and their placement in proper CMV subgroup. The sequence analysis, the isolates under study revealed 97–99 % sequence identities and close phylogenetic relationships with CMV strain of banana belonging to

subgroup IB; therefore, virus isolates from eggplant were identified as two strains of CMV of subgroup IB.

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