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Characterization of *Cucumber mosaic virus* infecting snake gourd and bottle gourd in India

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ABSTRACT

Snake gourd and bottle gourd are the common cucurbitaceous vegetables consumed in India that are prone to viral infections under field conditions. During our field survey in Southern India in 2012-14, the symptoms *viz.*, stunted growth, mosaic mottling, puckering and chlorosis in these crops were observed. Incidences of virus-like symptoms ranging between 63 and 88% were observed in three different locations. Based on the mechanical inoculations on their respective host, plants produced symptoms similar to those under field conditions. Samples tested with DAS-ELISA using CMV polyclonal antiserum were found positive for CMV. The occurrence of CMV was further confirmed by amplification of coat protein gene of CMV using RT-PCR assays. The sequence analysis of coat protein gene revealed highest nucleotide identity of > 92% with CMV subgroup IB isolates. Coding regions from CMV RNA 1, RNA 2 and RNA 3 genomic fragments were cloned and sequenced. Phylogenetic analysis of RNA1, RNA 2 and RNA 3 from CMV infecting snake gourd and bottle gourd showed close relatedness with Italian isolate that infect *Capsicum* rather than Asian isolates of CMV from India, Malaysia, China and Japan. The characterization of CMV from snake gourd and bottle gourd pertaining to biological, serological and molecular attributes are presented.

1. Introduction

Cucurbits are important vegetable crops belonging to the family Cucurbitaceae grown for domestic consumption and worldwide commercial trade. Out of 118 genera and 825 species of *Cucurbitaceae*, 36 genera and 100 species are found in India [1]. Around 5.6% of the total vegetable produced in India is contributed by cucurbitaceous crops [2]. Snake gourd also known as Chinese cucumber (*Trichosanthes cucumerina* L.) and bottle gourd (*Lagenaria siceraria* L) are important cucurbitaceous vegetables grown and consumed in Southern India, Bangladesh, and Nepal. Several bacterial, fungal and viral diseases in these species cause severe yield and economic losses to the farmers. Cucurbitaceous crops in India were reported to be infected by viruses belonging to the genera *Begomovirus, Cucumovirus*, *Potyvirus, Tobamovirus, Tospovirus,* etc. [3–7].

Cucumber mosaic virus (CMV), belongs to genus *Cucumovirus* in the family *Bromoviridae*. It has broader host range and infects more than 1200 plant species across 100 plant families with an average yield penalties ranging 10–20% [8]. CMV is widespread in Europe, Asia, Australia, Africa and North America. CMV has tripartite single-stranded positive sense RNA genome (RNAs 1, 2 and 3). Transmission of CMV mainly occurs through aphids in a non-persistent manner. Additionally, CMV can be transmitted by mechanical means [9] and through seeds in many cucurbit species *viz.*, pumpkin, cucumber, wild cucumber, etc. [10]. Based on the serological properties, CMV is categorized into subgroups I and II. Subgroup I was divided into IA and IB based on the phylogenetic analysis of coat protein gene and 5′ untranslated region (5′ UTR) [11]. CMV subgroups IA and II are present worldwide, while subgroup IB is originated from Asia [12]. Our survey on mosaic diseases of cucurbitaceous crops in Tamil Nadu confirmed the natural occurrence of CMV on snake gourd and bottle gourd. Here we report biological and molecular characterization of CMV isolates infecting snake gourd and bottle gourd in India.

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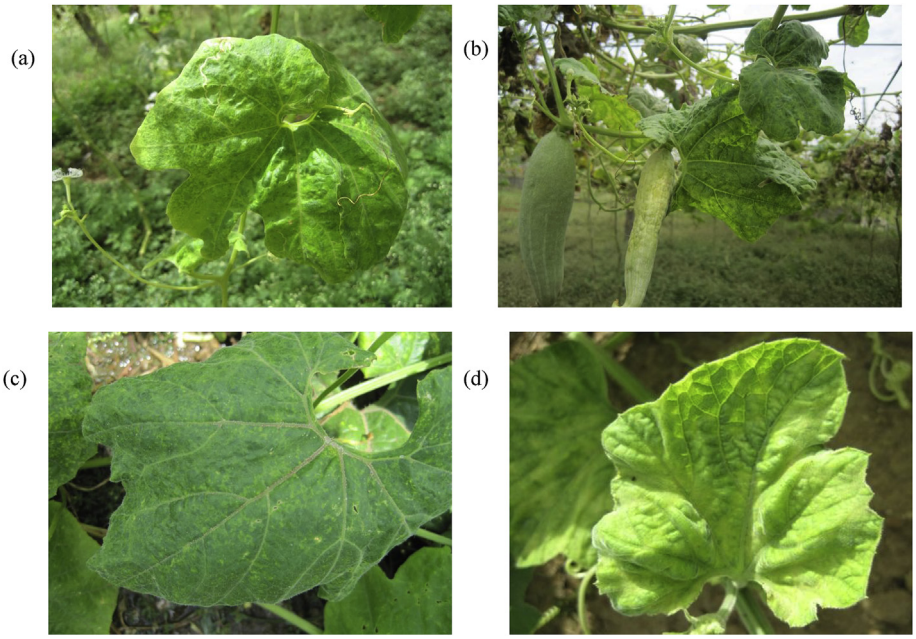

Fig. 1. Symptoms of CMV infected snake gourds plants showing mosaic mottling (a) puckering (b) and chlorotic spots (c) and chlorosis and mosaic mottling on bottle gourd leaf (d).

2. Materials and methods

2.1. Collection and maintenance of virus inoculum

Extensive surveys were conducted in the cucurbits cultivating areas of 12 districts in Tamil Nadu State, India, to study the occurrence of mosaic disease in cucurbitaceous crops during the growing season of 2012–2014. Naturally infected snake gourd and bottle gourd plants exhibiting virus like symptoms were collected from surveyed areas. Preliminary screening for the presence of CMV in these samples was done by Double Antibody Sandwich enzyme linked immunosorbent assay (DAS-ELISA) using polyclonal antibody against CMV that was obtained from DSMZ, Germany. Among them, CMV infected snake gourd (TN TNAU SG1 and TN TNV SG1) and bottle gourd (TN NGK BoG1) samples were mechanically inoculated on the snake gourd and bottle gourd plants, respectively. Virus isolates were maintained in insect-proof cages separately at the green houses of Department of Plant of Pathology, Tamil Nadu Agricultural University, Coimbatore (India) for further studies.

2.2. Host range study

CMV positive isolate (TN-TNAU-SG1) maintained on the snake gourd plants were mechanically sap inoculated onto 16 different hosts *viz.*, *Vigna unguiculata* L., *Nicotiana glutinosa* L., *Nicotiana plumbaginifolia* Viv., *Chenopodium amaranticolor* L., *Trianthema portulacastrum* L., *Datura stramonium* L., *Solanum lycopersicum* L., *Luffa aegyptiaca* Mill., *Lagenaria siceraria* L., *Cucurbita moschata* L., *Cucumis sativus* L., *Cucumis anguria* L., *Trichosanthes cucumerina* L., *Benincasa hispida* Thunb., *Luffa acutangula* L. and *Citrullus lanatus* Thunb. L. using 0.1 M sodium phosphate buffer (pH 7.0), 0.1% β-mercaptoethanol under greenhouse conditions to study the viral host range. The plants were maintained at 22–25 °C and observed for symptom development 30 dpi to record the local and systemic infection. Virus transmission was confirmed by RT-PCR using coat protein (CP) based primer pair, GK CMV F/R (Supplementary Table 1).

2.3. Cloning and sequencing of the genome

Total RNA was extracted from infected 100 mg leaf tissue of snake gourd and bottle gourd samples using Trizol reagent (Sigma Aldrich, USA). The integrity and quality of the total RNA was checked on 1% agarose gel. For the amplification of different ORFs 1a, 2a, 2 b, 3a and 3 b specific primers were designed by multiple alignments of different isolates of CMV nucleotide sequences available in NCBI GenBank using clustalW (Supplementary Table 1). First strand cDNA synthesis was carried out using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) as per manufacturer's instructions. Reactions were performed at 42 °C for 60 min followed by incubation at 70 °C for 5 min. Amplifications were carried out using Master mix (Bangalore Genei, Bengaluru, India) in a thermocycler (Eppendorf, Germany) with initial denaturation of 2 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing and extension temperature being specific for particular amplicon and finally an extension time of 10 min at 72 °C (Supplementary Table 1). PCR products were ligated into pGEMT-easy vector (Promega, Madison, USA) according to the manufacturer's instructions and then transformed into *E. coli* DH5α. Recombinant colonies were screened by restriction digestion. For each amplicon, at least three clones were sequenced at Excelris Pvt. Ltd., Ahemedabad, India. Sequences were assembled using CLUSTAL W2 and assembled sequences of RNA 1 (ORF 1a), RNA 2 (2a and 2 b) and RNA 3 (3a and 3 b) were deposited in the NCBI GenBank.

2.4. Phylogenetic analysis

Nucleotide sequences of CMV isolates belonging to subgroup IA, IB and II reported from different parts of the world were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov) and analyzed using MEGA 6.0 version. Phylogenetic relationships between these isolates were inferred from the nucleotide sequence alignment by Maximum likelihood method (1000 bootstrap replicates). To check the potential recombination events within CMV isolates, we used RDP4 program with default settings with all the CMV sequences available in GenBank database. Sequence identity percentage was calculated using Bioedit sequence alignment editor version 7.0.9 [13–15].