

Natural occurrence of *Cucumber mosaic virus* on lemongrass (*Cymbopogon citratus*), a new record

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Abstract. The natural occurrence of *Cucumber mosaic virus* (CMV) on *Cymbopogon citratus* was detected by enzyme linked immunosorbent assay using antiserum raised against CMV and reverse transcription polymerase chain reaction using specific primers for the coat protein. Sequence analysis of the PCR product showed 93–97% identities and a close phylogenetic relationship with CMV isolates of subgroup Ib.

Lemongrass (*Cymbopogon citratus*, family Poaceae) is a perennial herb, commercially cultivated in an ~3000 ha area in Kerala, Karnataka, Uttar Pradesh and Assam states of India, with an annual production of 300–350 t (<http://hortbizindia.org/Horticulture%20Crops%5CLemongrass%5CLemongrass1.htm>). The essential oil obtained from this herb has been reported to have antimicrobial activity, and is being used in medicines (as a central nervous system depressant), cosmetics and confectionary (Simon *et al.* 1984). Severe yellow stripes and tip necrosis of leaves were observed on *C. citratus* growing naturally at Chattha Research Farm of IIM, Jammu-Tawi, India in 2005–06. The causal pathogen was transmitted by mechanical inoculations using sap of infected leaves, which resulted in

local necrotic lesions on *Chenopodium amaranticolor* and systemic mosaic on *Nicotiana tabacum* cv. White Burley. The virus reacted positively with the antiserum raised against *Cucumber mosaic virus* (PVAS 242a, American Type Culture Collection, USA) in enzyme linked immunosorbent assay tests indicating the association of CMV with the leaf stripe disease of lemongrass.

To identify the virus isolate at the molecular level, total RNA was isolated by the method of Spears and Longhurst (1993) from infected and apparently healthy leaf tissues of *C. citratus* and the reverse transcription polymerase chain reaction (RT-PCR) was performed using the primers for the coat protein gene region of CMV (AM108922/AM108923;

Table 1. Coat protein gene-based sequence identity of the lemongrass isolate (EF153737) with strains of *Cucumber mosaic virus* (CMV) reported from India and elsewhere at nucleotide (nt) and amino acid (aa) level, using the Genomatix DiAlign programme

PSV, *Peanut stunt virus*; –, host not defined

Accession no	Strain/Abbreviation	Natural host	Location/Country	Subgroup	% Identities	
					nt	aa
DQ285569	CMV-PL	<i>Piper longum</i>	Lucknow, India	Ib	98	99
AY125575	CMV-Ban	<i>Musa paradisiaca</i>	Kerala, India	Ib	96	98
DQ006805	CMV-TN	<i>Thevetia nerefolia</i>	Italy	Ib	95	99
AY690621	CMV-PL	<i>Piper longum</i>	Kerala, India	Ib	95	98
AJ810260	CMV-Ch	<i>Capsicum annuum</i>	India	Ib	95	96
AY861397	CMV-WP	<i>Vanilla tahitensis</i>	New Zealand	Ib	95	98
AF350450	CMV-H	<i>Hyocymus muticus</i>	Lucknow, India	Ib	94	96
DQ914877	CMV-RS	<i>Rauvolfia serpentina</i>	Lucknow, India	Ib	94	97
AF198622	CMV-Amar	<i>Amaranthus tricolor</i>	Lucknow, India	Ib	93	94
AY541691	CMV-Tom	<i>Lycopersicon esculentum</i>	Greece	Ib	93	99
AF281864	CMV-Dat	<i>Datura innoxia</i>	Lucknow, India	Ib	93	95
AY545924	CMV-PN	<i>Piper nigrum</i>	Karnataka, India	Ib	93	97
AB042294	CMV-IA	–	Indonesia	Ia	92	98
D10538	CMV-Fny	–	NY, USA	Ia	91	97
AJ585086	CMV-Li	<i>Lilium longifolium</i>	India	II	66	78
L15336	CMV-Trk7	–	Hungary	II	68	79
NC_002040	PSV	<i>Vigna unguiculata</i>	USA	Out-group	42	57

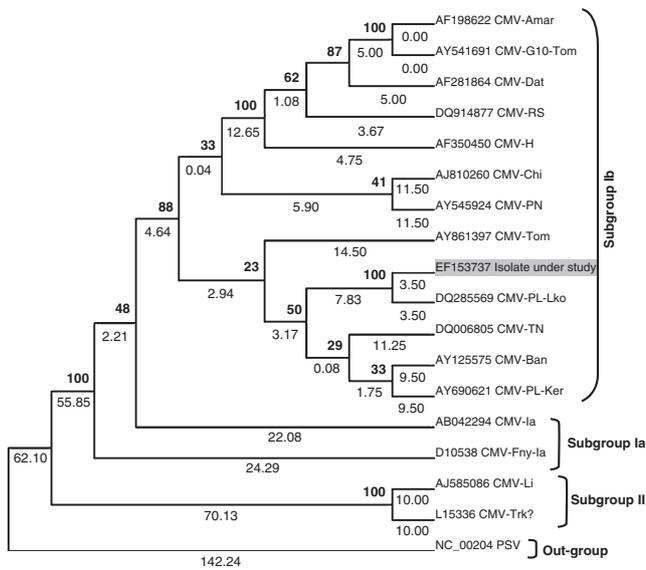


Fig. 1. Phylogenetic relationship of the virus isolated from lemongrass (EF153737) with strains of *Cucurbit mosaic virus* subgroups I (Ia & Ib) and II based on the nucleotide sequence alignment using the Mega 2.1 neighbour-joining tree method. Tree was rooted on *Peanut stunt virus* (PSV; NC_002040) as an out-group. The bootstrap values are adjacent to the joining lines (highlighted in bold) and branch length is beneath the line.

Srivastava *et al.* 2004). Electrophoresis of the RT-PCR products resulted in an amplicon of the expected size (~650 bp) in the infected but not in the healthy samples. The RT-PCR amplicon was cloned and sequenced (GenBank Accession number EF153737). Sequence analysis extended over the complete coat protein open reading frame of 657 nucleotides, encoding 218 amino acid residues. Genomatix DiAlign analysis of accession EF153737 with twelve other CMV isolates reported

from India and elsewhere in the world revealed 93–98% identities at the nucleotide and 95–99% at the amino acid level (Table 1). The lemongrass virus isolate showed closest phylogenetic relationships with CMV isolates belonging to subgroup Ib (Fig. 1).

There is no published record of any virus disease on *C. citratus* available except the rust and leaf spot diseases caused by *Puccinia nakanishikii* in California (Koike and Molinar 1999) and *Curvularia verruciformis* in India (Barua and Bodoloi 1983). To our knowledge this is the first record of the natural occurrence of CMV on *C. citratus*.

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