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First report of *Pepper leaf curl Bangladesh virus* strain associated with bitter gourd (*Momordica charantia* L.) yellow mosaic disease in India

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Abstract. A severe yellow mosaic disease with a significant disease incidence was observed on bitter gourd (*Momordica charantia* L.) during a survey of different locations around Gorakhpur, Uttar Pradesh, India in 2008. Polymerase chain reaction (PCR) was carried out using total DNA isolated from infected leaf samples and a pair of begomovirus-specific primers. The expected size (~1300 bp) amplicon was detected from all four symptomatic samples but not from samples of healthy plants, indicating the presence of a begomovirus infection. A PCR amplicon was cloned and sequenced (GenBank Accession reference EU888908). Basic local alignment search tool analysis revealed the newly derived sequence had the highest identities (99–97%) with *Pepper leaf curl Bangladesh virus* (PepLCBV) sequences. The phylogenetic analysis of the virus sequences with selected begomovirus sequences also revealed the closest relationship with PepLCBV. These results suggest an association of PepLCBV with yellow mosaic disease of bitter gourd; PepLCBV on bitter gourd (*M. charantia*) is a new record in India.

Bitter gourd (*Momordica charantia* L.) of the family *Cucurbitaceae*, also known as bitter melon is widely grown in China, India and throughout South-east Asia. It is also grown in small acreages in California and Florida USA, as a food and for medicine. Bitter gourd has been used for centuries in the ancient traditional medicines of India, China, Africa and Latin America. Its extract possesses anti-oxidant, anti-microbial, anti-viral, anti-hepatotoxic and anti-ulcerogenic properties and has the ability to lower blood sugar levels (Behera *et al.* 2009).

Bitter gourd is extensively cultivated in the north-eastern region of Uttar Pradesh (UP), India as one of the major vegetable crops and has great economic importance (Yadav *et al.* 2004). It has been reported to be a natural host of many viruses which affects its cultivation worldwide. During a survey in August 2008, a severe yellow mosaic disease was observed on bitter gourd growing in different locations of eastern UP, India. The incidence of the disease within crops was significant (10–20%) and symptoms consisted of a severe yellow mosaic and slight curling of leaf tissues (Fig. 1). Infected plants had less fruit, which were smaller in size as compared with those produced by healthy plants. An abundant population of the whitefly species *Bemisia tabaci* was also observed on the crop and also in the vicinity. The characteristic disease symptoms and presence of whitefly indicated the possibility of a begomovirus infection. Leaf samples from infected and healthy bitter gourd plants were collected.

To determine the identity of begomovirus, total nucleic acids were extracted from 100 mg of leaf tissue from samples of infected and healthy plants by a method described by Dellaporta *et al*. (1983) and resuspended in 20 µL TE (Tris + EDTA) buffer, quantified by spectrophometry then stored at –20°C. The extracts were tested by polymerase chain reaction (PCR) using the begomovirus genus-specific primers, PALIv 1978 and PARIc 496 (Rojas *et al*. 1993). Each 50 µL PCR contained 20 ng DNA, 1× PCR buffer (Bangalore Genei Pvt. Ltd, Bangalore, India), 200 µM of each dNTP, 1.5 mM $MgCl_2$, 25 pmole of each forward and reverse primers and 3 U *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd). Amplifications were performed in a Peltier thermal cycler (MJ Research, Watertown, MA, US), using an incubation regime of 94°C for 5 min once, followed by 30 cycles of 94°C for 50 s, 52°C for 60 s and 72°C for 90 s then a final incubation of 7 min at 72°C. The amplified DNA fragments were evaluated by electrophoresis with DNA marker (Lambda DNA double digested with *Hind*III and *Eco*RI; Bangalore Genei Pvt. Ltd) using a 1% agarose gel. An expected size amplicon of *ca*.1300 bp was observed from all four symptomatic samples (Fig. 2) but not from healthy samples collected from the same location.

The amplicon obtained from one of the symptomatic samples was cloned into a pGEM-T easy cloning vector (Promega, Madison, WI, USA) and three clones were sequenced from both directions. The 1313 bp consensus sequence without ambiguities was deposited in NCBI GenBank under Accession reference EU888908. The translation of this into amino acids indicated presence of partial rep protein (AC1), complete C4 protein (AC4) in complementary sense and pre-coat protein (AV2) and partial coat protein (AV1) genes in virion sense.

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Fig. 1. Naturally infected bitter gourd plant showing yellow mosaic disease in the field and showing yellow mosaic and leaf curl symptoms (*a* and *b*) as compared with a leaf from an uninfected plant (*c*).

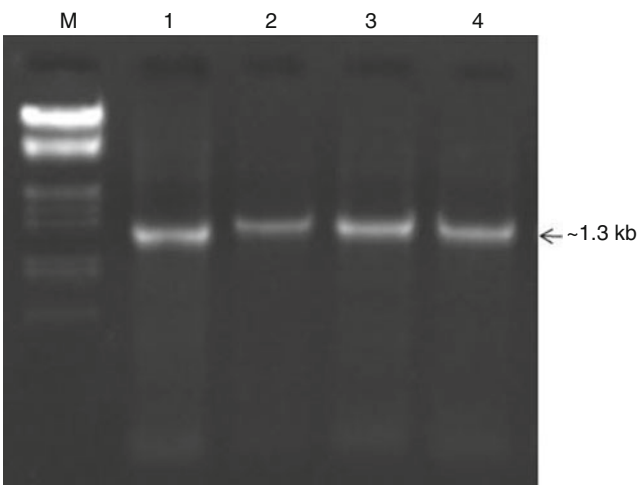

Fig. 2. Amplicons of ~1300 bp obtained by PCR from naturally infected bitter gourd leaf samples (lanes 1–4). M = Lambda DNA digested with *Eco*RI and *Hind*III (Bangalore Genei Pvt. Ltd, Bangalore, India) as a DNA marker.

Basic local alignment search tool (BLASTn) analysis of the 5′ end (1–811) of the newly derived sequence (GenBank EU888908) was 99% identical with *Pepper leaf curl Bangladesh virus* (PepLCBV, GenBank AF314531), 92% with *Chilli leaf curl Pakistan virus* isolate Multan (ChLCPV, GenBank DQ116877), ChLCPV isolate Khanewal (GenBank DQ116879 and DQ114477) and 89–88% with PepLCBV (GenBank DQ116881, AM404179). While sequence identities of 3′ end sequences (812–1313) of the sequence were 97% with PepLCBV (GenBank AF314531), 94% with *Chilli leaf curl virus*-[Jessore] (GenBank AM087118 and AM087119) and 91–90% with *Tomato leaf curl Joydebpur virus* (GenBank DQ673859, AJ875159, FJ345401, EU431116 and FJ345402).

Phylogenetic analysis of nucleotide sequence of the virus isolate with selected begomovirus isolates which were all at least 88% identical, was performed using the molecular evolutionary genetics analysis (MEGA) version 4.0 software (Tamura *et al.* 2007). The analyses revealed closest relationship of the virus isolate with PepLCBV (GenBank AF314531) and also close relationships with two isolates of PepLCBV (GenBank

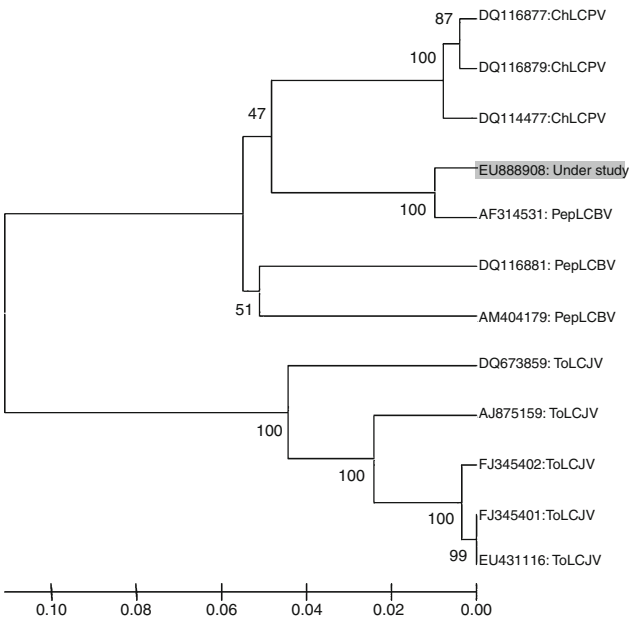

Fig. 3. Phylogenetic relationships of the newly derived virus sequence (GenBank EU888908) with selected begomovirus sequences reported from India and abroad. Phylogenetic tree generated in MEGA 4.0 version using 100 bootstrap values.

DQ116881 and AM404179) and three isolates of ChLCPV (GenBank DQ116877, DQ116879 and DQ114477). However, it did not show any relationship with *Tomato leaf curl Joydebpur virus* isolates considered in this study (Fig. 3). The criteria for identification of begomoviruses lists a demarcation value of 89%, above which virus isolates are considered to belong definitively to a single species (Fauquet *et al.* 2008). Given the newly derived sequence clearly exceeds the demarcation value for species identification, as it is 99% and 97% identical at the 5′ end and at 3′ end sequence, respectively, to known PepLCBV sequences, the virus detected in bitter gourd with yellow mosaic disease was identified as an isolate of *Pepper leaf curl Bangladesh virus* (PepLCBV).

The possibility of other viruses, particularly potyviruses which are commonly found infecting cucuribits, was checked by sap inoculations on three seedlings each of *M. charantia*, *Cucurbita maxima*, *Cucumis sativus*, *Nicotiana tabacum* cv. White Burley, *Lycopersicon esculentum* and *Datura metal*. No local or systemic symptoms were observed in any of the test plants, indicating an absence of other viruses.

Many viruses have been reported to naturally infect bitter gourd. These include *Papaya ring spot virus* (Dahal *et al*. 1997), *Watermelon mosaic virus* (Tomar and Jitendra 2005), begomovirus (Khan *et al*. 2002; Raj *et al*. 2005), *Bitter gourd yellow mosaic virus* (Rajinimala *et al*. 2005), *Tomato leaf curl New Delhi virus* (Tahir and Haider 2005), *Indian cassava mosaic virus* (Rajinimala and Rabindran 2007) and *Melon yellow spot virus* (Takeuchi *et al*. 2009). However, no report of PepLCBV on *M. charantia* or any other host exists in India; therefore, it may be considered as a new report.

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