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Detection and molecular characterization of a new begomovirus associated with mosaic disease of *Malachra capitata* (Malvaceae)

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Abstract

During a survey from March 2013 to October 2015, in Barasat, West Bengal, India, symptoms suggestive of begomovirus infection were observed in *Malachra capitata*. The occurrence of begomovirus on *M. capitata* was confirmed by symptomatology, polymerase chain reaction (PCR), Southern blot analysis and nucleotide analysis of the whole genome sequence. Sequence analysis showed that the virus had 90.6% identity with available sequences of *Tomato leaf curl New Delhi virus* (ToLCNDV). We propose that the new begomovirus be named Malachra yellow mosaic virus (MalYMV). To the best of our knowledge, this is the first time the complete genome of the MalYMV, infecting *M. capitata* in West Bengal, India, has been sequenced.

Keywords Begomovirus · *Malachra capitata* · PCR · Southern blot · Detection · Genome

Begomoviruses (family Geminiviridae) are among the most economically important plant viruses. They are efficiently transmitted by the whitefly (*Bemisia tabaci*), resulting in crop losses estimated to exceed billions of U.S. dollars annually and threatening food security (Stansly and Naranjo 2010). Begomoviruses affect production of a wide range of important crops, including beans, cassava, cotton, squash, sweet potato, and tomato. Two of the most devastating diseases caused by begomoviruses are tomato leaf curl disease, widely distributed around the world (Lefeuvre et al. 2010), and cassava mosaic disease, which has reached pandemic levels in African countries (Legg et al. 2014). Weed species act as reservoir hosts for many economically important plant virus diseases. Weeds infected with begomoviruses have been reported from different geographic areas of the world and have been described as natural hosts of various begomoviruses (Hallan et al. 1998; Khan et al. 2012).

Malachra capitata (Family Malvaceae) is a common and frequently occurring weed species in India. It is widely distributed in cultivated and uncultivated land, wasteland, railway tracks, and road sides. It has some economic importance, as it is grown as a fibre plant in India, and formerly also in Cuba. The leaves and flowers are recorded in the Venezuelan Pharmacopoea. In some regions, roots and leaves are used as remedies for the many disease conditions such as pain, diarrhoea, convulsion, hepatic cirrhosis, inflammation, pyrexia, ulcer, dementia, healing of wounds (Ames et al. 1981; Rice-Evans et al. 1997; Bhowal and Yawalikar 2015). It is also used for bathing purposes (Tiwari et al. 1998).

During a survey in the summer seasons of 2013 and 2015, in Barasat, West Bengal, India, specimens of *M. capitata* exhibited the typical symptoms of a begomovirus infection, including leaf curling, leaf mosaic, leaf yellowing and stunted growth (Fig. 1). Symptomatic leaves were collected from different fields at Barasat and total DNA was extracted from the using the CTAB method (Ghosh et al. 2009) with some modification. Extracts were tested for the presence of begomoviruses by PCR using an indigenously designed begomovirus specific degenerate primer pair (Roy et al. 2015). Of 14 samples, 12 had positive PCR reactions. This indicates a begomovirus infection in symptomatic plants with amplification of parts of the AV1, AC3 and AC2 genes fragment of approx. ~760 bp length (Fig. 2a). For the Southern blot analysis, we designed a biotin labelled probe (5'-ATRRTHTGGATGGAYGARAACAT -3′). The 5′ end of the probe was biotinylated and used for the detection of geminiviruses from the total sap of infected plant samples. Briefly, about 5 μl of freshly prepared sap was blotted on a nitrocellulose membrane and air dried. The membrane was then UV-cross linked for 30 min under UV-Cross Linker

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Fig. 1 A symptomatic *M. capitata* plant showing leaf curling, yellow mosaic, vein clearing, leaf yellowing and stunted growth of the plants typical to that of begomovirus infection. The right hand picture shows a healthy plant

(GeNei$_{TM}$, India). Pre-hybridization, hybridization and washing of the membrane was done according to the Southern blot analysis protocol using biotin-labelled probes (Weigel et al. 2015). Of 12 samples, 10 were positive, whereas samples from non-symptomatic plants gave negative results (Fig. 2b). The strong signal found suggested that the virus titer in *M. capitata* was high. For host range and transmission studies, *B. tabaci* were fed on infected leaves of *M. capitata* for 24 h. After this acquisition access period, viruliferous *B. tabaci* were collected and batches of ten insects per plant were placed on healthy seedlings of *Lycopersicon esculentum* (tomato) for additional 24 h (Wang et al. 2012). Monitoring of virus was based on PCR amplification. The initial symptoms included curling of leaves with clearing and thickening of veins and the entire plant remained stunted. Begomovirus DNA was detected in inoculated tomato plants 14 d.p.i.

The complete viral genome was amplified by rolling circle amplification (RCA) using the Illustra Templiphi DNA Amplification Kit (GE Healthcare, USA) as per manufacturer's instructions. It was cloned and completely sequenced. The plasmids were isolated using a plasmid isolation kit, and sent for sequencing with different sets of primers covering the whole genome of the virus. Sequence results of the full genome were analysed with available begomovirus DNA-A sequences obtained from the GenBank database using BLASTn, and pairwise identity scores were calculated using SDTv1.2 (Sequences Demarcation Tool version 1.2). BLASTn sequence analysis showed that the virus shares 87% - 91% identity with other begomoviruses. SDTv1.2 analysis of the virus from *M. capitata* that the virus shares 90.6% identity with that of a *Tomato leaf curl New Delhi virus* isolate from Gujarat (GenBank Accession No. KX513856), 90% identity with *Pea leaf distortion virus* isolate from Nepal (GenBank Accession Nos. KY001640, and KY001641) and Malvastrum yellow vein Chitwan virus isolate from Nepal (GenBank Accession No. JQ327841). According to the 10th ICTV Report, the criterion for new species demarcation is <91% pairwise nt identity on the basis of a full-length genome of monopartite or full length DNA-A for bipartite begomoviruses (Brown et al. 2015). Here, we propose the name Malachra yellow mosaic virus. A phylogenetic tree was constructed using Vector NTI, BioEdit and maximum likelihood method with MEGA 6.06 software. *Beet curly top virus* (BCTV) GenBank Accession No. KX867052 was used as the outgroup and is marked as a solid square (Fig. 3). Our sequences are marked with solid triangles (Fig. 3). The phylogenetic analysis showed that two major clusters formed, and that the virus from *M. capitata* is distinct from other begomoviruses. The whole genome sequence of the virus was determined as 2739 nt and was submitted to the DDBJ sequence database as Accession No. LC080677.

To the best of our knowledge this is the first molecular evidence of a distinct new species of begomovirus infecting *M. capitata* in West Bengal, India. More detailed study of

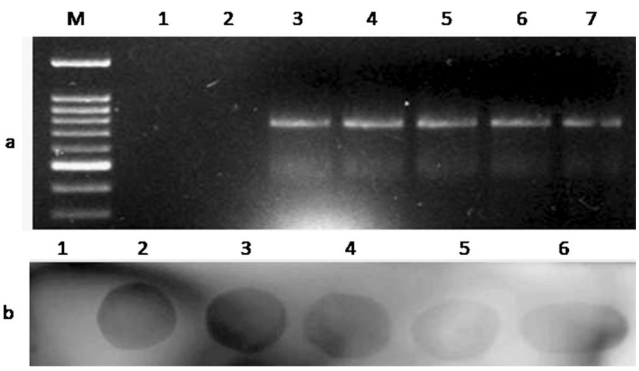

Fig. 2 **a** Detection of MalYMV from *M. capitata* leaf by using indigenously designed *begomovirus* specific degenerate primer pair which gave amplification approximately 760 bp. Lane M: 100 bp DNA marker (Black Bio), Lane 1–2: healthy *M. capitata* leaf, Lane 3–7: infected *M. capitata* leaf. **b** Southern hybridization detection of MalYMV by using biotin probe (lane 2–6 were positive in Southern hybridization and lane 1 was found negative)

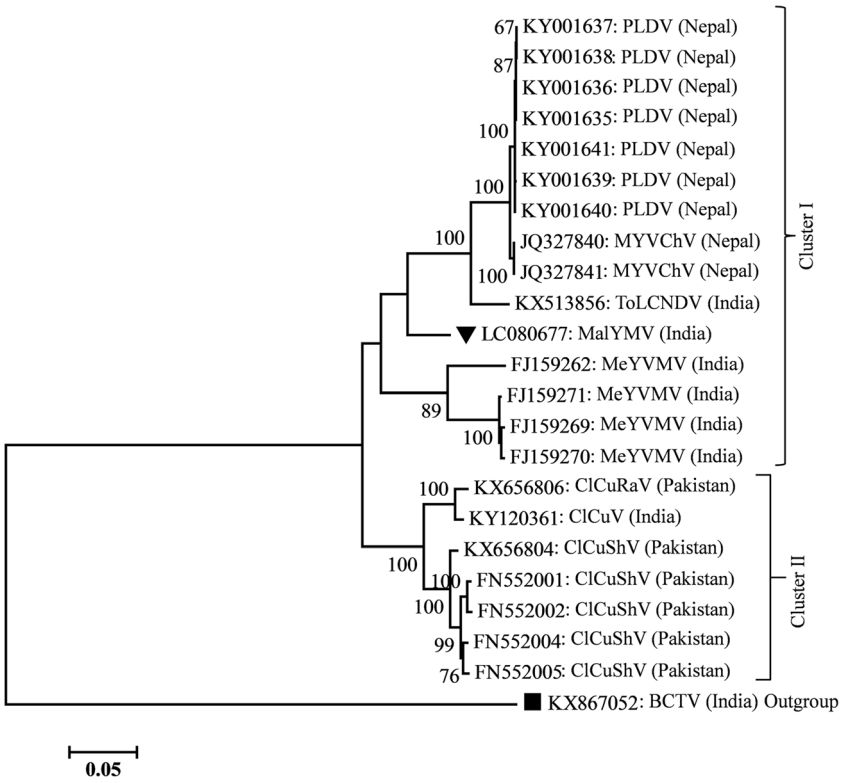

Fig. 3 Phylogenetic tree constructed from nucleotide sequences using the maximum likelihood method, with MEGA 6.06 software. Numbers below the branches indicate bootstrap value percentages from 1000 replications, but bootstrap values below 60% are not shown. *Beet curly top virus* (BCTV) Accession No. KX867052 was used as the outgroup and marked with a solid square. Our sequence (GenBank Accession No. LC080677) was marked with a solid triangle. CLCuShV, Cotton leaf curl Shadadpur virus; ToLCNDV, Tomato leaf curl New Delhi virus; CLCuRV, Cotton leaf curl Rajasthan virus; PLDV, Pea leaf distortion virus; MYVChV, Malvastrum yellow vein Chitwan virus; MeYVMV, Mesta yellow vein mosaic virus; MalYMV, Malachra yellow mosaic virus and BCTV, Beet curly top virus

Malachra yellow mosaic virus is needed, as it may be a threat to other cultivated crops. Many begomoviruses are already reported as emerging and re-emerging in recent years and infecting different hosts, including economically important crops which are susceptible to begomoviruses. The spread of the disease should be studied further, and characterized in detail at the molecular level. Interactions between the hosts and vectors of the virus should also be investigated. Present results indicate that, for effective management of viral diseases, it is essential to research the potential of different weeds in the field to act as viral reservoirs.

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References

Ames BN, Catheart R, Schwiers E, Hochstein P (1981) Uric acid provides as antioxidant defense in humans against oxidant and radical caused aging and cancer: a hypothesis. Proc Natl Acad Sci U S A 78(11):8658–6862

Bhowal M, Yawalikar N (2015) Antibacterial activity of *Malachra capitata* L. Indian J Plant Sci 4(3):96–99

Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Arch Virol 160(6):1593–1619

Ghosh R, Paul S, Ghosh SK, Roy A (2009) An improved method of DNA isolation suitable for PCR based detection of Begomoviruses from jute and other mucilaginous plants. J Virol Methods 159:34–39

Hallan V, Saxena S, Singh BP (1998) Ageratum, croton and malvastrum harbour geminiviruses: evidence through PCR amplification. World J Microbiol Biotechnol 14(6):931–932

Khan MS, Tiwari AK, Ji SH, Chun SC (2012) *Ageratum conyzoides* and its role in Begomoviral epidemics; *Ageratum enation* virus: an emerging threat in India. Vegetos 25(2):20–28

Lefeuvre P, Martin DP, Harkins G, Lemey P, Gray AJ, Meredith S, Lakay F, Monjane A, Lett JM, Varsani A, Heydarnejad J (2010) The spread of *Tomato yellow leaf curl virus* from the Middle East to the world. PLoS Pathog 6:e1001164

Legg JP, Shirima R, Tajebe LS, Guastella D, Boniface S, Jeremiah S, Nsami E, Chikoti P, Rapisarda C (2014) Biology and management of Bemisia whitefly vectors of cassava virus pandemics in Africa. Pest Manag Sci 70:1446–1453

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2(4):152–159

Roy B, Chakraborty B, Mitra A, Sultana S, Sherpa AR (2015) Natural occurrence of *Bhendi yellow vein mosaic virus* on *Litsea spp.* in India. New Dis Rep 31:7

Stansly PA, Naranjo SE (2010) Bemisia: bionomics and management of a global pest. Springer, New York

Tiwari SP, Jain RK, Nema RK, Rajagopal S (1998) Effect of feeding *Malachra capitata* leaves on rumen fermentation and blood constituents in kids. Indian J Anim Nutr 15(1):67–68

Wang J, Bing XL, Li M, Ye GY, Liu SS (2012) Infection of tobacco plants by a begomovirus improves nutritional assimilation by a whitefly. Entomol Exp Appl 144:191–201

Weigel K, Pohl JO, Wege C, Jeske H (2015) A population genetics perspective on geminivirus infection. J Virol 89:11926–11934