NEW RECORDS

First report of cucumber mosaic virus infecting antamul vine (*Tylophora indica*) in India

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Abstract *Tylophora indica* (Burm f.) Merrill; commonly known as antamul, is an important medicinal herb. Typical yellow rings or irregular yellow spot and in severe condition necrotic rings were observed on the leaves of the crop. The examination of symptomatic leaf samples under transmission electron microscopy revealed the presence of spherical virions confirmed the association of cucurmo like virus group. A novel degenerate primers pair was designed by multiple sequence alignment of RdRP region and used in RT-PCR to amplify a ~ 410 bp genomic fragment. The sequence of the amplified fragment shared 97–98% sequence identity with cucumber mosaic virus (CMV). This study is the first report of the association of CMV with the yellow ring symptom of antamul in India.

Keywords Antamul · *Cucumber mosaic virus* · Electron microscopy · Primer · RT-PCR

Medicinal plants are gaining importance in present scenario as the changing food habits bringing the complex health related ailments. *Tylophora indica* (Burm f.) Merrill; (Family: *Asclepidaceae*) commonly known as antamul is an important medicinal perennial climbing herb. It consist more than 60 species and distributed throughout the world in tropical and sub tropical regions. In India it normally found in Uttar Pradesh, West bangal, Orissa, Assam and

Himalayas and the other eastern hilly regions. The plant is used in medicinal purpose since centuries for the treatment of bronchial asthma, jaundice and inflammation.

Cucumber mosaic virus (CMV) is a type species of the genus *Cucumovirus* of the family *Bromoviridae*, reported to infect about 1287 plant species [2]. Recently, the cucumovirus-like symptoms of yellow chlorotic rings or irregular yellow spot and in severe condition necrotic rings were observed on the infected vine leaflets of the antamul (Fig. 1a) throughout the year in 2015–2016, in the herbal garden of ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat. The virus association with the disease was confirmed based on the electron microscopy observations. The symptomatic leaf tissue processed following the standard leaf dip procedure [1] was performed and spherical virus particles ranging from 28 to 32 nm size had been observed under the electron microscopy (Fig. 1b). Further, to identify the casual virus of genus *Cucumovirus*, a set of novel degenerate primers (RM07F/CMV-ATYCAYGGHGGTTATGAYATGGG and RM07R/CMV-CRAYRATYTTATASGTCATRAT) was designed and used for polymerase chain reaction (RT-PCR) to amplify ~ 410 bp of the RdRp genomic region. Total RNA was isolated from naturally infected diseased and healthy leaves of antamul using Ribozol RNA extraction reagent (Amresco). The expected size of ~ 410 bp amplicon was recovered from the diseased sample in the RT-PCR (Fig. 1c). The purified amplicon DNA was cloned using pGEM-T vector, sequenced and deposited to NCBI data (Accession no. MG011667). The Blast analysis of the nucleotides sequences showed 97–98% identity with various isolates of CMV (KF891357.1; KM272277.1). Based on these findings the virus infecting antamul has been identified as an isolate of CMV. According to best of our knowledge, this is first

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Fig. 1 Symptoms of *Cucumber mosaic virus* on naturally infected antamul (*Tylophora indica*) plants (a) spherical virus particles observed under the electron microscopy (b) and gel profile of detection of cucumber mosaic virus in symptomatic leaf samples by RT-PCR (c)

report of CMV infecting antamul in India. Antamul is a perennial climber which may serve as important alternate host for survival and source of primary inoculum for spreading the cucumoviruses.

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