First report of *Konjac mosaic virus* in elephant foot yam (*Amorphophallus paeoniifolius*) from India

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Abstract Natural association of *Konjac mosaic virus* (KoMV) with mosaic disease of elephant foot yam (*Amorphophallus paeoniifolius*) in India was identified by enzyme-linked immunosorbent assay using an antiserum raised against KoMV recombinant coat protein (CP) and immunocapture-reverse transcription-polymerase chain reaction using CP gene specific primers. Sequence analysis showed 77.2–98.9 % and 92.1–98.2 % identities at CP nucleotide and amino acid levels, respectively with the reported KoMV isolates.

Keywords Elephant foot yam · Mosaic · IC-RT-PCR · *Konjac mosaic virus*

Amorphophallus paeoniifolius (syn: *A. campanulatus*, elephant foot yam) is one of the important aroid tuber vegetable crops cultivated for its corms in tropical and sub-tropical regions of Asia (Ravi et al. 2009). This crop is mainly propagated through vegetative propagules (corms) and so any virus infecting the crop has quarantine relevance as the virus is vertically transmitted. Thus correct identification of viruses transmitted through vegetative propagules has significance. So far, *Dasheen mosaic virus* (DsMV) in India and *Konjac mosaic virus* (KoMV) in Japan have been reported to naturally infect *Amorphophallus* species (Shimoyama et al. 1992; Pandit et al. 2001; Babu et al. 2012). Recently, partial genome sequence of a potyvirus associated with infections on elephant foot yam in Kerala, India confirmed the virus as DsMV (Genbank A/C HQ207537). Nishiguchi et al. (2006) have determined the full-length genome sequence of KoMV-F isolate and confirmed its

identity as a distinct virus species in the genus *Potyvirus*, family *Potyviridae*. Further, they have suggested that *Zantedeschia mosaic virus*, previously identified as a new potyvirus (Kwon et al. 2002) based on partial genome sequence analysis, is now considered as a strain of KoMV. Padmavathi et al. (2011) have determined the partial genome sequence from 3' end (~1.6 kbp) of three potyvirus isolates and identified for the first time the association of KoMV with infections on *Colocasia*, *Caladium* and *Dieffenbachia* spp. from Andhra Pradesh, India.

In India, elephant foot yam is extensively cultivated in Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh and Jarkhand states (Ravi et al. 2009). Elephant foot yam showing initially prominent chlorotic spots followed by chlorotic vein banding and mosaic symptoms were observed in Andhra Pradesh during commercial field surveys in 2011 (Fig. 1). Symptomatic leaf samples (26) collected from different locations in Andhra Pradesh tested positive (A_{405} values: infected - 0.94–1.35, healthy - 0.13–0.15) for KoMV by DAC-ELISA (Clark and Bar-Joseph 1984) using polyclonal antibodies produced to recombinant coat protein (rCP) of KoMV-Col-AP isolate infecting taro (Padmavathi 2012). IC-RT-PCR was performed by the procedure of Nolasco et al. (1993) for amplification of CP gene from symptomatic *Amorphophallus* leaves using rCP KoMV-Col-AP antibodies and CP specific primers of KoMV-Col-AP isolate (KoMV-Col-CP-F - 5' CAAGGGATCCAGTGGAGAGGAAGA GAAG 3'; KoMV-Col-CP-R-5'CTGAGAATTCCTAAATAGCGCGCACAC 3'), and it yielded an amplicon of the expected size of 849 nucleotides (Fig. 2). The CP amplicon was cloned into pTZ57/RT vector (Fermentas, USA), sequenced and deposited in GenBank DNA database (Accession No. JQ773440). Sequence analysis (BioEdit v. 7.0.5) and comparison with reported KoMV isolates showed 77.2–98.9 % and 92.1–98.2 % identities at CP gene nucleotide and amino

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Fig. 1 **a** Healthy elephant foot yam. **b** Elephant foot yam leaves showing chlorotic vein banding and mosaic symptoms

acid levels, respectively. The present virus isolate CP has DAG triplet in its N-terminus like several other aphid-transmitted potyviruses. The other conserved motifs identified in the CP are WCIEN, AFDF, RQ, RAREA, QMKA, LDG and TERHT (Adams et al. 2005). Phylogenetic relationships with other potyviruses infecting aroid plants were determined based on deduced coat protein amino acid sequence and the present virus isolate clustered with KoMV isolates recently reported from Andhra Pradesh, India (Fig. 3; Padmavathi et al. 2011).

Based on the molecular criteria suggested for discrimination of unknown potyvirus isolates (Adams et al. 2005), the present virus isolate associated with chlorotic spots, chlorotic vein banding and mosaic symptoms on *A. paeoniifolius* in Andhra Pradesh, India was identified

Fig. 2 IC-RT-PCR amplification of KoMV-Amor-Chi-AP isolate coat protein (CP) gene using KoMV CP specific primers. Lane M, Gene Ruler 1Kb DNA ladder (Fermentas, Canada). Lane 1, 2, *Amorphophallus* leaves with mosaic symptoms. Lane 3, 4, healthy *Amorphophallus* leaves

for the first time as a strain of KoMV and we named it as KoMV-Amor-Chi-AP. This is the second potyvirus naturally associated with *A. paeoniifolius* in India. This work can help to design multiplex diagnostic methods for parallel screening and differentiation of potyviruses associated with elephant foot yam germ plasm and mother plants meant for micropropagation.

Fig. 3 Neighbor-joining phylogenetic tree showing the relationships of CP amino acid sequence of KoMV-Amor-Chi-AP isolate with other potyviruses infecting aroid crops. The phylogenetic tree was constructed using MEGA 4.0. The values at the forks indicate the number of trees that this grouping occurred after bootstrapping the data. The scale bar represents a genetic distance of 0.02. Virus acronyms: KoMV - Konjac mosaic virus, JHMV - Japanese hornwort mosaic virus, TuMV - Turnip mosaic virus, BYMV- Bean yellow mosaic virus, DsMV - Dasheen mosaic virus, ZaMMV - Zantedeschia mild mosaic virus, BCMV- Bean common mosaic virus, WMV-2 - Watermelon mosaic virus 2, CLLV- Calla lily latent virus, SMV- Soybean mosaic virus

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