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Identification of *Tomato aspermy virus* as the cause of yellow mosaic and flower deformation of chrysanthemums in India

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Abstract. Using indirect ELISA, RT-PCR and coat protein gene sequence analysis, *Tomato aspermy virus* is identified as the cause of yellow mosaic and flower deformation of *Chrysanthemum morifolium* cultivars in India.

Chrysanthemum (*Chrysanthemum morifolium*, family Asteraceae) is a popular ornamental plant worldwide and is one of the most commercially important flower crops in India. Symptoms of yellow mosaic, green vein-banding, stunting and flower deformation were observed on various chrysanthemum cultivars growing in the gardens and nursery of the National Botanical Research Institute, Lucknow, India, between September 2004 and February 2005 (Fig. 1). *Cucumber mosaic virus* (CMV), *Tomato aspermy virus* (TAV) and *Chrysanthemum virus B* (CVB) have previously been reported to infect chrysanthemum (Bouwen and van Zaayen 1995) and, therefore, indirect ELISAs to detect these viruses were performed using the method of Srivastava *et al*. (1992) and antisera from the American Type Culture Collection. A positive reaction ($A_{405\,nm}$ four times greater than the healthy chrysanthemum control) was observed with antiserum to TAV (PVSA-24) but not with antiserum to CMV (PVAS 242a) or CVB (PVAS 349).

To prepare template for use in reverse transcription–polymerase chain reaction (RT-PCR), total RNA was isolated from healthy and naturally infected leaf tissue of chrysanthemum plants using the method of Spears and Longhurst (1993). Complementary DNA was synthesised using 200 U of RevertAid H-minus MMuLV reverse transcriptase (Fermentas, USA) and the reverse primer 5′-TCACACCGGGAGCGTTGAAGC GGAA-3′ and PCR performed using 3 U *Pfu* DNA polymerase

Fig. 1. *Chrysanthemum morifolium* showing yellow mosaic, stunting and flower deformation.

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Table 1. Coat protein gene-based analysis of the TAV-Lucknow isolate (DQ191798) based on the Genomatix DiAlign programme

Accession number	Location	Abbreviation used	% Identity based on Genomatix DiAlign[A]	
			Nucleotide	Amino acid
L79972	Australia	TAV-V	97 (0.923)	95 (0.928)
L15335	Hungary	TAV-P	97 (0.923)	95 (0.928)
AJ277269	USA	TAV-B	97 (0.915)	95 (0.928)
AJ237849	South Korea	TAV-KC	97 (0.912)	95 (0.928)
AM055753	New Delhi	TAV-ND	97 (0.918)	94 (0.924)
AM055758	Haryana	TAV-HR	97 (0.916)	94 (0.920)
AJ580841	Uttar Pradesh	TAV-UP	97 (0.911)	94 (0.924)
AJ582718	Punjab	TAV-PJ	96 (0.914)	94 (0.925)
AJ970532	Sikkim	TAV-SIK	96 (0.897)	93 (0.908)
AJ965491	Maharastra	TAV-MH	96 (0.888)	93 (0.912)
AM055755	Karnataka	TAV-KR	96 (0.879)	93 (0.906)
AJ586134	Himanchal Pradesh	TAV-HP	95 (0.854)	94 (0.922)
AM055757	Bihar	TAV-BR	94 (0.848)	93 (0.899)
AM055754	Andhra Pradesh	TAV-AP	92 (0.840)	94 (0.920)
AJ965492	TamilNadu	TAV-TN	93 (0.830)	94 (0.913)
AM049404	Assam	TAV-Asm	94 (0.833)	91 (0.870)
AM049403	Arunanchal Pradesh	TAV-ArP	94 (0.818)	93 (0.895)
AM055756	Madhya Pradesh	TAV-MP	93 (0.810)	88 (0.822)
AM055591	Gujarat	TAV-GR	90 (0.749)	86 (0.793)
AM055592	Uttaranchal	TAV-UT	89 (0.710)	84 (0.785)

[A]The % identity denotes number of identical sequences (in % of shorter sequence) and value given in brackets is the similarity (relative to the maximum similarity).

(MBI Fermentas, USA) using the same reverse primer combined with the forward primer 5′-ATGGCCCAAAACGG TACGGGAGGAG-3′; reactions were set up as described in the manufacturer's instructions. An amplicon of the expected size (~650 bp) was obtained, which was cloned using the pGEM-T Easy vector system (Promega, USA). Sequencing was conducted at Genei (Bangalore Genei Pvt. Ltd, India) and the sequence submitted to GenBank (Accession number DQ191798). When analysed using the DiAlign 2 module of GEMS Launcher (Genomatix), DQ191798 was 97% identical at the nucleotide level and 95% at amino acid level with seven other TAV isolates from India and elsewhere in the world (Table 1).

We therefore conclude that the cause of the yellow mosaic and flower deformation in the chrysanthemums was TAV. The occurrence of TAV in chrysanthemum in India has been reported previously on the basis of biophysical properties, aphid transmissibility and Ouchterlony gel double diffusion tests (Sastry 1964; Gupta and Singh 1981; Raj *et al*. 1991). However, to the best of our knowledge this is the first report of the molecular characterisation of a TAV isolate infecting chrysanthemums in India.

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