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Identification of *Mungbean yellow mosaic India virus* infecting *Vigna mungo* var. *silvestris* L.

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Summary. Yellow mosaic of *Vigna mungo* var. *silvestris*, a wild relative of blackgram (*Vigna mungo* [L.] Hepper), was noticed at the Indian Institute of Pulses Research, Kanpur, India during 2008–2010, with an incidence of 100 per cent. The observed symptoms, consisting of veinal yellowing and scattered bright yellow spots, were suggestive of infection with a begomovirus. To characterize the virus, several sets of primer pairs were designed to amplify the targeted DNA fragments of the causal virus. The sequence data revealed that the coat protein (AV1) gene of the begomovirus under study contained a single open reading frame with 774 nucleotides, coding for 257 amino acids. Comparative analysis of the coat protein (AV1) gene of the virus under study (FJ821189) showed a 97 and 99% similarity with *Mungbean yellow mosaic India virus* (MYMIV)-Mungbean strain at the nucleotide and the amino acid levels respectively. Sequence homology of different genes (AC1, AC2, AC3 and AC4) of the isolate under study (FJ663015) with MYMIV-Mungbean (EU523045) was 94–97% for the nucleotides and 91–99% for the amino acids sequence. Therefore, the begomovirus infecting *V. mungo* var. *silvestris* at Kanpur is to be considered a strain of MYMIV and is designated as MYMIV-VSKN. This appears to be the first report of the molecular characterization of MYMIV infecting *V. mungo* var. *silvestris*.

Key words: begomovirus, MYMIV, *Vigna*, whitefly.

Introduction

Yellow mosaic affects many legumes in India and other south Asian countries and is caused by whitefly (*Bemisia tabaci* Genn.) transmitted geminiviruses. Four geminivirus species in various leguminous species cause yellow mosaic: *Mungbean yellow mosaic virus* (MYMV, genus *Begomovirus*, family *Geminiviridae*), *Mungbean yellow mosaic India virus* (MYMIV, genus *Begomovirus*, family *Geminiviridae), Dolichos yellow mosaic virus* (DoYMV, genus *Begomovirus*, family *Geminiviridae*) and *Horsegram yellow mosaic virus* (HgYMV, genus *Begomovirus*, family *Geminiviridae*). All these, referred as legume yellow mosaic viruses (LYMVs), are bipartite begomoviruses and they have geminate (twin) particles, 18–20 nm in diameter, 30 nm long, apparently consisting of two incomplete icosahedra joined together in a structure with 22 pentameric capsomeres and 110 identical protein subunits (Qazi *et al*., 2007).

Yellow mosaic occurs across the Indian subcontinent and is a major constraint to cultivation of most warm season legumes, particularly mungbean, urdbean and soybean. Estimates of actual losses due to yellow mosaic in farmers' fields are difficult to make since they vary from year to year and from variety to variety. However, based on the incidence of MYMV in mungbean, urdbean and soybean, an annual loss of over US$ 300 million is estimated in these crops (Varma *et al*., 1992). Yellow mosaic occurs in a number of legu-

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minous plants such as mungbean, urdbean, cowpea (Nariani, 1960, Nene, 1973), soybean (Suteri, 1974), horsegram (Muniyappa *et al*., 1975), lablab bean (Capoor and Varma, 1948) and French bean (Singh, 1979).

Vigna mungo var. *silvestris* L. (syn. *V. silvestris*), a wild relative of urdbean (*Vigna mungo* [L.] Hepper) is used in breeding programmes to create variability in plant characters and exploit them in developing new plant types with a wider adaptability and a higher yield (Reddy and Singh, 1989). During the rainy season of 2008, 2009 and 2010, an accession of *V. mungo* var. *silvestris* (IPUW-07) grown at Indian Institute of Pulses Research, Kanpur, India, showed yellow mosaic symptoms characteristic of LYMVs infection in cultivated species of *Vigna*, particularly mungbean and urdbean, and many other leguminous crops. *Vigna mungo* var. *silvestris* is known to be affected by yellow mosaic, but there appears to be no report characterizing a begomovirus (MYMIV) from *V. mungo* var. *silvestris*. The present paper deals with the characterization of the virus causing yellow mosaic in *V. mungo* var. *silvestris*.

Materials and methods

Transmission

Whiteflies (*Bemisia tabaci* Genn.) feeding on yellow mosaic diseased *V. mungo* var. *silvestris* plants grown in the field were collected using an aspirator and were released (40–50 whiteflies/5 plants) on 10–15 day old healthy *V. mungo* var. *silvestris* plants grown in a net house and covered with a plastic jar. Viruliferous whiteflies were allowed inoculation feeding on healthy plants for 48 hours. Whitefly-inoculated plants were maintained under insect-free conditions for 30 days.

Isolation and amplification of targeted genes

Young leaves showing the characteristic yellow mosaic symptoms were collected from field-infected plants of *V. mungo* var. *silvestris,* brought to the laboratory, washed in distilled water and dried by blotter paper. Leaf tissue (100 mg) was used for isolation of total DNA using the EZNA® Plant DNA Miniprep Kit (Norcross, Georgia, USA) according to the manufacturer's instructions. The total DNA obtained was used as a template to amplify the coat protein gene AV1, the replication initiator protein gene AC1, the transcription activation gene AC2, the replication enhancer gene AC3 and the AC4 gene, using the specific primer pairs AV1PF -5'GTA TTT GCA (GT)CA (AT)GT TCA AGA3'/AV1PR - 5'AGG (AGT)GT CAT TAG CTT AGC3', AC1PF -5'AGT TGA TAT GGA TGT AAT AGC3'/AC1PR - 5'ACA AAA ACG ACT TCA AAT ATG CCA A3', AC2PF - 5'AGC TAA TGA CCC CTA AAT TAT3'/AC2PR - 5'GAG TAC TTG GAT GAA GAG AAC3', AC3PF -5'TTA TGA TTC GAT ATT GAA TTA ATA3'/AC3PR - 5'CTG AAG TGT GGG TGT AGC TAT3' , AC4PF -5' CAA ATT ACA ATT TAA GTT ATG 3'/AC4PR -5' ACT TCT AGC CTT GTC AAC ACC AG3' derived from several isolates of MYMIV sequences in a polymerase chain reaction (PCR). Samples from five plants showing characteristic yellow mosaic symptoms and one healthy plant were processed. One sample from a plant that became infected as a result of whitefly inoculation was also used for virus detection.

Amplification of target genes by PCR

PCR was performed in a T1 Thermocycler, Biometra® (Goettingen, Germany), programmed for 35 cycles with one step of initial denaturation for 2.5 min., and denaturation for 45s at 94°C, 1 min. annealing at 54°C (for primer pair AV1PF/AV1PR), 49°C (for primer pairs AC1PF/AC1PR and AC2PF/AC2PR) and 48°C (for primer pairs AC3PF/AC3PR and AC4PF/AC4PF), and a 1 min. extension at 72°C followed by a one step final extension for 10 min. at 72°C. PCR assays were conducted with an Easy-Do™ PCR PreMix (SBS Genentech Co. Ltd., Beijing, China) in total reaction mixture volume of 50 µL that contained DNA template (50 ng μL^{-1})-2 µL, Primer (50 pmole μL^{-1})-1 µL each and dH_2O - 46 µL. The experimental control was a PCR master mix in which the template DNA was 5 µL of healthy *V. silvestris* DNA.

Electrophoresis, sequencing and analysis

PCR amplicons were separated by 1% agarose gel electrophoresis in Tris-acetate EDTA (TAE) containing 0.1% ethidium bromide. The gel was examined under a UV trans-illuminator and photographed using a digital camera (Sony DSC-W270). The PCR products of the target genes were purified using the EZNA® Gel Extraction Kit (Norcross, Georgia, USA), were sequenced direct-

ly, and were used for extracting the target genes. Sequence data were blasted using the NCBI data base. Multiple sequence alignment and the phylogram were generated using CLUSTAL W version 1.83. Sequences of AV1, AC1, AC2, AC3, AC4 genes of the begomovirus that infected *V. mungo* var. *silvestris* at Kanpur were compared with other begomoviruses (Table 1).

Results

Transmission

Symptoms of yellow mosaic started appearing 10 days after whitefly inoculation. Thirty days after inoculation, out of the 15 whitefly inoculated plants, 7 plants (46.7%) developed yellow mosaic symptoms similar to those seen in the field indicating that the causal virus was transmitted by the whiteflies.

Field symptoms and disease incidence

Infected *V. mungo* var. *silvestris* (accession No. IPUW-07) plants in the field showed yellow mosaic symptoms typical of the begomoviruses that cause yellow mosaic in leguminous plants. Initially, only a few yellow patches appeared in the inter-veinal areas, but by the time the crop reached maturity, more than 70% of the leaf area had turned yellow. Disease incidence was 70 to 100 per cent in different plots.

Table 1. Details of viruses and their genes used for comparison of sequence data.

Name of the virus[a]	Accession	AV1 (nt/aa)[b]	AC1 (nt/aa)	AC2 (nt/aa)	AC3 (nt/aa)	AC4 (nt/aa)
MYMIV-VSKN	FJ821189 FJ663015	774/257	1089/362	453/150	405/134	300/99
MYMIV*	EU523045	774/257	1089/362	411/136	405/134	300/99
MYMV	AY271896	777/257	1089/362	414/135	405/134	300/99
HgYMV	NC_005635	774/257	1086/361	408/135	405/134	300/99
DYMV	AY309241	774/257	1098/365	429/142	411/136	246/81
CGMV	AF029217	762/253	1059/352	408/135	405/134	294/97

[a] MYMIV-VSKN, *Mungbean yellow mosaic India virus*, this study; *, Type isolate; MYMV, *Mungbean yellow mosaic virus* (Mungbean isolate); HgYMV, *Horsegram yellow mosaic virus*; DYMV, *Dolichos yellow mosaic virus*; CGMV, *Cowpea golden mosaic virus*.
[b] nt, nucleotides; aa, amino acids.

Amplification and sequence analysis

PCR tests yielded amplified DNA fragments of the expected sizes, ~1100 bp, ~480 bp, ~450 bp, ~390 bp and ~950 bp, corresponding respectively to genes AC1, AC2 AC3, AC4 and AV1 of MYMIV in the five field samples and in the symptomatic leaf sample collected from whitefly-inoculated plants. A gel photograph of PCR amplified products of all the genes along with a healthy sample is shown in Figure 1.

The PCR amplified products were sequenced directly and used to extract the sequences of the targeted genes. Genes AV1, AC1, AC2, AC3 and AC4 ORF contained 774, 1089, 453, 405 and 300 nucleotides (nt) respectively, which coded for 257, 362, 136, 134 and 99 amino acids (aa) respectively. The sequence data were submitted to GenBank under accession number FJ821189 (AV1) and FJ 663015 (annotated sequence of AC1, AC2, AC3 and AC4). A comparison of the coat protein gene (AV1) sequences of begomovirus infecting *Vigna mungo* var. *silvestris* with the coat protein gene of other begomoviruses indicated that the begomovirus under study (MYMIV-VSKN) had a nucleotide sequence similarity level of 97% with MYMIV-Mungbean (EU523045). The Nucleotide similarity with other begomoviruses was 80% with MYMV-Soybean (AY271896), 81% with HgYMV (NC_005635), 70% with DoYMV (AY309241) and 68% with *Cowpea golden mosaic virus* (CGMV)

Table 2. Per cent nucleotide and amino acid sequence identity of different genes of MYMIV-VSKN with other type members of the begomoviruses.

Name of the virus [a]	AV1 (nt/aa)[b]	AC1 (nt/aa)	AC2 (nt/aa)	AC3 (nt/aa)	AC4 (nt/aa)
MYMIV VSKN	-	-	-	-	-
MYMIV*	97/99	96/96	94/91	95/93	96/93
MYMV	80/85	84/84	87/85	86/79	85/74
HgYMV	81/84	83/81	85/77	85/80	83/66
DYMV	70/77	62/61	66/59	67/57	60/24
CGMV	68/69	65/68	53/42	62/39	68/37

[a, b,] See Table 1.

(AF029217). Similarly, a comparison of deduced amino acid sequences indicated that the begomovirus under study had a maximum similarity of 99% with MYMIV-Mungbean (EU523045) followed by 85% with MYMV-Soybean (AY271896), 84% with HgYMV (NC_005635), 77% with DoYMV (AY309241) and 69% with CGMV (AF029217) (Table 2).

Genes AC1, AC2, AC3 and AC4 of MYMIV-VSKN had the highest nucleotide sequence similarity to the corresponding genes of MYMIV at 96, 94, 95 and 96% respectively, and amino acid sequence levels of 96, 91, 93 and 93% similarity, followed by MYMV (84, 87, 86, 85% at nt level, and 84, 85, 79,74% at aa level) and HgYMV (83, 85, 85, 83% at nt level and 81, 77, 80, 66% at aa level).

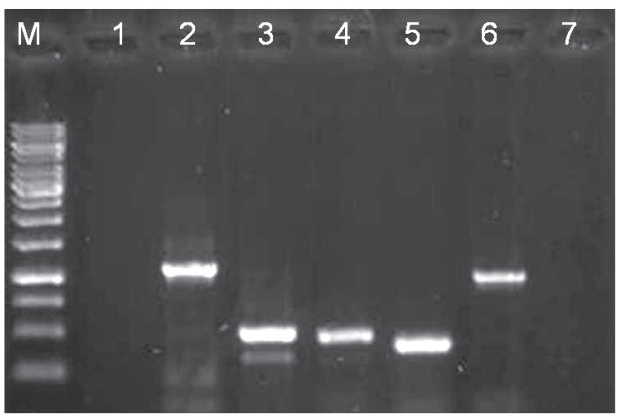

Figure 1. Gel photograph showing amplified product of begomovirus genes. Lane M, 1 kb DNA ladder; lane 1, DNA from virus free leaves; lane 2, *AC1*; lane 3, *AC2*; lane 4, *AC3*; lane 5, *AC4*; lane 6, *AV1*; lane 7, DNA from virus free leaves.

The cluster phylogram based on the multiple alignment of deduced amino acid sequences of the coat protein gene (AV1) of MYMIV-VSKN and some begomoviruses also indicated that the former is an isolate of MYMIV, since it clustered with MYMIV (EU523045) (Figure 2). MYMV, HgYMV, DoYMV and CGMV formed separate clusters. The cluster phylogram based on the sequences of genes AC1, AC2, AC3 and AC4 indicated the same trend (Figure 3).

A comparison of the AV1, AC2, AC3 gene sequences of MYMIV-VSKN with the gene sequences of DoYMV and CGMV revealed that at nt/aa level it was more similar to the sequences of the corresponding genes of DoYMV than to the sequences of CGMV. However, the AC1 and AC4 gene sequences of the present virus were closer to the sequences of the corresponding genes of CGMV (65/68%, 68/37%) than to the sequences of DoYMV (62/61%, 60/24%).

Discussion

Because of its high degree of conservation, the coat protein ORF (CP or AV1) is the only bego-

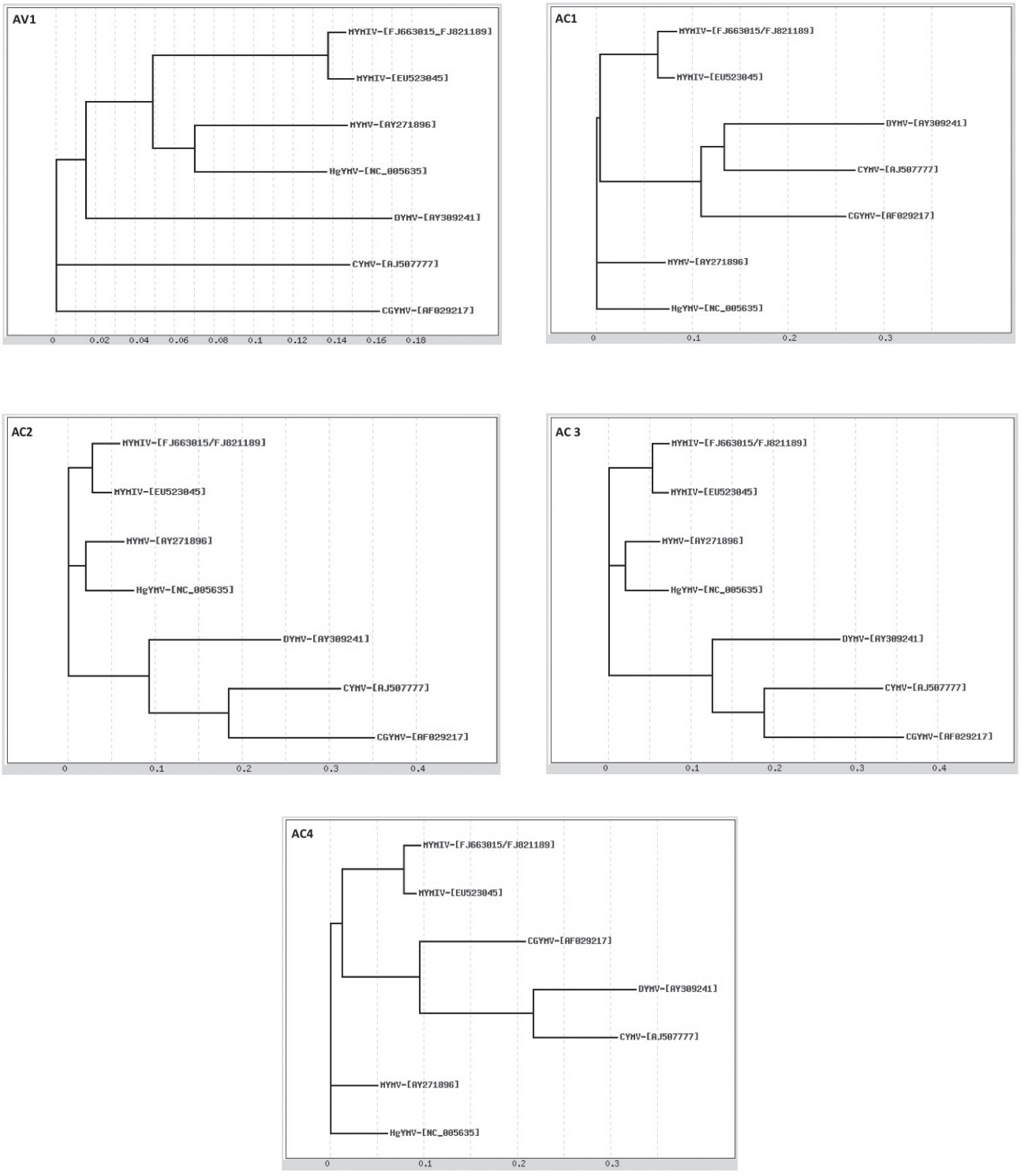

Figure 2. Cluster phylogram illustrating the phylogenetic relationship based on the multiple alignments of nucleotide sequences of genes AV1, AC1, AC2, AC3 and AC4 of the MYMIV isolate under study with the corresponding genes of some known begomoviruses.

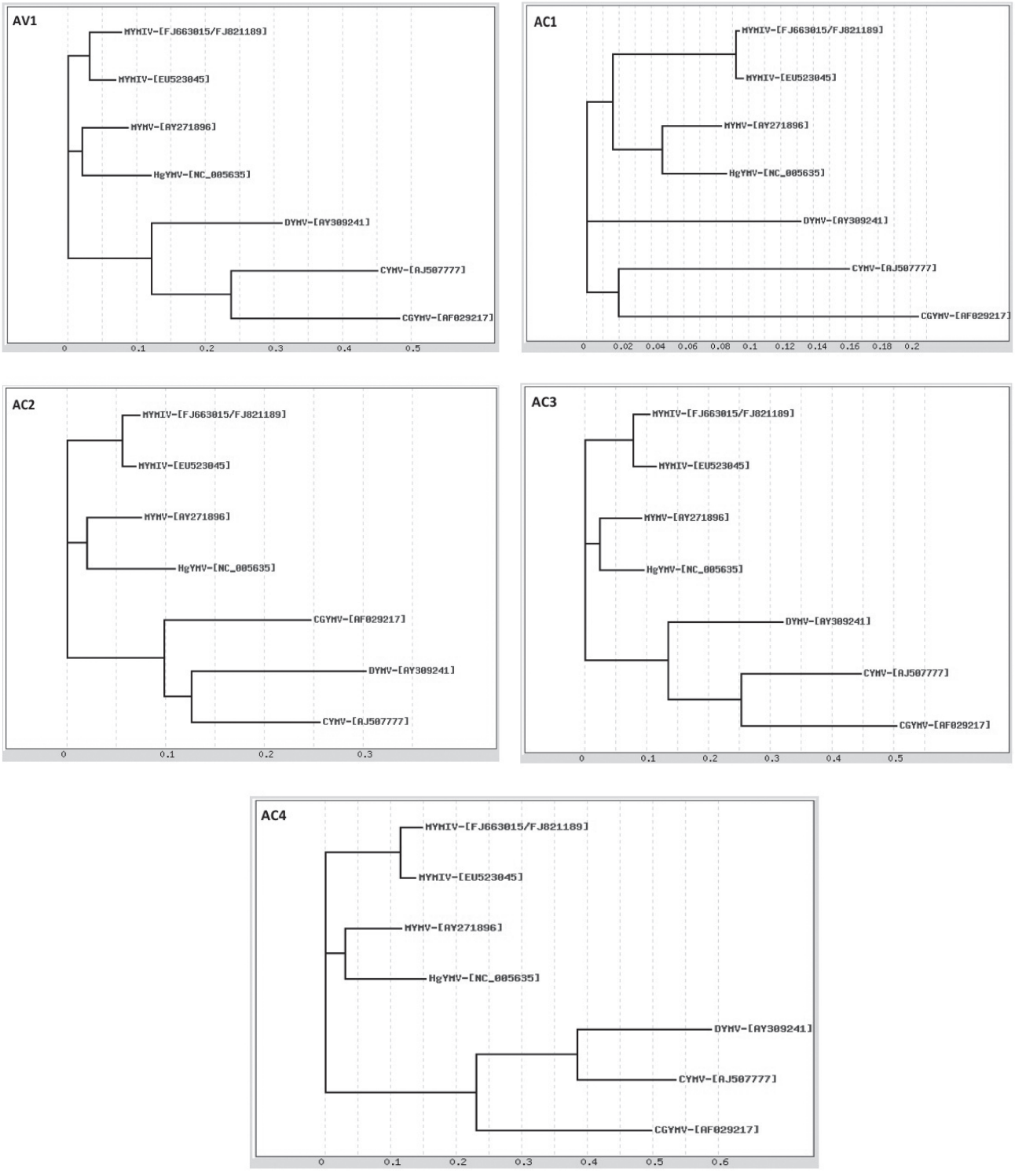

Figure 3. Cluster phylogram illustrating the phylogenetic relationship based on the multiple alignments of the deduced amino acid sequences of genes AV1, AC1, AC2, AC3 and AC4 of the MYMIV isolate under study with the corresponding genes of some known begomoviruses.

movirus sequence approved by the International Committee on Taxonomy of Viruses for ascertaining the identity of a begomovirus (Mayo and Pringle, 1998), and the sequence comparison has been used to identify and classify geminiviruses (Padidam *et al*., 1995; Brown *et al*., 2001). The sequence data of the coat protein gene of the present begomovirus indicated a 97% similarity with the MYMIV-Mungbean strain at the nt level, whereas a 90 per cent nucleotide sequence similarity has been suggested as a guideline for predicting the identity of a distinct begomoviral species (<90 percent) or a viral strain (>90 percent) (Rybicki, 1998). Therefore, the begomovirus infecting *V. mungo* var. *silvestris* at Kanpur is a strain of MYMIV and is designated as MYMIV-VSKN. The findings indicate that the primers specific to genes other than the coat protein gene may also be used for detection of MYMIV infection in plants. The data in Table 2 further indicate that the AV1 gene is most highly conserved, followed by AC1; and that among the viruses used for comparison, gene AC4 has a high degree of variability. *Vigna mungo* var. *silvestris* is known to be affected by yellow mosaic, but the present study seems to be the first report characterizing a begomovirus, MYMIV from this host.

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