Eur J Plant Pathol (2010) 127:41–51
DOI 10.1007/s10658-009-9569-1

ORIGINAL RESEARCH

Characterization of a begomovirus causing horsegram yellow mosaic disease in India

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Received: 7 April 2009 / Accepted: 23 November 2009 / Published online: 11 December 2009

Abstract The virus causing horsegram (*Macrotyloma uniflorum*) yellow mosaic disease has been shown to be a typical Old World bipartite begomovirus. The viral origin of the disease has been established through agroinoculation of horsegram using partial tandem repeat clones of both DNA-A and DNA-B. The DNA-A genome shows less than 89% identity with the corresponding sequences of all the begomoviruses in the databases earlier to this sequence submission (AJ627904). Therefore *Horsegram yellow mosaic virus* (HgYMV-[IN:Coi]) can be considered to be a new species of the genus *Begomovirus* (family *Geminiviridae*). Phylogenetic analysis shows that this virus is part of the cluster of mungbean yellow mosaic viruses of legumes from South and South East Asia.

Keywords Horsegram yellow mosaic virus · Phylogenetic analysis · Agroinoculation

Introduction

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) is an indigenous plant cultivated in India, other Asian countries and Africa (Jayan and Maya 2001). Since it is a hardy drought resistant plant, it has been cultivated as a low input agricultural crop of the marginal lands. Year after year the cultivation area of this crop is increasing in Tamil Nadu State of India. In 2005–2006 the yield was 22023 tonnes, an increase of 9.5% compared to the previous year. The cost also has escalated 22.5% within a year (Government of T.N. Report 2006). A little moisture from dew is enough to have the harvest in 90–110 days (Sharma 1995). Large numbers of poor farmers sow horsegram in small areas during the month of November. The increasing spread of the yellow mosaic disease due to *Bemisia tabaci* population increases results in almost complete loss of the crop during summer (Muniyappa et al. 1978). The disease causes yellow discoloration on the leaves that leads to irregular, small, greenish yellow mosaic symptoms. Severe infection stunts the plant and reduces the leaf size (Muniyappa et al. 1976).

The causative agent of the yellow mosaic disease of horsegram is the whitefly-transmitted begomovirus, (HgYMV), as listed by Fauquet et al. (2008). Isolation, purification, electron microscopic and serological studies of HgYMV have been reported (Muniyappa et al. 1987). However, the present study is the first report of the molecular characterization of the genome components of HgYMV.

Materials and methods

Source of virus and total DNA extraction

Horsegram plants (Co-1 variety) showing severe mosaic and mottling symptoms were collected from Tamil

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Nadu Agricultural University (TNAU), Coimbatore, India. Total nucleic acid was extracted from the symptomatic leaves by a modified CTAB method (Porebski et al. 1997).

PCR amplification, cloning and sequencing

A pair of degenerate primers RuGEMF1 and RuGEMR1 (Table 1) derived from the conserved region of the coat protein (CP) gene of bipartite begomoviruses was used for amplifying a ~500 bp fragment. The amplified product was cloned in pXcmKn12 (Cha et al. 1993) digested with *Xcm*I to derive pXHCP. The cloned DNA fragment was further characterized by restriction digestion and sequencing. Full-length amplification of DNA-A of HgYMV-[IN:Coi] was achieved by a pair of abutting primers (HgYMVAF and HgYMVAR; Table 1), derived from the sequence of pXHCP clone. Before cloning and sequencing, the viral origin of the PCR product was verified by Southern hybridization using the core region of the coat protein gene as a probe. The amplicon was cloned into pXcmKn12 to derive pXHA and confirmed by restriction digestion and sequencing. Amplification of the full-length DNA-B component of HgYMV-[IN:Coi] was also achieved in two steps. First a ~500 bp fragment was amplified using a set of primers (DNABF and DNABR; Table 1) derived from the sequence of the BC1 open reading frame of legume-infecting begomoviruses (Fig. 1) from South and South-East Asia. The PCR product was cloned into pGEM-T Easy vector (Promega) and sequenced (pGHBC). A pair of abutting primers (HgYMVBF and HgYMVBR; Table 1) was designed from the derived sequence of pGHBC and the full-length DNA-B of HgYMV-[IN:Coi] was amplified. The amplicon was cloned into pTZ57R (MBI Fermentas) and the clone, pTHB was sequenced.

Sequence analysis

Sequences of DNA-A and DNA-B components were independently subjected to FASTA analysis (http://www.ebi.ac.uk) (Table 2). Based on the close sequence identity and the length of the sequences, DNA-A and DNA-B sequences of various begomoviruses were downloaded from Gen Bank with the accession numbers provided by the FASTA output and were fed into ClustalX (Thompson et al. 1997). The multiple sequence alignment was used to derive a phylogenetic tree. Boot strapping was applied to statistically validate the tree using NJ-plot (Perrière and Gouy 1996). The Bioedit program (Hall 2005) was used to construct the maps and also for analyzing sequence identities.

Table 1 Primers used for PCR

Primer	Sequences	Position
RuGEMF1	5′ - GTATATGGCATGTACTCATGC - 3′	CONSERVED REGION OF AV1
RuGEMR1	5′ - TGTGAGGGCCCATGTAAGGTTC - 3′	CONSERVED REGION OF AV1
BgDNABF.	5′ - TTGTAGCCAAGTCTCWC - 3′	CONSERVED REGION OF BC1
BgDNABR	5 ′- ACAAAGAGTTGCGAATAC - 3	CONSERVED REGIONOF BC1
HgYMVAF	5′ - ATCATACTGAGAACGCTTTG - 3′	HgYMVA-(IN:Coi)0965-0984
HgYMVAR	5′ - TGTCATACTTCGCAGCTTC - 3′	HgYMVA-(IN:Coi)0945-0964
HgYMVBF	5′ - AAGAACGACGAAGAGA - 3′	HgYMVB-(IN:Coi)1815-1830
HgYMVBR	5′ - GTGTCGTCCTTGAGGGAA - 3′	HgYMVB-(IN:Coi)1797-1814
HgPTRAF	5′ - ACCTTTGGTTACGTGTGTGG -3′	HgYMVA-(IN:Coi)2386-2405
HgPTRAR	5′ - ACACAGGGTTGCCAAGAAG - 3′	HgYMVA-(IN:Coi)1549-1567
HgPTRBF	5′ - GTCTGTTGAGTGTTTCGCTGA - 3′	HgYMVB-(IN:Coi)1688-1708
HgPTRBR	5′ - AGAAGCACATCCAACGAAC - 3′	HgYMVB-(IN:Coi)0975-0993
HgYMVASq.P.1	5′ - ATCAGGAGAGCGATACAACC - 3′	HgYMVA-(IN:Coi)0473-0492
HgYMVASq.P.2	5′ - TCCTCTAGCCGATCTTCC - 3′	HgYMVA-(IN:Coi)2234-2251

IUPAC code for nucleotide: R,A/G;N,A/C/G/T;H,ACT;W,A/T

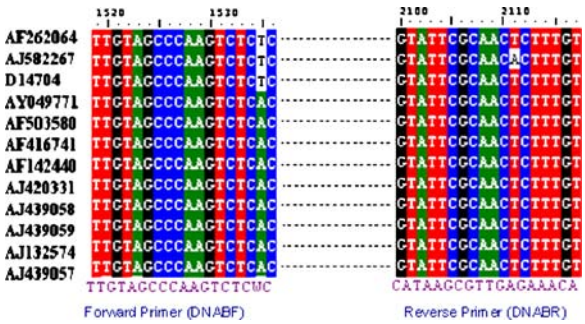


Fig. 1 Alignment showing the conserved BC1 region of begomoviruses used for the design of primers to amplify a ~500 bp from DNA-B of HgYMV

Construction of infectious clones of DNA-A and DNA-B

Restriction maps (Fig. 2) of DNA-A (AJ627904) and DNA-B (AJ627905) were used to construct the partial tandem repeat (PTR) clones of the respective DNA components. A PCR based strategy was adapted for the construction of partial tandem repeats. The full length DNA-A of HgYMV-[IN:Coi], was amplified from pXHA (in order to get an uninterrupted coat protein gene) using a high fidelity DNA Polymerase (Phusion; Finnzymes) that does not add the terminal base 'A'. The PCR amplicon was gel-purified, phosphorylated and self- ligated. The self-ligated full-length DNA-A was used as the template for amplifying a 1.9 kb fragment with the primers (HgPTRAF and HgPTRAR; Table 1). The PCR amplicon, covering the sequence from 2386 to 1567 in the clockwise direction (Fig. 2a) was cloned into pTZ57R to generate pTZPTRA1.9. The insert of pTZPTRA1.9 was released with *Pst*I and *Kpn*I and cloned into the corresponding sites of pOKHA—a sub clone of pXHA. Thus the PTR clone pOKHPTRA4.1 of HgYMV-[IN:Coi] DNA-A was derived. The PTR was released from pOKHPTRA4.1 with *Xba*I and cloned into the corresponding sites of pMON binary vector (Monsanto) to derive pMHPTRA4.1. The correct construct was identified by digestion with *Hpa*I, which released the full-length 2.7 kb DNA-A component.

To construct the PTR of DNA-B of HgYMV-[IN:Coi], the full-length DNA-B was amplified from pTHB (in order to get an uninterrupted movement protein gene) using the DNA polymerase Phusion (Finnzymes). The amplicon was gel-purified, phosphorylated and self-ligated. The self-ligated full-length DNA-B was amplified with primers (HgPTRBF and HgPTRBR; Table 1). The 1.9 kb amplified product covering sequences from 1689 to 993 (Fig. 2b) was cloned into pTZ57R to yield pTHPTRB1.9, from which the insert was released with *Nco*I and *Kpn*I and cloned into pTHB to derive the partial tandem repeat pTHPTRB4.5. The PTRB from the pTHPTRB4.5 was released with *Sma*I and *Sac*I and was cloned into pBin19 (Bevan 1984) to yield pB19HPTRB4.5. The correct construct was identified by digestion with *Bam*HI, which released the full-length 2.7 kb DNA-B component.

Agroinoculation

The binary vectors harboring PTRs of DNA-A (pMHPTRA4.1) and DNA-B (pB19HPTRB4.5) of HgYMV-[IN:Coi] were mobilized into *Agrobacterium tumefaciens* EHA105 (Hood et al. 1993) by triparental mating (Ditta et al. 1980) and the transconjugants were selected on kanamycin (50 µg/ml) and rifampicin (10 µg/ml). Horsegram seeds (Co-1 variety) were surface-sterilized and grown for 2 days on Petri plates lined with cotton soaked in sterile water. Germinated seedlings were pricked at the cotyledonary node and hypocotyl regions. The entire seedlings were soaked for 20 hrs in 1OD culture of *Agrobacterium* mobilized with pMHPTRA4.1 and pB19HPTRB4.5, either separately or mixed together. A hundred seedlings for each category were washed and sown on autoclaved vermiculite. Plants were grown in a growth chamber set at 25ºC temperature, 65% humidity and a photoperiod of 16 h and were analyzed for symptoms at 25 days post inoculation.

Results

Genome organization of DNA-A and DNA-B

Analyses of HgYMV-[IN:Coi] DNA-A and DNA-B sequences showed typical features of bipartite begomoviruses characterized by six conserved open reading frames in DNA-A and two in DNA-B (Fig. 2).

Comparison of the complete DNA-A sequence of HgYMV-[IN:Coi] with other bipartite begomoviruses revealed 84–85% identity (Table 2) with isolates of *Mungbean yellow mosaic virus* (MYMV) and 98–99% identity with the recent accessions of horsegram,

Table 2 Percentage identity of HgYMV with other begomoviruses

Virus name	DNA-A		DNA-B	
HgYMV-[IN:Coi]	AJ627904	100.0	AJ627905	100
HgYMV-[IN:Ban:04]	AM932427	98.6	AM932428	97.0
HgYMV-[IN:Fb:04]	AM932425	98.3	AM932426	97.2
HgYMV-[IN:Lb:04]	AM932429	98.1	AM932430	96.9
MYMV-[IN:Vam:05]	DQ400848	84.5		
MYMV-[IN:Vam:KA28]			AJ439058	72.1
MYMV-[IN:Vam:KA21]			AJ439059	72.1
MYMV-[IN:NamB1:05]			DQ865202	72.2
MYMIV-[IN:ND:Cp:04]			AY939925	73.1
MYMV-[IN:Vam:KA34]			AJ439057	71.6
MYMV-[IN:Mad:Sb]			AJ582267	70.0
MYMV-[IN:Vam:KA27]			AF262064	70.0
MYMV-[IN:NamB2:05]			DQ865203	70.5
MYMV-[IN:Har:01]	AY271896	84.4		
MYMV-[IN:Mah:Sb:99]	AF314530	84.4		
MYMV-[IN:Nam:06]	DQ865201	84.3		
MYMV-[IN:Vig]	AJ132575	84.2	AJ132574	70.8
MYMV-[KH:PP:03]	AY271892	84.4		
MYMV-[IN:Mad:Sb]	AJ421642	84.4	AJ867554	96.6
MYMV-[PK:Isl:Sb:00]	AY269991	84.2		
MYMV-[TH:Mg2]	AB017341	84.8		
MYMIV-[PK:Isl:00]	AY269992	84.7		
MYMIV-[PK:130.7]	AJ512496	82.6		
MYMIV-[PK:130.12]	AJ512497	82.5		
MYMIV-[PK:14]	AJ512495	82.5		
MYMIV-[IN:Kan:Cp:06]	DQ389154	82.4		
MYMIV-[IN:ND:Cp:05]	DQ389153	82.5		
MYMIV-[IN:Jab:SbTN]	AJ416349	82.3	AJ420331	73.1
MYMIV-[PK:Cp:00]	AY269990	82.7		
MYMIV-[IN:Var:Dol]	AY547317	82.7	DQ061273	72.9
MYMIV-[IN:Ako]	AY271893	82.2	AY271894	73.6
MYMIV-[NP:Lal]	AY271895	82.1		
MYMIV-[PK:106	AJ512498	82.3		
MYMIV-[IN:Var:Cp]	AY618902	81.9		
MYMIV-[IN:Pun:05]	DQ400847	81.8		
MYMIV-[BD:98]	AF314145	81.5		
MYMIV-[IN:Sb]	AY049772	82.7	AY049771	73.3
MYMIV-[IN:Cp]	AF481865	82.6	AF503580	73.5
MYMIV-[Mg]	AF416742	82.6	AF416741	73.3
MYMIV-[IN:Bg3]	AF126406	82.1		

limabean and Frenchbean isolates of HgYMV from Bangalore. Table 2 presents the values for begomovirus species with the highest identities. The comparison of individual open reading frames gave similar results (Barnabas 2007). An analysis by Qazi et al. (2007) establishes that legume yellow mosaic viruses are genetically isolated begomoviruses. The phylogenetic tree built using the DNA-A of HgYMV-[IN:Coi]

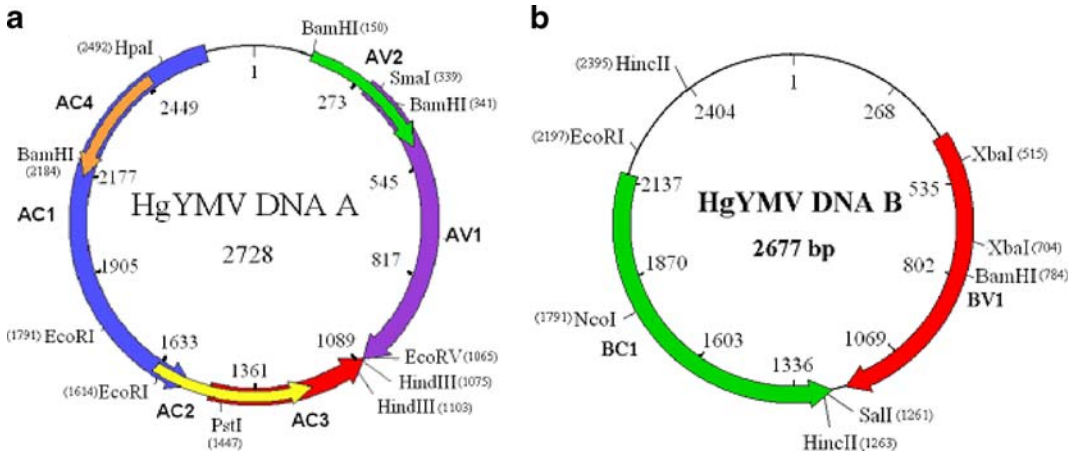


Fig. 2 Maps of the HgYMV genomic DNA-A (**a**) and B (**b**) components with some restriction sites. The open reading frames are defined by bar arrows with the head showing the 3′- terminus. The number denotes the nucleotide position

showed three clusters (Fig. 3a), with HgYMV-[IN:Coi] falling in cluster I, MYMV isolates in cluster II and *Mungbean yellow mosaic India virus* (MYMIV isolates) in cluster III. The multiple sequence alignment based on the *ori* region (the region between and including the repeated putative Rep binding motif and stem–loop sequences (Fontes et al. 1994; Lazarowitz et al. 1992)) of HgYMV-[IN:Coi] DNA-A (Fig. 4) shows that there is a four base deletion between the TATA box and the stem–loop region. Such a feature is found in MYMIV also.

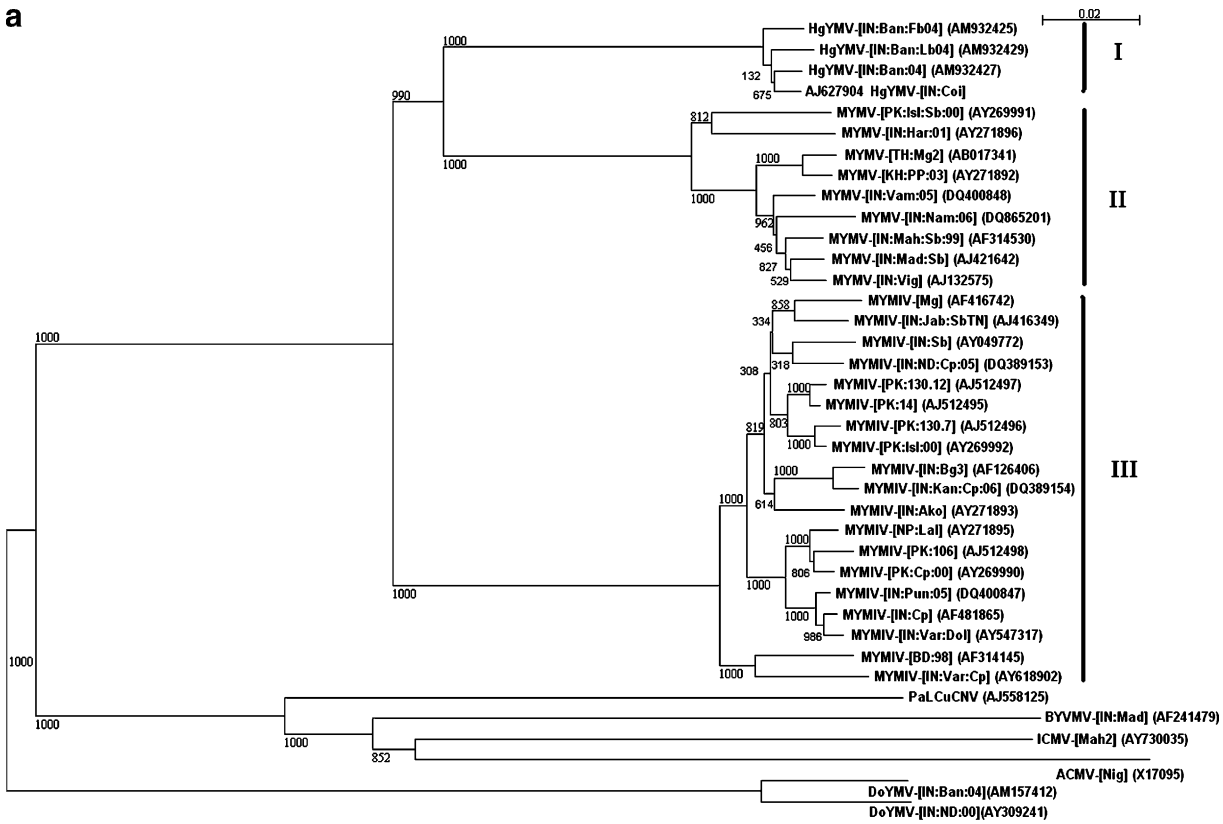
Comparison of the complete DNA-B sequence of HgYMV-[IN:Coi] with other bipartite begomoviruses revealed 97% identity with the recent accessions of HgYMV isolates and also with one DNA-B component of MYMV-[IN:Mad:Sb]. However, the rest of the DNA-B components of isolates of MYMV and MYMIV showed only 70–74% identity with HgYMV (Table 2). The phylogenetic tree (Fig. 3b) based on the DNA-B sequence of begomoviruses showed that all the isolates of HgYMV formed a separate cluster that includes MYMV-[IN:Mad:Sb]. Analysis of the aligned *ori* sequences of DNA-B (Fig. 5) also reflected the uniqueness of all HgYMV with an insertion around position 27–28 and another insertion TAC in the putative stem–loop region. However HgYMV-[IN:Coi] also has some sequence identity with MYMV in the region around position 80 (Fig. 5). In both the genomic components, HgYMV-[IN:Coi] has the iteron GGTGA, the putative Rep binding region, which is different in the chosen and aligned begomoviruses (where it is GGTGT) except in the DNA-A of MYMV-[IN:Har:01] and MYMV-[IN:Nam:06]. In spite of sharing a number of features with MYMIV and MYMV, the *ori* of HgYMV-[IN:Coi] DNA-B has unique sequences particularly at a region between the TATA box and the adjacent Rep binding site and at the stem–loop region, which are however highly conserved between the corresponding regions of the cognate DNA-A.

Agroinoculation of horsegram with the tandem repeat constructs of DNA-A and DNA-B components of HgYMV-[IN:Coi]

When agroinoculation was performed with PTRs of DNA-A and DNA-B together, characteristic yellow mosaic symptoms were observed 25dpi on 27 out of the 100 plants inoculated (Fig. 6). Plants agroinoculated with DNA-A or DNA-B individually did not develop symptoms. Southern analyses (data not shown) of the agroinoculated plants confirmed the presence of the replicated HgYMV-[IN:Coi] genomic DNA components.

Discussion

The first report of the yellow mosaic disease of horsegram was by William et al. (1968). Later preliminary electron microscopic studies revealed geminate particles (Muniyappa et al. 1987) from the infected leaves. Swanson et al (1992) showed that monoclonal antibodies raised against *African cassava mosaic virus*


a
0.02
HgYMV-[IN:Ban:Fb04] (AM932425)
HgYMV-[IN:Ban:Lb04] (AM932429)
HgYMV-[IN:Ban:04] (AM932427)
AJ627904 HgYMV-[IN:Coi]
MYMV-[PK:Isl:Sb:00] (AY269991)
MYMV-[IN:Har:01] (AY271896)
MYMV-[TH:Mg2] (AB017341)
MYMV-[KH:PP:03] (AY271892)
MYMV-[IN:Vam:05] (DQ400848)
MYMV-[IN:Nam:06] (DQ865201)
MYMV-[IN:Mah:Sb:99] (AF314530)
MYMV-[IN:Mad:Sb] (AJ421642)
MYMV-[IN:Vig] (AJ132575)
MYMIV-[Mg] (AF416742)
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MYMIV-[IN:Sb] (AY049772)
MYMIV-[IN:ND:Cp:05] (DQ389153)
MYMIV-[PK:130.12] (AJ512497)
MYMIV-[PK:14] (AJ512495)
MYMIV-[PK:130.7] (AJ512496)
MYMIV-[PK:Isl:00] (AY269992)
MYMIV-[IN:Bg3] (AF126406)
MYMIV-[IN:Kan:Cp:06] (DQ389154)
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MYMIV-[NP:Lal] (AY271895)
MYMIV-[PK:106] (AJ512498)
MYMIV-[PK:Cp:00] (AY269990)
MYMIV-[IN:Pun:05] (DQ400847)
MYMIV-[IN:Cp] (AF481865)
MYMIV-[IN:Var:Dol] (AY547317)
MYMIV-[BD:98] (AF314145)
MYMIV-[IN:Var:Cp] (AY618902)
I
II
III
PaLCuCNV (AJ558125)
BYVMV-[IN:Mad] (AF241479)
ICMV-[Mah2] (AY730035)
ACMV-[Nig] (X17095)
DoYMV-[IN:Ban:04](AM157412)
DoYMV-[IN:ND:00](AY309241)
b
0.02
ICMV-[IN:Mah2:88] (AY730036)
ToLCNDV-IN[IN:HD:Sw:92] (U15017)
MYMIV-[IN:Jab:SbTN](AJ420331)
MYMIV-[IN:Ako] (AY271894)
MYMIV-[Mg] (AF416741)
MYMIV-[IN:ND:Cp:04] (AY939925)
MYMIV-[IN:Sb] (AY049771)
MYMIV-[IN:Var:Dol] (DQ061273)
MYMIV-[IN:Cp] (AF503580)
MYMV-[IN:NamB1:05] (DQ865202)
MYMV-[IN:Vam:KA21] (AJ439059)
MYMV-[IN:Vam:KA28] (AJ439058)
MYMV-[IN:Vig] (AJ132574)
MYMV-[IN:Vam:KA34] (AJ439057)
MYMV-[IN:NamB2:05] DQ865203
MYMV-[IN:Vam:KA27] (AF262064)
MYMV-[IN:Mad:Sb] (AJ582267)
MYMV-[IN:Mad:Sb] (AJ867554)
HgYMV-[IN:Ban:Lb04] (AM932430)
HgYMV-[IN:Ban:Fb04] (AM932426)
HgYMV-[IN:Ban:04] (AM932428)
HgYMV-[IN:Coi] (AJ627905)
ACMV-[Nig] (X17096)

Fig. 3 Phylogenetic tree obtained from aligned DNA-A sequences **(a)**, and DNA-B sequences **(b).** The dendrograms are calculated using neighbor-joining and bootstrap (1000 replications) options of CLUSTALX 1.8 version and NJ Plot. The numbers at the nodes indicate the number of times in which the given branch is supported. Vertical distances are arbitrary. Horizontal distances reflect number of nucleotide differences between branch nodes. Accession numbers of the Begomoviruses used for the analyses are given as follows :- **DNA-A and DNA-B:** Horsegram yellow mosaic virus—[India:Coimbatore](HgYMV-[IN:Coi]) AJ627904 & AJ627905; Horsegram yellow mosaic virus—[India:Bangalore: Frenchbean 04] (HgYMV-[IN:Ban:Fb04]) AM932425& AM932426; Horsegram yellow mosaic virus—[India:Bangalore 04] (HgYMV-[IN:Ban:04]) AM932427& AM932428; Horsegram yellow mosaic virus—[India:Bangalore:Limabean 04] (HgYMV-[IN:Ban:Lb04]) AM932429& AM932430; Mungbean yellow mosaic virus—[India:Vigna] (MYMV-[IN:Vig]) AJ132575 and AJ132574; Mungbean yellow mosaic India virus—[India:Jabalpur:Soybean TN] (MYMIV-[IN:Jab:SbTN]) AJ416349 & AJ420331; Mungbean yellow mosaic India virus—[India:Akola] (MYMIV-[IN:Ako]) AY271893 & AY271894; Mungbean yellow mosaic India virus—[India:Soybean] (MYMIV-[IN:Sb]) AY049772 & AY049771; Mungbean yellow mosaic India virus—[India:Cowpea] (MYMIV-[IN:Cp]) AF481865 & AF503580; Mungbean yellow mosaic India virus—[Mungbean] (MYMIV-[Mg]) AF416742 & AF416741; African cassava mosaic virus-[Nigeria] (ACMV-[Nig]) X17095 & X17096: Indian cassava mosaic virus isolate Mah-2 (ICMV-[IN:Mah2:88]) AY730035 & AY730036 ; Dolichos yellow mosaic virus (DoYMV) AM157412& AY309241. **DNA-A**: Mungbean yellow mosaic India virus—[India:Blackgram 3] (MYMIV-[IN:Bg3]) AF126406; Mungbean yellow mosaic virus—[India:Madurai:Soybean] (MYMV-[IN:Mad:Sb]) AJ421642; Mungbean yellow mosaic virus [India:Maharashstra: Soybean: 1999] (MYMV-[IN:Mah:Sb:99]) AF314530; Mungbean yellow mosaic virus—[Pakistan:Islamabad:Soybean:2000] (MYMV-[PK:Isl:Sb:00]) AY269991; Mungbean yellow mosaic virus—[Thailand:Mungbean 2] (MYMV-[TH:Mg2]) AB017341; Mungbean yellow mosaic India virus—[Pakistan: Islamabad:2000] (MYMIV-[PK:Isl:00]) AY269992; Mungbean yellow mosaic India virus—[Pakistan:130.7] (MYMIV-[PK:130.7]) AJ512496; Mungbean yellow mosaic India virus—[Pakistan:14(MYMIV-[PK:14])] AJ512495; Mungbean yellow mosaic India virus—[Pakistan:130.12] (MYMIV-[PK:130.12]) AJ512497; Mungbean yellow mosaic India virus—[Pakistan: Cowpea:2000] (MYMIV-[PK:Cp:00]) AY269990; Mungbean yellow mosaic India virus—[India: Varanasi:Dolichos] (MYMIV-[IN:Var:Dol]) AY547317 & DQ061273 ; Mungbean yellow mosaic India virus—[Nepal: Lalitpur] (MYMIV-[NP:Lal]) AY271895 ; Mungbean yellow mosaic India virus—[Pakistan:106] (MYMIV-[PK:106]) AJ512498; Mungbean yellow mosaic India virus—[Bangladesh:1998] (MYMIV-[BD:98]) AF314145; Mungbean yellow mosaic virus—[India:Haryana:2001] (MYMV-[IN:Har:01]) AY271896; Mungbean yellow mosaic India virus –[New Delhi:Cowpea] (MYMIV-[IN:ND:Cp:05]) DQ389153; Mungbean yellow mosaic virus-[Cambodia: Phnom Penh] MYMV-[KH:PP:03] AY271892;Mungbean yellow mosaic virus-[India : Vamban:05](MYMV-[IN:Vam:05]) DQ400848; Mungbean yellow mosaic virus-[India:Namakkal:mothbean:06](MYMV-[IN: Nam:06]) DQ865201; Mungbean yellow mosaic India virus –[India:Punjab:05] (MYMIV-[IN:Pun:05])DQ400847; Mungbean yellow mosaic India virus—[India:[Kanpour:Cowpea] (MYMIV-[IN:Kan:Cp:06]) DQ389154;Mungbean yellow mosaic India virus—[India:Varanasi:Cowpea](MYMIV-[IN: Var:Cp]) AY618902; Bhendi yellow vein mosaic virus-[Madurai] (BYVMV-[In:Mad] AF241479; Papaya leaf curl China virus (PaLCuCNV)AJ558125; Tomato leaf curl New Delhi virus-Severe (ToLCNDV-IN[IN:ND:Svr:92]) U15017. **DNA-B:** Mungbean yellow mosaic virus—[India:Vamban: KA28] (MYMV-[IN:Vam:KA28]) AJ439058; Mungbean yellow mosaic virus—[India:Vamban:KA21] (MYMV-[IN:Vam: KA21]) AJ439059; Mungbean yellow mosaic virus—[India: Vamban:KA34] (MYMV-[IN:Vam:KA34]) AJ439057; Mungbean yellow mosaic virus—[India: Madurai:Soybean] (MYMV-[IN:Mad:Sb]) AJ582267; Mungbean yellow mosaic virus—[India:Vamban:KA27] (MYMV-[IN:Vam:KA27]) AF262064; Mungbean yellow mosaic India virus—[New Delhi:Cowpea] (MYMIV-[IN:ND:Cp:04]) AY939925; Mungbean yellow mosaic virus—[India:Namakkal:Mothbean:DNA-B2] (MYMV-[IN: NamB2:05]) DQ865203

reacted with extracts from yellow mosaic diseased horsegram leaves, indicating the geminivirus origin of the disease. However the present work describes the first molecular characterization of the genomic components of HgYMV-[IN:Coi]. Currently there are at least three sequences available that show a close identity of 98% with HgYMV-[IN:Coi]. Agroinfection of horsegram with the DNA-A and DNA-B infectious clones produced the typical yellow mosaic disease symptoms, indicating that both these components are essential for disease induction. Koch's postulate was thus fulfilled.

Phylogenetic analyses (Fig. 3a) clearly indicate that DNA-A of HgYMV-[IN:Coi], HgYMV-[IN: Ban:04]), (HgYMV-[IN:Ban:Fb04]) and (HgYMV-[IN:Ban:Lb04]), though originating from Coimbatore and Bangalore in southern India, shows close relationship with MYMV infecting *Vigna radiata* of Haryana and soybean of Maharashtra and Pakistan, which are geographically distant. It had been established that o*ri* is a hot-spot for recombination (Stanley 1995). HgYMV-[IN:Coi] with the members of its group shares homology at the *ori* region with various strains of MYMIV infecting mungbean of Pakistan, Bangladesh, Nepal and Akola (in India), cowpea of Pakistan and soybean of Jabalpur (in India) and Pakistan (Fig. 4). Nevertheless it shows a close overall sequence identity even with the Thailand isolate of MYMV infecting mungbean (Table 2). Although the *ori* region of DNA-

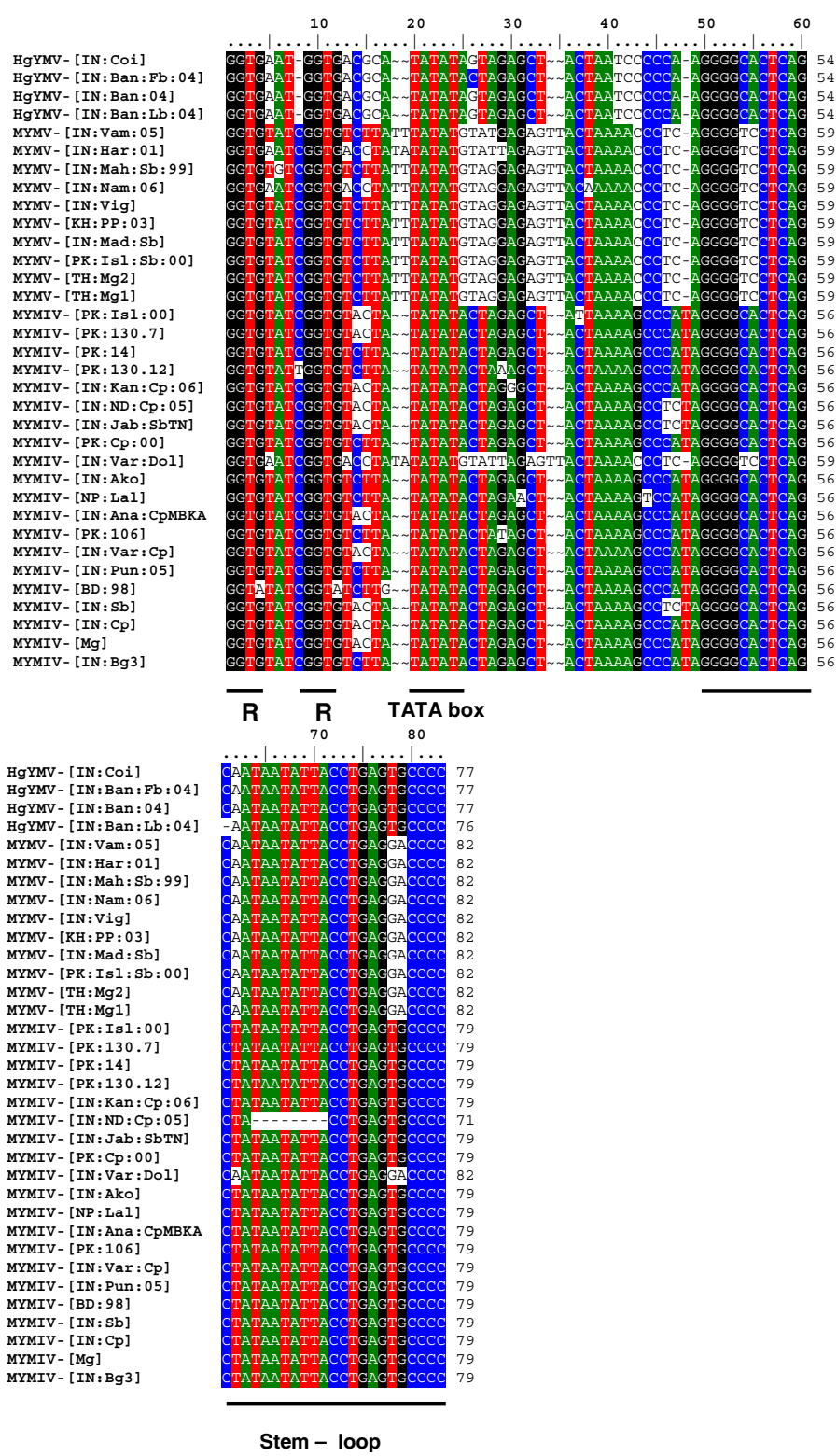

Fig. 4 Multiple alignment of *Ori* sequences from DNA-A of various isolates of legume infecting begomoviruses. The common region is underlined. Putative rep binding motif is R. TATA box and common stem—loop regions are underlined

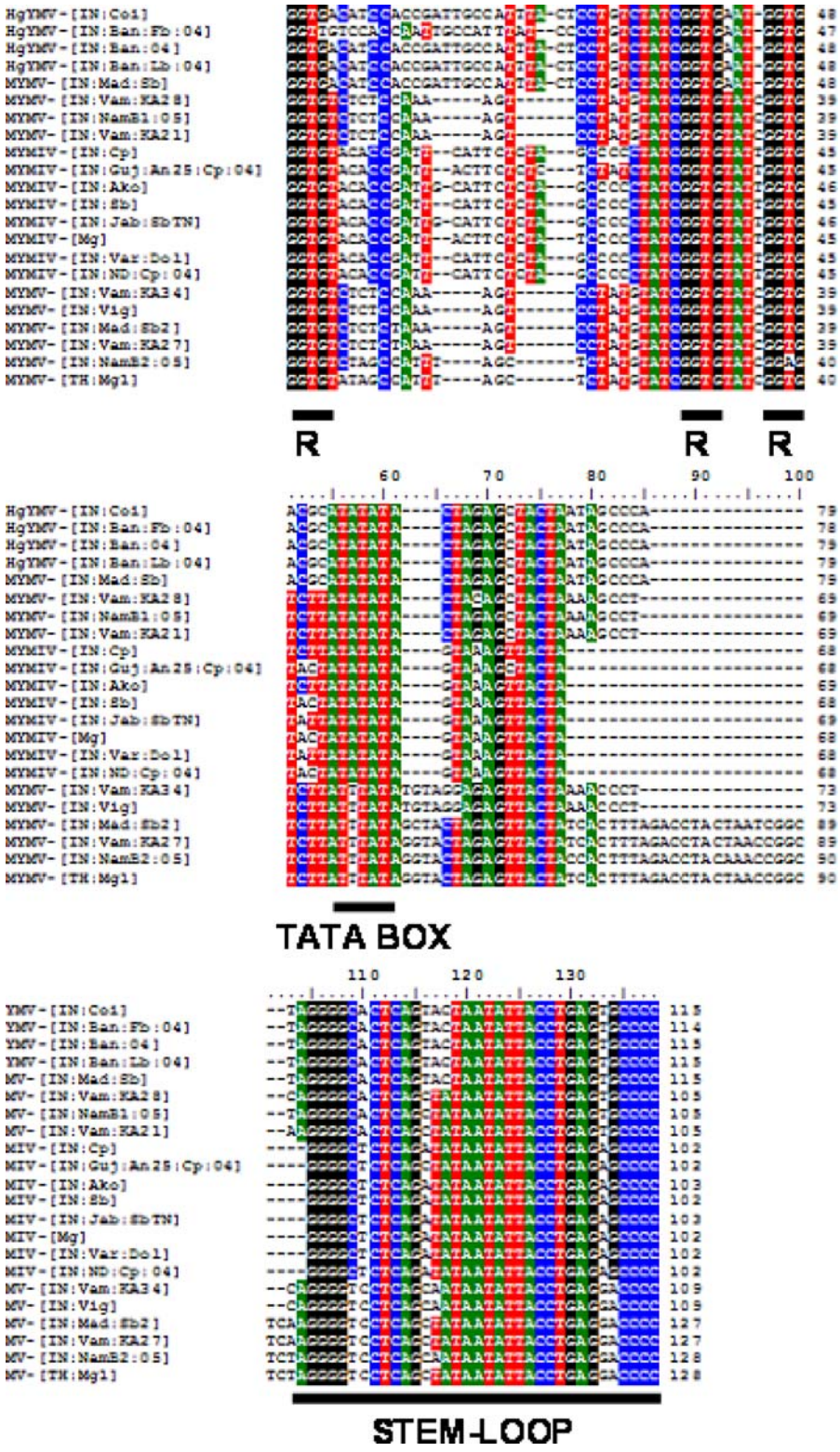

Fig. 5 Multiple alignment of *Ori* sequences from DNA-B of various isolates of legume infecting begomoviruses. The common region is underlined. Putative rep binding motif is R. TATA box and common stem—loop regions are underlined

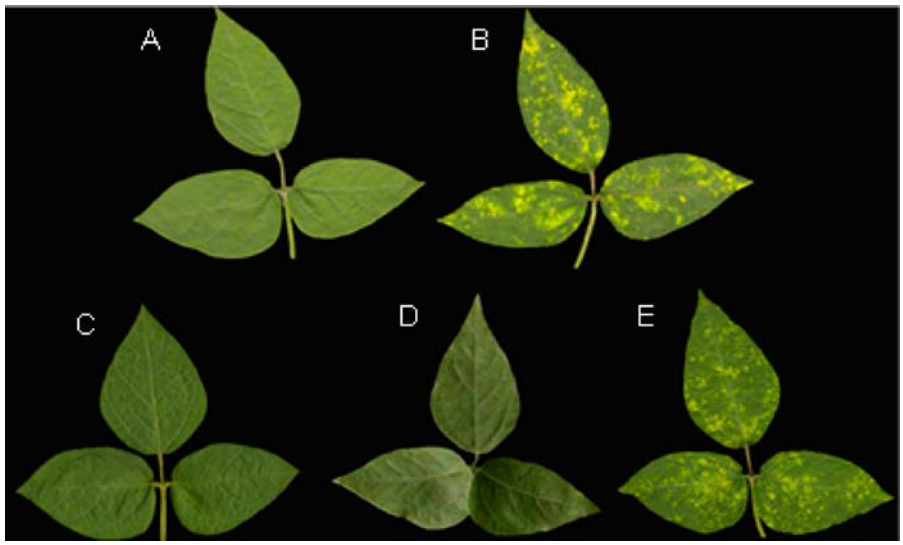

Fig. 6 Agroinoculation of Horsegram with DNA-A and DNA-B of HgYMV—[IN:Coi]. Trifoliate leaves are from healthy plants (A), A naturally infected plant (B) and plant inoculated with either DNA-A (C), DNA-B (D) or DNA-A and DNA-B together (E)

A (Fig. 4) shares many common features with that of MYMV and MYMIV, the full-length DNA-A forms a separate clade (Fig. 3a). It is likely that the differences in the common region sequences of these viruses could reflect mutations introduced during the course of evolution. The presence of one of the DNA-Bs of MYMV-[IN:Mad:Sb] in the cluster along with HgYMV DNA-Bs could be an event of pseudorecombination as suggested by Qazi et al. (2007).

Sequence analyses of the DNA-B *ori* region revealed (Fig. 5) that there is a divergence due to nucleotide substitution, deletion and insertion. There is a high degree of identity in the common region (CR) between DNA-A and DNA-B of HgYMV-[IN:Coi] as against other yellow mosaic viruses of mungbean (Girish and Usha 2005).

Comparison of the complete DNA-A and DNA-B sequences of HgYMV-[IN:Coi] with other bipartite begomoviruses revealed identities of 84–85% and 70–74% respectively in the databases at the time of the submission of the viral sequences. Full-length DNA-A sequence analyses of more than 200 viruses have led to a guideline to identify a begomovirus species (Fauquet et al. 2003). According to this stricture, a species should have less than 89% full-length sequence identity with the DNA-A of other begomoviruses to have a status of a species. Comparative epitope profile studies show that HgYMV falls into the group of isolates from black-gram, cowpea, Frenchbean, pigeonpea, soybean, *Indigofera hirsuta* and isolates from mungbean (Swanson et al. 1992). The present study confirms that *Horsegram yellow mosaic virus* (HgYMV-[IN:Coi]) belongs to the group of yellow mosaic virus of legumes and that HgYMV represents a distinct begomovirus species.

Acknowledgements We acknowledge funding from Department of Biotechnology, Government of India. ADB thanks University Grants Commission for FIP fellowship and GKR thanks Council of Scientific and Industrial Research for SRF. We are grateful to Tamil Nadu Agricultural University for the horsegram samples and Dr. V. G. Malathi for EHA105. We thank School of Biotechnology, Madurai Kamaraj University for the facilities.

References

Barnabas, A. D. (2007). Molecular characterization of Horsegram yellow mosaic virus and efforts towards the development of transgenic soybean expressing truncated replicase gene of Soybean yellow mosaic virus. PhD thesis submitted to Madurai Kamaraj University.

Bevan, M. (1984). Binary Agrobacterium vectors for plant transformation. *Nucleic Acid Research, 12*, 8711–8721.

Cha, J., Bishai, W., & Chandrasegaran, S. (1993). New vectors for direct cloning of PCR products. *Gene, 136*, 369–370.

Ditta, G., Stanfield, S., Corbin, D., & Helinski, D. R. (1980). Broad host range cloning system for Gram-negative bacteria: construction of a gene bank of *Rhizobium meliloti*. *Proceedings of National Acadamy of Sciences, 77*, 7347–7351.

Fauquet, C. M., Bisaro, D. M., Briddon, R. W., Brown, J. K., Harrison, B. D., Rybicki, E. P., et al. (2003). Revision of taxonomic criteria for species demarcation in the family *Geminiviridae* and an updated list of begomovirus species. *Archives of Virology, 148*, 405–421.

Fauquet, C. M., Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., et al. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology, 153*, 783–821.

Fontes, E. P. B., Gladfelter, H. J., Schaffer, R. L., Petty, I. T. D., & Hanley-Bowdoin, L. (1994). Geminivirus replications have a modular organization. *The Plant Cell, 6*, 405–416.

Girish, K. R. & Usha, R. (2005). Molecular characterization of two soybean-infecting begomovirus from South-East Asia. *Virus Research, 108*, 167–176.

Government of T. N. (2006). Department of Economics and Statistics, Seasons and Crop Report 2005–2006. Retrieved September 09.2009, from http://www.tn.gov.in/crop/AreaProduction.htm.

Hall, T. (2005). http://www.mbio.ncsu.edu/RNaseP/info/programs/BIOEDIT/bioedit.html.

Hood, E. E., Gelvin, S. B., Melchers, L. S., & Hoekema, A. (1993). New *Agrobacterium* helper plasmid for gene transfer to plants. *Transgenic Research, 2*, 208–218.

Jayan, P. K. & Maya, C. N. (2001). Studies on the germplasm collection of Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.). *Indian Journal of Plant Genetic Resources, 14*, 43–47.

Lazarowitz, S. G., Wu, L. C., Rogers, S. G., & Elmer, J. S. (1992). Sequence specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. *The Plant Cell, 4*, 799–809.

Muniyappa, V., Reddy, H. R., & Shivashankar, G. (1976). Studies on the yellow mosaic disease of horsegram (Dolichos biflorus Linn.). II.Host range studies. *Mysore. Journal of Agricultural Science, 10*, 610–614.

Muniyappa, V., Reddy, H. R., & Mustak Ali, T. M. (1978). Studies on the Yellow Mosaic disease of horsegram *(Dolichos biflorus*), IV.Epidemeology of disease. *Mysore Journal of Agricultural Science, 12*, 277–279.

Muniyappa, V., Rajeshweri, R., Bharathan, N., Reddy, D. V. R., & Nolt, B. L. (1987). Isolation and characterization of a geminivirus causing yellow mosaic disease of Horsegram (*Macrotyloma uniflorum*(Lam.)Verdc.) in India. *Indian. Journal of Phytopathology, 119*, 81–87.

Perrière, G. & Gouy, M. (1996). WWW-Query: an on-line retrieval system for biological sequence banks. *Biochimie, 78*, 364–369.

Porebski, S., Bailey, L. G., & Baum, B. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter, 15*, 8–15.

Qazi, J., Ilyas, M., Mansoor, S., & Briddon, R. W. (2007). Legume yellow mosaic viruses: genetically isolated begomoviruses. *Molecular Plant Pathology, 8*, 343–348.

Sharma, K. R. (1995). Nature of variation and association among grain yield and yield components in horsegram. *Crop improvement, 22*, 73–76.

Stanley, J. (1995). Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. *Virology, 206*, 707–12.

Swanson, M. M., Varma, A., Muniyappa, V., & Harrison, B. D. (1992). Comparative epitope profiles of the particle proteins of whitefly-transmitted geminiviruses from nine crop legumes in India. *Annals of applied Biology, 120*, 425–433.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research, 24*, 4876–4882.

William, F. J., Grewal, J. S., & Amin, K. S. (1968). Serious and new disease of pulse crops in India in 1966. *Plant Disease Reporter, 52*, 300–304.