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Full Length Research Paper

Characterization of Sunn hemp begomovirus and its geographical origin based on *in silico* structural and functional analysis of recombinant coat protein

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Sequence alignment of the 897 bp amplicon obtained from a diseased sunn hemp (*Crotalaria juncea* L.) plant DNA revealed a complete 771 bp coat protein (CP) gene flanked by 3' regions of the AV2 and AC3 genes. Southern hybridization using (α-32P) dCTP labeled (CP) gene probe of Indian tomato leaf curl virus (IToLCV) demonstrated the association of begomovirus with the leaf curl disease of sunn hemp. Phylogenetic data suggested that, the AV2, CP and AC3 genes have closest genetic relationship with begomovirus isolates from India, China and Bangladesh, respectively. *In silico* recombination analysis elucidated a 297 nucleotides hot spot (346 to 643 nucleotides) within AV2 overlapping region of CP gene, amenable to genetic rearrangements, with lineage from tomato leaf curl virus Bangalore (ToLCuVB) and Indian cassava mosaic virus-Ind (ICMV) as major and minor parents, respectively. Thus, it is concluded that the recombinant CP genes related to begomoviruses are evolved from the Indian isolates, causing broad host specificity and molecular diversity among the related begomoviruses across the geographical limits of Southeast Asia.

Key words: *Begomovirus*, sunn hemp, coat protein, recombination, phylogenetic analysis*, in silico* analysis.

INTRODUCTION

Sunn hemp (*Crotalaria juncea* L.) is a sub-tropical annual legume that belongs to the family of *Fabaceae*. It is regarded as a renewable fiber and pulp crop with the potential of biological nitrogen fixation, drought tolerance and management of weeds and nematodes (Cook and White, 1996; Bhardwaj et al., 2005). This crop is susceptible to different pathogens including several viruses such as Cowpea mosaic virus (Lister and Thresh, 1955), Tobacco mosaic virus, Crotalaria mosaic virus (Kassanis and Varma, 1975), Sunn hemp rosette virus (Verma and Awasthi, 1976) and Cowpea mosaic virus (Ladipo, 1988). Among the major diseases of sunn hemp, the leaf curl disease is the more serious problem with economic implications. Limited studies are reported on the etiology of leaf curl disease and its association with begomovirus as a causative agent (Khan et al., 2002; Raj et al., 2003). The genus Begomovirus (family *Geminiviridae*) typically have bipartite, circular single-stranded DNA (ssDNA) genomes with all functions required for virus replication, control of gene expression and encapsidation encoded on DNA-A and genes involved in intra and inter-cellular movement encoded on DNA-B (Bowdoin et al., 1999).

Begomoviruses exhibit a great deal of geographic dependent but host independent genomic variations (Padidam et al., 1995; Harrison and Robinson, 1999). A key contributor to the genomic diversification and evolution of begomoviruses is the inter-specific homologous recombination (Zhou, et al., 1997; Harrison and Robinson, 1999; Padidam et al., 1999; Sanz, et al., 1999). Consequently, the detailed characterization of recombination amongst the sunn hemp infecting and

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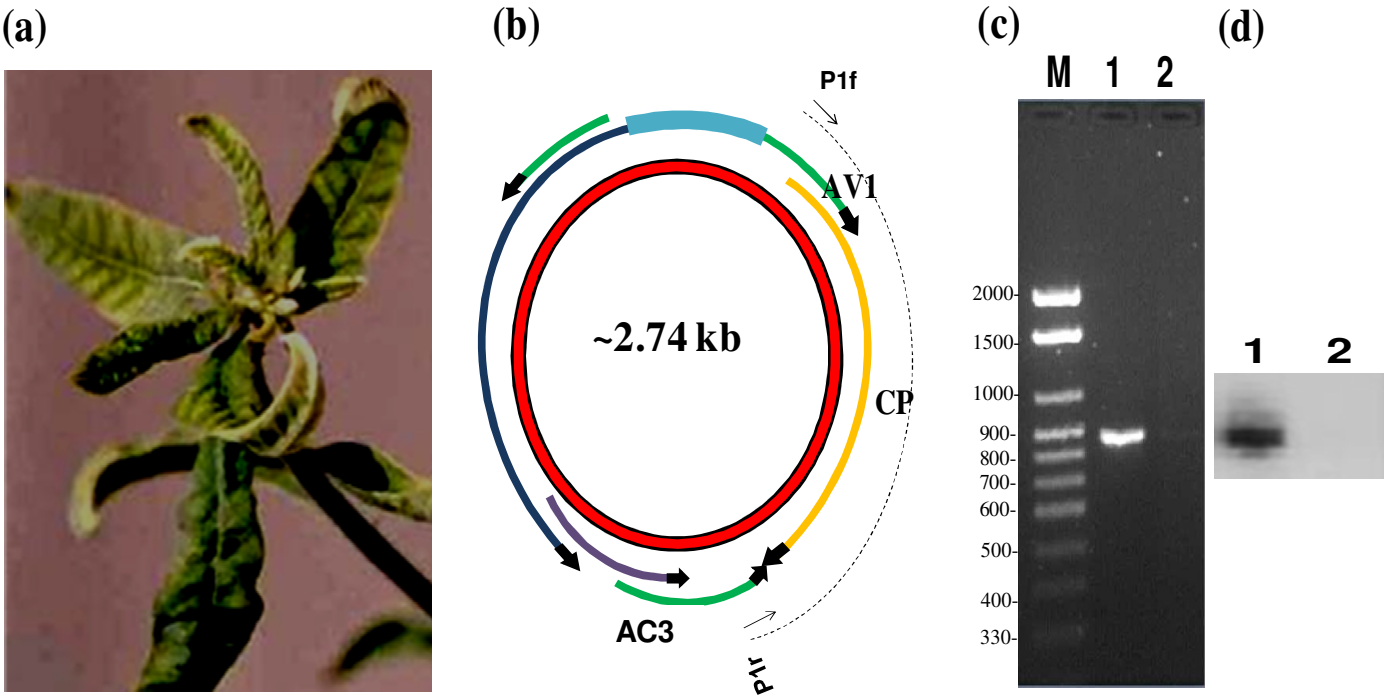

Figure 1. Morphological and molecular characterization of symptomatic sunn hemp plant: Panel a, depicts the naturally infected Sunn hemp leaf with disease symptoms; Panel b, genome organization of the Sunn hemp begomovirus; Panel c, PCR based detection of causative agent of sunn hemp leaf curl disease using the specific primers. Lane M, Molecular DNA ladder (2 kb); Lane 1, diseased sunn hemp leaf tested for begomovirus isolate; Lane 2, healthy (asymptomatic) plant; Panel d, southern hybridization of amplified DNA fragment containing complete CP and partial AV2 and AC3 genes from infected leaf, probed with (α-32P)dCTP labeled CP gene of Indian tomato leaf curl virus (IToLCV).

related begomoviruses is important for understanding as to how these important pathogens are evolving. The specific recombination events including the recombination breaks and hot spots have not been reported so far in sunn hemp-infecting begomoviruses. It is also currently unknown as to whether the sequences in particular parts of the begomovirus genomes are exchangeable between different species and/or members of the same genus from different geographical locations. Also, the Southeast Asian begomovirus diversity is so sparse that we do not yet fully appreciate the geographical range of many of the isolates. Therefore, molecular characterization and understanding of the genomic analysis of the sunn hemp-infecting begomovirus is imperative for the pathogen diagnosis and disease management.

MATERIALS AND METHODS

Plant DNA isolation and polymerase chain reaction (PCR) amplification

Total genomic DNA was extracted from leaf tissues of sunn hemp plant infected with leaf curl disease using the procedure of Bendahmane et al. (1995). The purified genomic DNA was used as a template for PCR amplification using the self-designed primers P1-f 5' GGTCACGATTTAATGAGGGA 3' and P1-r 5' GTTGATCA TGTATTGTTTGATGTA 3' corresponding to the 5' region of AV2 and 3' region of AC3 genes, respectively (Figure 1). Amplification was performed in a total of 50 µl PCR mixture contained 0.20 mM dNTPs, 25 pmole primers, 5 µl 10X buffer, 2.0 mM $MgCl_2$ and 1.5U *Taq* DNA polymerase with the following parameters: 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1min with an initial denaturation and final extension 5 and 10 min at 94 and 72°C, respectively. An aliquot of 5 µl of PCR product was analysed by electrophoresis in 1.5% agarose gel containing ethidium bromide and documented on universal hood II gel documentation system (Bio-Rad Laboratories Inc. CA, USA).

Southern hybridization

The specificity of amplicon obtained from diseased sunn hemp leaves was ascertained by Southern hybridization. The amplicon was transferred to Hybond N membrane (Amersham Biosciences, USA) following the capillary method (Sambrook et al., 1989). The membrane was subjected to UV crosses linking. The CP gene specific (α-32P) dCTP labeled probe was prepared from cloned CP gene of Indian tomato leaf curl virus (IToLCV) (Srivastava et al., 1995) following the random priming extension method (Feinberg and Vogelstein, 1983), using random primer labeling kit (Genie, Bangalore, India), which also cross hybridize with the amplicon under high stringency conditions. The membrane was subjected to prehybridization at 42°C for 1 h following hybridization with the radio-labeled probe (0.5 x 10^6 dpm/ml) at 65°C for overnight in hybridization oven. The membrane was washed twice in 2X SSC, 0.1% SDS and 1X SSC, 0.1% SDS for 5 and 15 min, respectively followed by another wash in 1 X SSC, 0.1% SDS for 15 min. All the

washing steps were carried out at room temperature. Autoradiography was performed using Fuji X-ray film on Fluor-S ™ Multi-Imager (Bio-Rad Laboratories Inc. CA, USA).

Cloning and sequencing

The amplicon obtained from the symptomatic leaf was purified using QIAEX II agarose gel extraction kit (Qiagen Inc., USA) and sub-cloned into pUC 19 vector at *Sma* I site using SureClone ligation kit (Amersham Biosciences, USA). The recombinant vector was transfected into *Escherichia coli* strain DH5α cells and plated on ampicillin (100 µg/ml) supplemented Luria-Bertani agar plates containing X-gal (5-bromo 4-chloro-3 indolyl-β-D-galactoside) and IPTG (isopropyl β-D-thiogalactopyranoside). The white colonies were picked up from the master plates and sub-cultured in presence of ampicillin for purification of recombinant plasmid DNA using the miniprep method of Birnboim and Doly (1979). The gene insert in the recombinant plasmid of the clones was confirmed by the PCR and dot blot hybridization. The recombinant plasmid was denatured in boiling water bath for 5 min and immediately chilled on ice. An aliquot of 1 µl was spotted on Hybond N membrane (Amersham Biosciences, USA) and probed with (α-32P) dCTP labeled homologous probe prepared from the amplicon obtained from the symptomatic leaves. The randomly selected three positive clones were sequenced using an automated ABI-Prism 377 DNA sequencer (Applied Biosystems, CA, USA). The sequence has been deposited in NCBI GenBank Accession No. EF119337 as *C. juncea* begomovirus coat protein (CP) gene.

Multiple sequence alignments and phylogenetic analysis

The DNA sequence obtained was compared with the sequences of other begomovirus isolates in GenBank using Blastn (Altschul et al., 1997). Multiple sequence analysis was performed by using CLUSTALW with default parameters (Thompston et al., 1994). The phylogenetic trees were constructed by the Neighbour-Joining (NJ) method (Saitou and Nei, 1987) with nucleotide pair-wise genetic distances corrected by Kimura two-parameter method (Kimura, 1980) using TreeCon tool. The reliability of tree topologies were subjected to bootstrap tests and numbers at nodes indicate bootstrap support values as a percentage of 1000 replications. All branches with <60% bootstrap support were judged as inconclusive and were collapsed and branch lengths for all trees were normalized to 0.02% divergence.

In silico recombination analysis of CP

Recombination analysis was performed using Recombination Detection Program version 3.0 (RDP3Beta42) on Windows operating system (Martin et al., 2005), which detects and analyzes recombination points in a set of aligned DNA sequence. The begomovirus isolates specified in Table 1 were subjected to recombination analyses considering the complete CP and partial AV2 and AC3 genes using Chimaera method. Analysis was allowed by employing Bonferroni correction method with confidence greater than 95% (*P* value 0.05).

RESULTS AND DISCUSSION

In this study, a begomovirus causing leaf curl disease in sunn hemp plants was investigated and characterized using molecular and computational approaches. Earlier investigations based on PCR and hybridization studies have indicated the associations of begomovirus with leaf curl disease in sunn hemp plants (Khan et al., 2002; Raj et al., 2003). However, the molecular characterization of the virus and its *in silico* genomic analysis based on the CP gene/protein homology, phylogenetic analysis, mapping of recombination sites and its significance in diversity and evolution of the begomovirus have not been systematically explored. This has prompted us to perform a comprehensive study in order to ascertain the genetic characteristics, origin and explicit role of begomovirus in causing the leaf curl disease in sunn hemp plants. The symptoms of leaf curl disease in a representative sunn hemp plant are shown in Figure 1a. The PCR amplification of DNA from the infected leaves resulted in a 897 bp amplicon consisting of partial AV2 and AV3 genes (conserved motifs) flanking the complete CP gene of begomovirus, as depicted in Figures 1b and c. The DNA from normal uninfected leaves has not shown the amplification of any region under identical conditions. The amplicons was subjected to Southern hybridization using the (α-32P)dCTP radio-labeled probe prepared from a cloned CP gene of Indian tomato leaf curl virus (IToLCV) (Srivastava et al., 1995) (Figure 1d), which has unequivocally suggested the association of the begomovirus with the disease.

In order to establish the sequence homology of the isolate, the amplicon was cloned and sequenced. The sequence of the amplicons in Figure 2 revealed a complete CP region of 771 nucleotides and the partial AV2 and AC3 overlapping gene fragments of 246 and 81 nucleotides, respectively. The complete CP and the partial AV2 and AC3 genes encode for 256, 81 and 26 amino acids, respectively. The nucleotides and amino acids sequence analyses of the CP and partial AV2 and AC3 genes exhibited highest level of sequence similarity with the reference begomovirus isolates reported from China, Bangladesh, and India, respectively (Table 1). This corroborates well with the earlier report suggesting that the viruses originating from the China and Indian subcontinent are more closely related to each other (Prasanna and Rai, 2007). Based on the phylogenetic analysis the nucleotide sequence of CP gene showed closest genetic relatedness with Tobacco curly shoot virus (GU199584) (Figure 3), whereas the AV2 gene exhibited close relationship with tomato leaf curl virus Bangalore (U38239) and tomato leaf curl Karnataka virus (AY754812) (Figure 4). However, the AC3 gene showed the close relationship with the tomato leaf curl Bangladesh virus (AF188481) with a low bootstrap value (Figure 5). The phylogenetic data suggested that, the CP, AV2 and AC3 genes of sunn hemp begomovirus belong to different geographic regions and the begomovirus isolates originating from the China, Bangladesh and India are more closely related to each other. The nucleotide sequences of these genes have some scattered substitutions, which do not significantly influence the

Table 1. Nucleotide sequence percent homology of CP, AV2 and AC3 genes of sunn hemp infecting begomovirus with the reference isolates.

Begomoviruse	Acronym	Accession numbers	CP	AV2	AC3
Tobacco curly shoot virus	**TCSV**	U199584	**96.64**	98.35	92.59
Ageratum enation virus Gorakhpur	AEV	GQ268327	96.11	98.35	93.82
Tobacco curly shoot virus [Y35]	ToCSV	AJ420318	95.86	97.94	92.59
Tobacco curly shoot virus-[Y282]	ToCSV	AJ971266	95.86	97.53	92.59
Tomato leaf curl Bangladesh virus	**ToLcBV**	AF188481	94.57	97.15	**95.06**
Euphorbia leaf curl virus	EuLCV	EU194914	94.83	97.54	88.88
Papaya leaf curl virus	PaLCV	AJ436992	94.56	96.28	88.88
Papaya leaf curl virus	PaLCV	FM955601	93.14	95.86	87.65
Cotton leaf curl Burewala virus	CLCuBV	AM421522	94.17	95.52	90.12
Chilli leaf curl India virus	ChLCuIV	FM877858	90.33	94.21	87.65
Tomato leaf curl Karnataka virus	ToLCuKV	FJ514798	89.44	93.08	87.65
Pedilathus leaf curl virus-Mul	PLCuV	AM712436	90.07	94.02	86.41
Tomato leaf curl Pakistan virus	ToLCPV	FM164938	89.80	94.62	86.41
Tomato leaf curl Karnataka virus	**ToLCKV**	AY754812	89.41	**98.37**	88.88
Tomato leaf curl virus Bangalore	ToLCuVB	U38239	87.51	97.96	87.65
Pepper leaf curl Bangladesh virus	PeLCuBV	AM691745	84.91	94.21	70.37
Pepper leaf curl virus- Varanasi	PeLCV	EF190217	85.49	92.14	62.97
Papaya leaf curl virus	PaLCV	DQ989326	85.47	91.32	70.66
Tomato leaf curl Bangalore virus (Kolar)	ToLCBV	AF428255	81.46	76.44	86.41
Tomato leaf curl Bangalore virus (Ban-5)	ToLCBV	AF295401	81.33	76.03	86.41
Indian cassava mosaic virus-Ind	ICMV	AJ314739	82.15	88.22	72.23
Cotton leaf curl Multan virus	CLCuMV	AJ132430	80.84	80.65	75.30
Bhendi yellow vein mosaic virus	BhYVMV	FJ179373	80.71	81.81	73.80
Crassocephalum yellow vein virus	CYVV	EF165536	80.86	96.74	90.12
Tobacco leaf curl virus Thailand	ToLCV	DQ871221	81.21	91.52	72.21
Papaya leaf curl China virus-Gu	PaLCCV	AY650283	81.84	83.26	73.75
Malvastrum leaf curl Guangdong	MLCG	AM503104	80.53	82.04	ND
Ageratum yellow vein China virus	AYVCV	AJ849916	80.47	81.30	75.32
Stachytarpheta leaf curl virus	SLCV	AJ810156	80.27	80.64	ND
Honeysuckle yellow vein mosaic virus	HYVMV	AB178947	72.47	79.23	74.39

The values in bold face represent the highest percent homology of the begomovirues with the corresponding genes.

encoded amino acids. This pattern of variation typically occurs due to point mutations in different isolates of a begomoviruses often causing genetic drifts. Comparative nucleotide homology of CP and partial AV2 and AC3 genes sequences indicate that, each of these genes have different evolutionary origin in the sunn hemp begomo-virus. The plausible reason for this difference could be the genetic recombination between begomo-viruses (Zhou et al., 1998). Nawaz-ul-Rehmana et al. (2009) have also suggested the Southeast Asia as a possible center of origin for molecular diversity of geminiviruses.

Using the CP gene as a target, the computational analysis of structural and functional analysis of the gene and its protein product was subjected to in depth *In silico* analysis for mapping of recombinant points within gene and other functional domains. Population scaled recom-bination (RHO) and mutation rate (Theta) were assessed within the sequenced region. The recombination pattern obtained with chimaera, region between the nucleotides 346 to 643 exhibited recombination in the sunn hemp begomovirus and other related isolates (Figure 6a) from the major parent (ToLCuVB) and minor parent (ICMV). Mapping of the recombination site on CP revealed the breakpoints at position 346 (position 350 in alignment) and position 643 (position 647 in alignment) (Figure 6b). Recombination sites have been reported in CP gene of both the DNA and RNA viruses (Prasanna and Rai, 2007; Gagarinova et al., 2008). Presumably, the different patho-types can simultaneously infect a host cell and exchange genetic materials through recombination. The recombi-nation observed between geographically separated iso-lates probably represents older events, which may have occurred before their present separation. Movement of vectors and/or infected plant materials could be another factor for the gene flow between the widely separated locations (Rojas et al., 2005). Indeed, the prerequisites

Figure 2. Nucleotide sequence map of the complete CP, partial AV2 and AC3 genes within 897 bp PCR amplicon of the sunn hemp begomovirus.

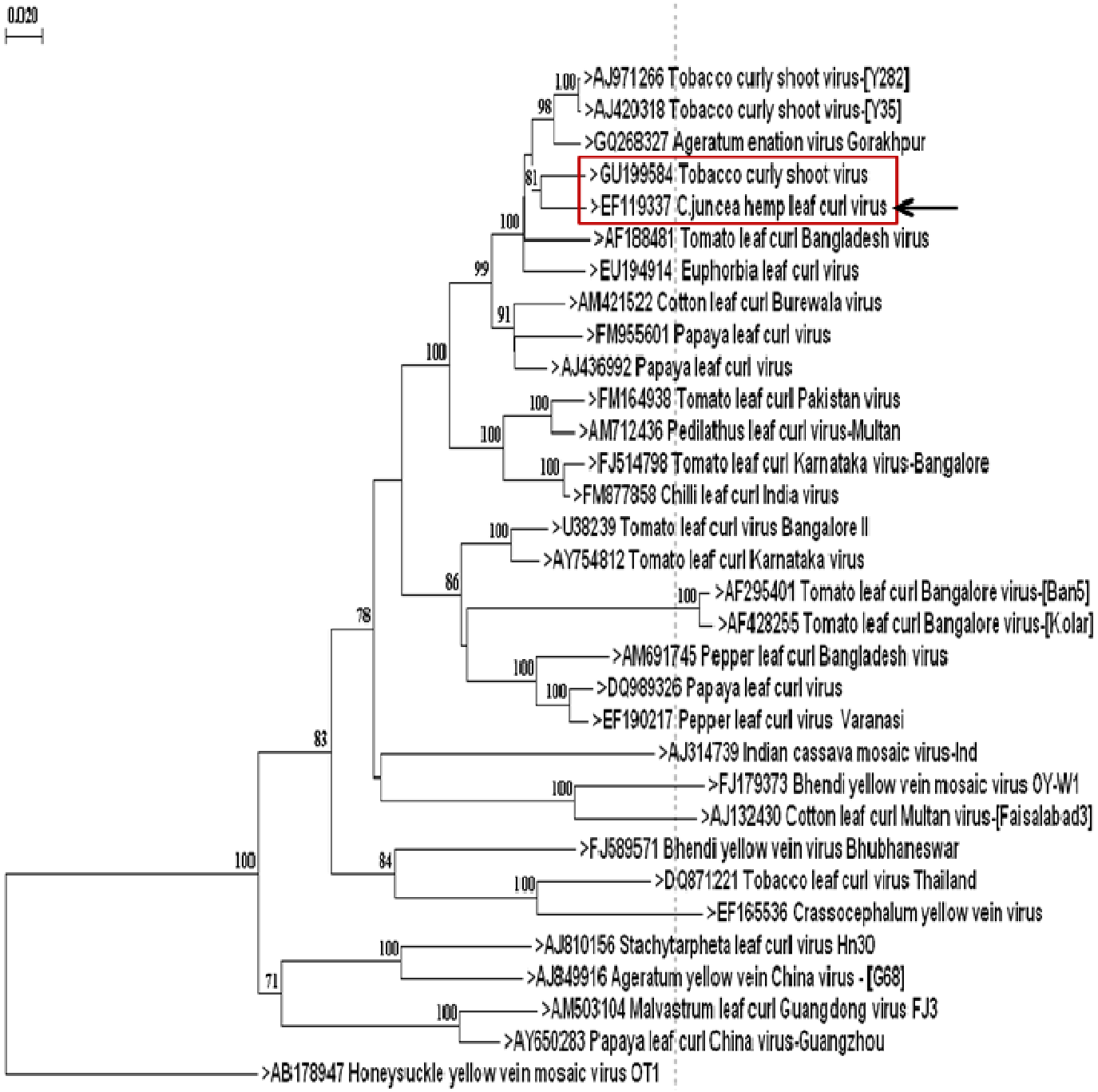

Figure 3. Phylogenetic relationships between the sunn hemp begomovirus and reference isolates sequence retrieved from NCBI GenBank, based on CP gene nucleotide sequences. All bootstrap values of 60% or greater are indicated on the tree. The scale bar indicates the numbers of nucleotide substitutions per site.

for recombination between begomoviruses include: (1) shared host ranges; (2) ability to co-infect the same cells (Sanchez-Compos et al., 1999; Stonor et al., 2003; Chowda Reddy et al., 2005); (3) high levels of viral replication (Accotto et al, 1993); (iv) overlapping geographical ranges.

The phylogenetic analysis based on Bayesian method using the recombinant region (350 and 647nucleotide) suggested the evolution of the begomovirus isolates including the sunn hemp begomovirus from India (Figure 7). Recombination is known to occur in geminiviruses and is probably the most important molecular mechanism for developing genetic changes that allow exploitation of new ecological niches. The computational analysis has

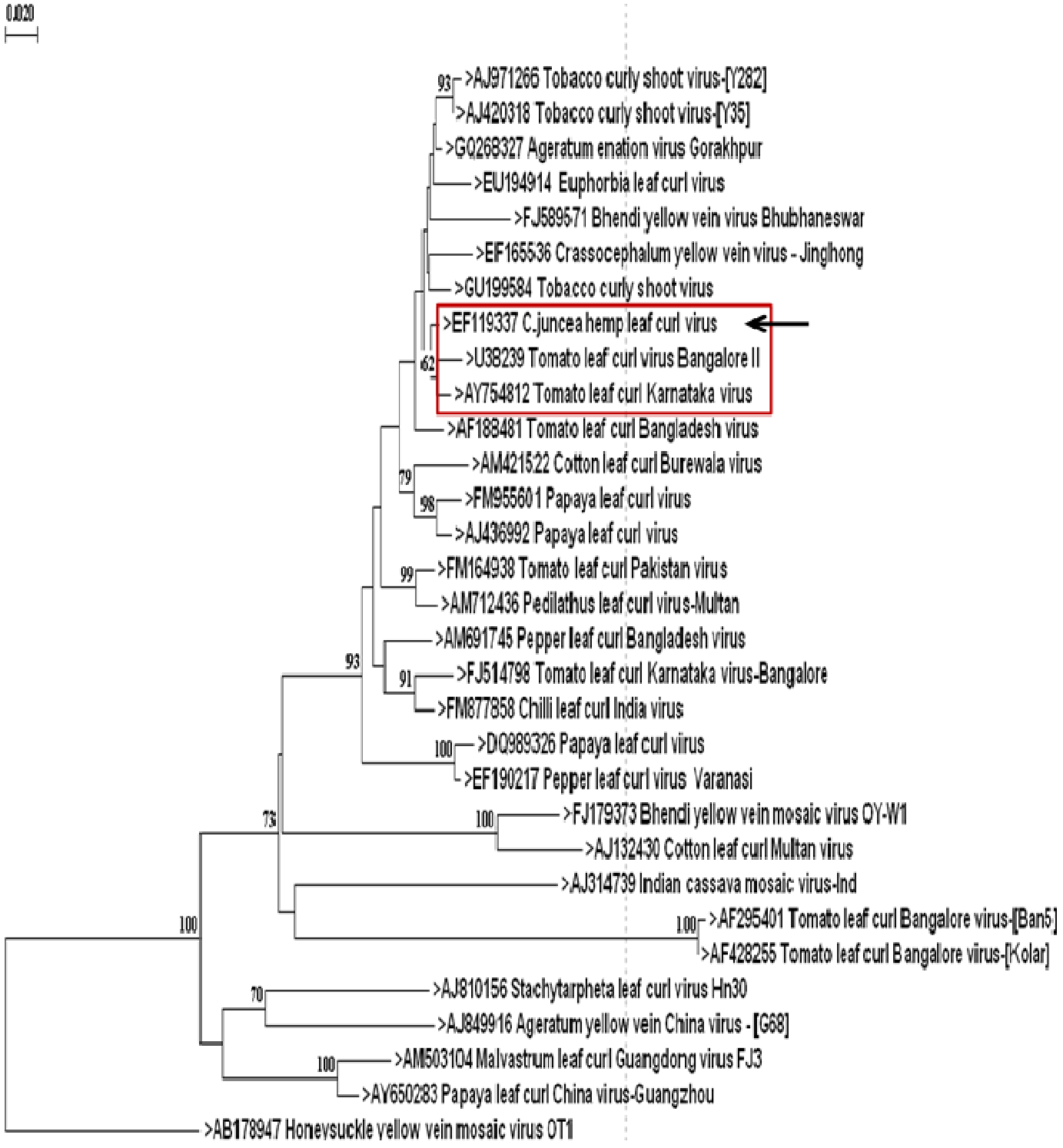

Figure 4. Phylogenetic relationships between the sunn hemp begomovirus and reference isolates sequence retrieved from NCBI GenBank based on AV2 gene nucleotide sequences. All bootstrap values of 60% or greater are indicated on the tree. The scale bar indicates the numbers of nucleotide substitutions per site.

suggested that, the sunn hemp begomovirus and other related isolates are the recombinants, developed from the major parent (Tomato leaf curl virus Bangalore) and minor parent (Indian cassava mosaic virus-Ind). This corroborate with the reports of recombination in gemini-viruses between the members belonging to the same

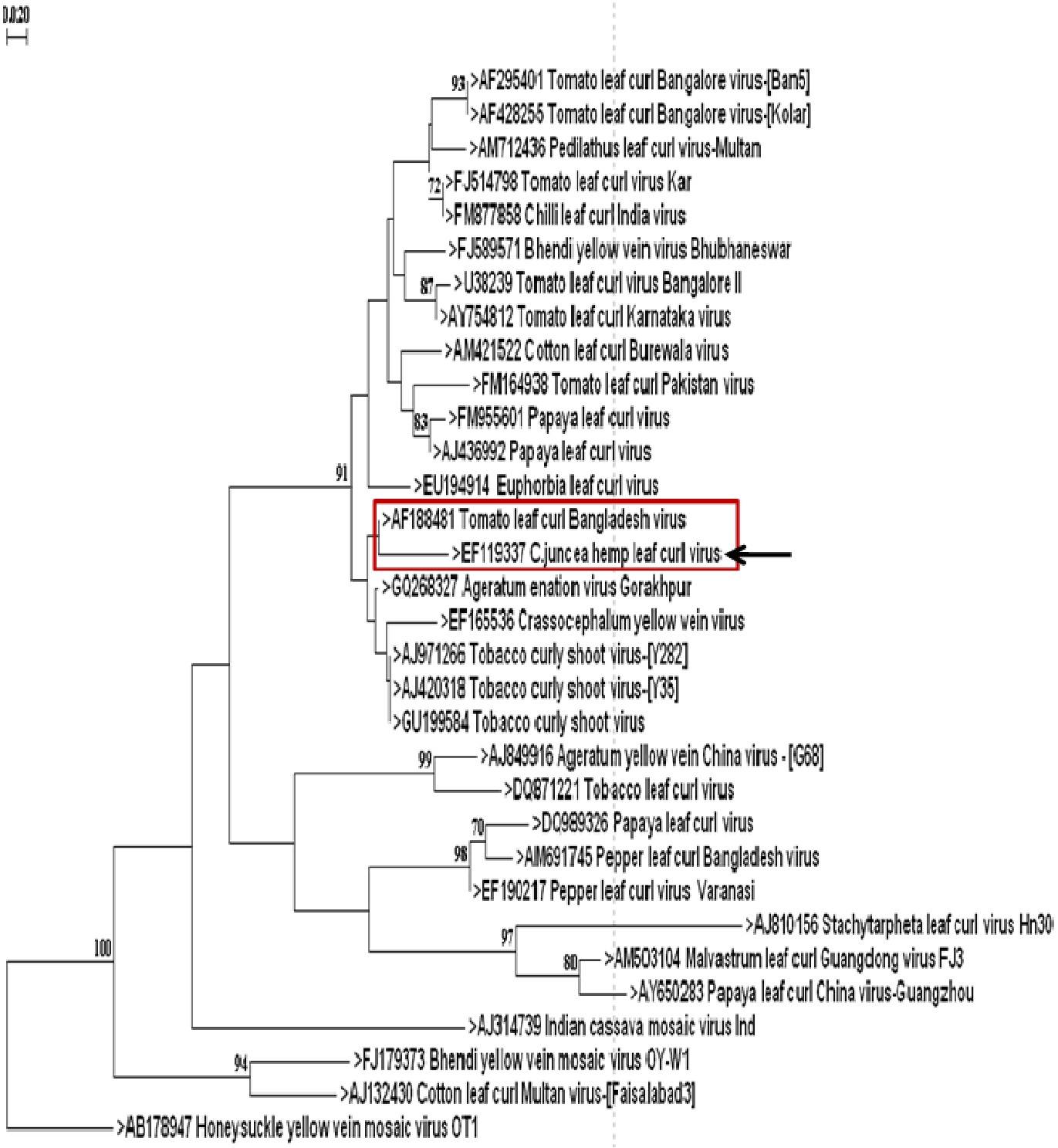

Figure 5. Phylogenetic relationships between the sunn hemp begomovirus and reference isolates sequence retrieved from NCBI GenBank based on AC3 gene nucleotide sequences. All bootstrap values of 60% or greater are indicated on the tree. The scale bar indicates the numbers of nucleotide substitutions per site.

genus (Bull et al., 2006; García-Andrés et al., 2006) and of different genera (Briddon et al., 1996).

Thus, it is concluded that, such a recombination may result in significant changes in the biological properties of virus isolates with the ability to adopt and sustain in different environmental conditions. Perhaps this is the

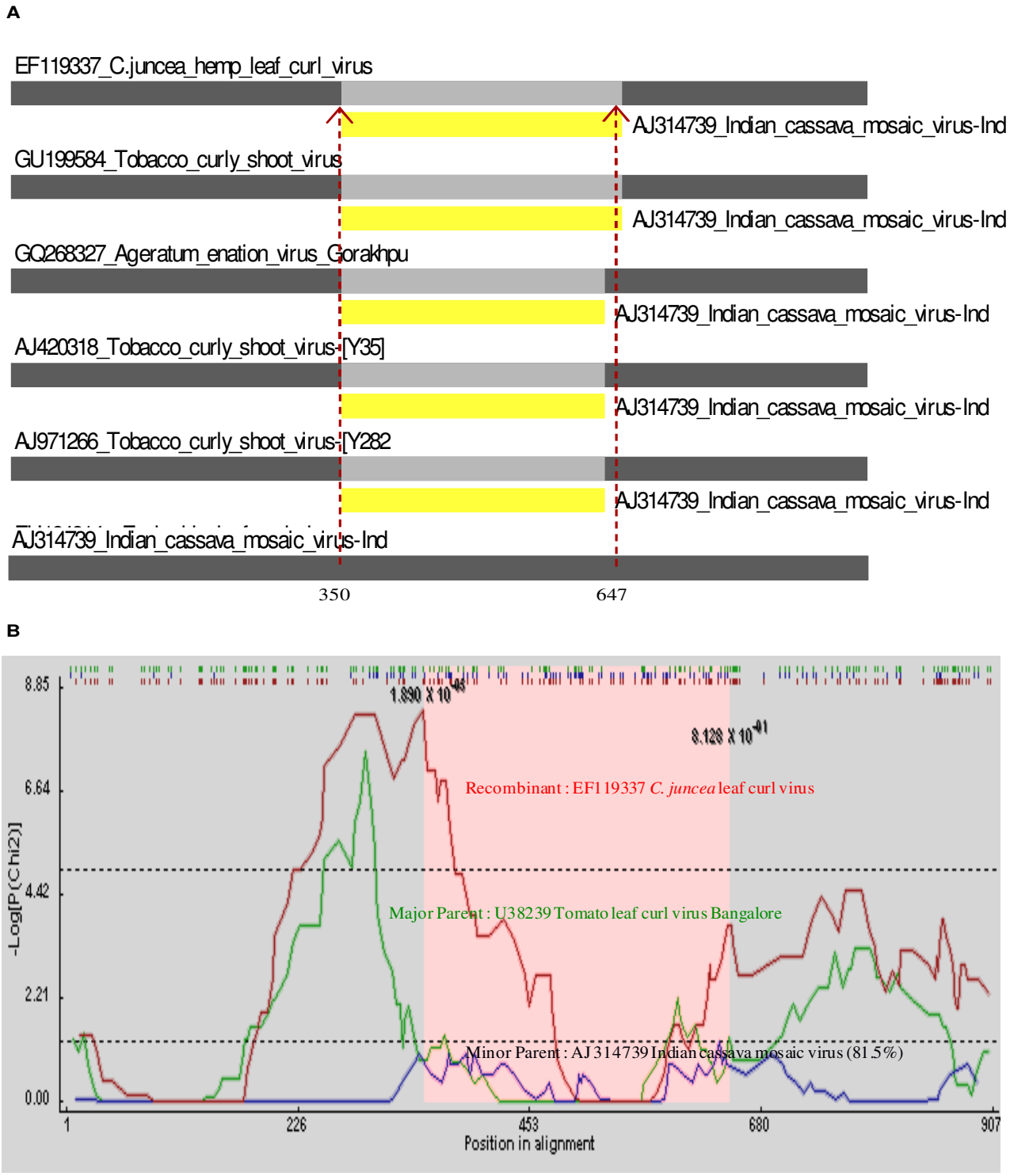

Figure 6. Assessment of recombination and mapping of hot spots within CP genes. Panel a, shows the schematic linearized map of putative recombinant fragments within the CP gene of the sunn hemp begomovirus and related begomovirus isolates. Each horizontal line represents the genotype of one virus isolate and the color-coded boxes represent the tentative origins of the putative recombinant fragments. The vertical arrows indicate the position of putative "hot spots" for recombination. Panel b, shows the graphical plot indicating the recombination break points with alignment positions.

first report of recombination in sunn hemp-infecting begomovirus, which would provide significant information for understanding the diversity and evolution of begomoviruses in Southeast Asia. Furthermore, the *in silico*

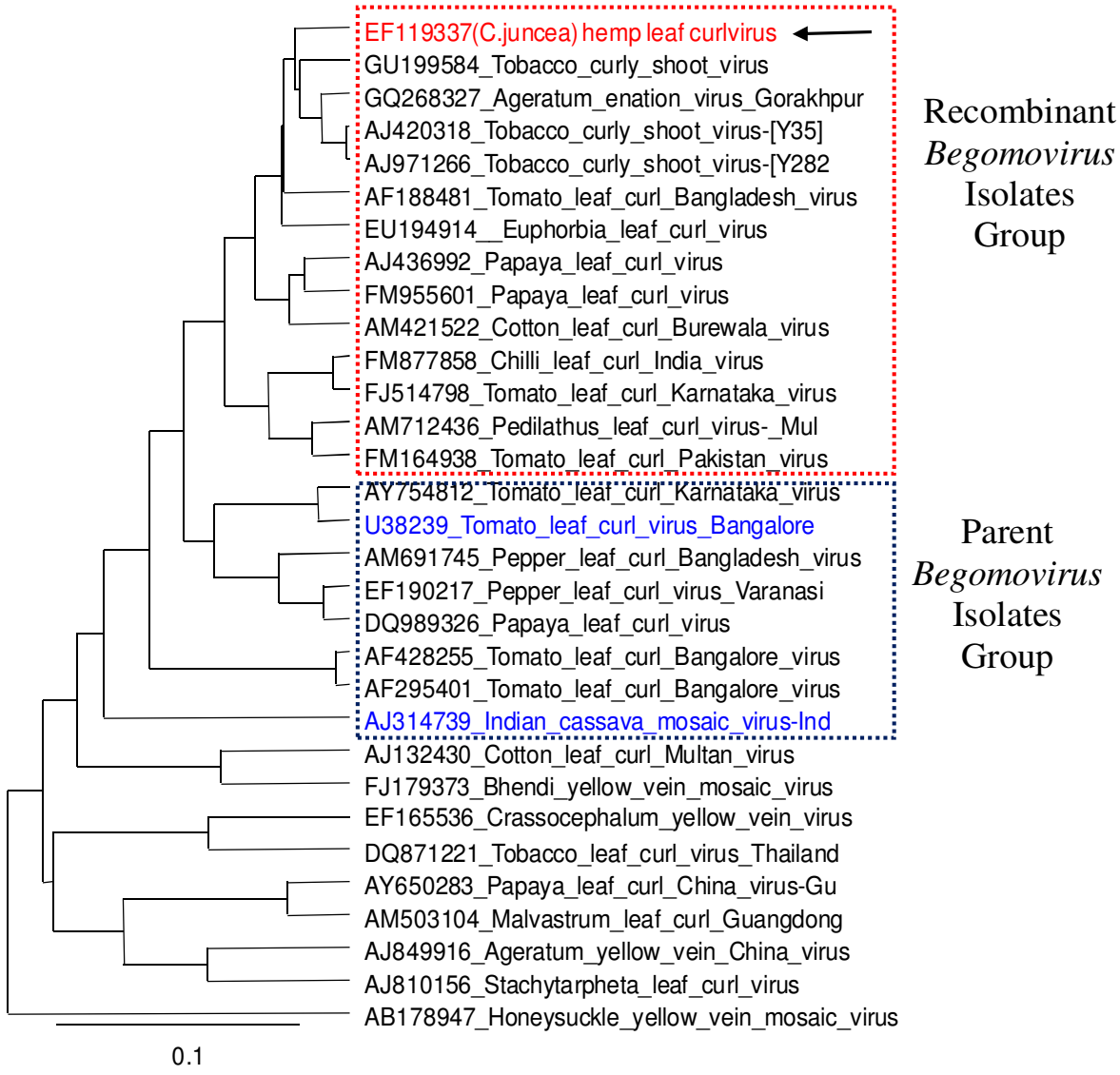

Figure 7. Bayesian phylogenetic analysis based on recombinant region (350 to 647) between the sunn hemp begomovirus and reference sequences retrieved from the NCBI GenBank.

structural and functional analysis of CP suggested that, the related begomovirus isolates from different geographical regions share common ancestry and have originated from Tomato leaf curl virus Bangalore, India.

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REFERENCES

Accotto GP, Mullineaux PM, Brown SC, Marie D (1993). Digitaria streak geminivirus replicative forms are abundant in S-phase nuclei of infected cells. Virology, 195: 257-259.

Altschul SF, Thomas LM, Alejandro AS, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSIBLAST, a new generation of protein data base search programs. Nucleic Acids Res. 25: 3389-3402.

Bendahmane M, Schalk HJ, Gronenborn B (1995). Identification and characterization of wheat dwarf virus from France using a rapid method for geminivirus DNA preparation. Phytopathology, 85: 1449-1455.

Bhardwaj HL, Webber CL III, Sakamoto GS (2005). Cultivation of kenaf and sunn hemp in the mid-Atlantic United States. Ind. Crops Prod. 22: 151-155.

Birnboim HC, Doly J (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7: 1513-1523.

Bowdoin LH, Settlage SB, Orozco BM, Nagar S, Robertson D (1999). Geminiviruses: models for plant DNA replication, transcription and cell cycle regulation. Crit. Rev. Biochem. Mol. Biol. 18: 71-106.

Briddon RW, Bedford ID, Tsai JH, Markham PG (1996). Analysis of the nucleotide sequence of the treehopper-transmitted geminivirus,

tomato pseudo-curly top virus, suggests a recombinant origin. Virology, 291: 387-394.
Bull SE, Briddon RW, Sserubombwe WS, Ngugi K, Markham PG, Stanley J (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. J. Gen. Virol. 87: 3053-3065.
Chowda Reddy RV, Muniyappa V, Colvin J, Seal SE (2005). Diversity and distribution of begomoviruses infecting tomato in India. Arch. Virol. 150: 845-867.
Cook CG, White V (1996). *Crotalaria juncea*: A potential multi-purpose fiber crop. In: Janick J (ed.). Progress in new crops. ASHS Press, Arlington, VA. pp. 389-394.
Feinberg AP, Vogelstein B (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132: 6-13.
Gagarinova AG, Babu M, Strömvik MV, Wang A (2008). Recombination analysis of Soybean mosaic virus full-length genome sequences reveals evidence of RNA recombination between distinct pathotypes. Virol. J. 4: 111-120.
García-Andrés S, Monci F, Castillo JN, Moriones E (2006). *Begomovirus* genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. Virology, 350: 433-442.
Harrison BD, Robinson DJ (1999). Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). Annu. Rev. Phytopathol. 37: 369-398.
Kassanis B, Varma A (1975). Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses, No. 53.
Khan JA, Siddiqui MK, Singh BP (2002). The natural occurrence of a begomovirus in sunn hemp (*Crotalaria juncea*) in India. Plant Pathol. 51: p. 398.
Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences J. Mol. Evol. 2: 111-20.
Ladipo JL (1988). Viruses associated with a mosaic disease of *Crotalaria juncea* in Nigeria: I. Cowpea mosaic virus. J. Phytopathol. 121: 8-18.
Lister RM, Thresh JM (1955). A mosaic disease of leguminous plants caused by a strain of *Tobacco mosaic* virus. Nature, London. 175: 1047-1048.
Martin DP, Williamson C, Posada D (2005). RDP2: Recombination detection and analysis from sequence alignments. Bioinformatics, 21: 260-262.
Nawaz-ul-Rehman MS, Mansoor S, Briddon RW, Fauquet CM (2009). Maintenance of an old world betasatellite by a new world helper begomovirus and possible rapid adaptation of the betasatellite. J. Virol. 83: 9347-9355.
Padidam M, Beachy RN, Fauquet CM (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. J. Gen. Virol. 76: 249-263.
Padidam M, Sawyer S, Fauquet CM (1999). Possible management of new geminiviruses by frequent recombination. Virology, 265: 218-225.
Prasanna HC, Rai M (2007). Detection and frequency of recombination in tomato-infecting begomoviruses of South and Southeast Asia. Virol. J. 4: 111-120.
Raj SK, Singh R, Pandey SK, Singh BP (2003). Association of a geminivirus with a leaf curl disease of sunn hemp (*Crotalaria juncea*) in India. Eur. J. Plant Pathol. 109: 467-470.
Rojas MR, Hagen C, Lucas WJ, Gilbertson RL (2005). Exploiting chinks in plant's armor: Evolution and emergence of geminiviruses. Annu. Rev. Phytopathol. 43: 361-394.
Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-25.
Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning, 2nd edn. Cold Spring Harbor Laboratory Press, New York, USA.
Sanchez-Compos SJ, Navas-Castillo R, Camero C, Soria JA, Diaz , Moriones E (1999). Displacement of tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. Phytopathology, 89: 1038-1043.
Sanz AI, Fraile A, Gallego JM, Malpica JM, García-Arenal FJ (1999). Genetic variability of natural populations of cotton leaf curl geminivirus, a single-stranded DNA virus. Mol. Evol. 49: 672-681.
Srivastava KM, Hallan V, Raizada RK, Chandra G, Singh BP, Sane PV (1995). Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating super coiled replicative form of viral DNA. J. Virol. Methods, 51: 297-304.
Stonor J, Hart P, Gunther M, DeBarro P, Rezaian MA (2003). Tomato leaf curl geminivirus in Australia: occurrence, detection, sequence diversity and host range. Plant Pathol. 52: 379-388.
Thompston JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: Improving the sensitivity of multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.
Verma HN, Awasthi LP (1976). Sunn hemp rosette: A new virus disease of sunn hemp, Curr. Sci. 45: 642-643.
Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape GW, Robinson DJ, Harrison BD (1997). Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. J. Gen. Virol. 78: 2101-2111.
Zhou X, Liu Y, Robinson DJ, Harrison BD (1998). Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. J. Gen. Virol. 79: 915-923.