MOLECULAR CHARACTERIZATION OF A STRAIN OF CUCUMBER MOSAIC VIRUS BASED ON COAT PROTEIN AND MOVEMENT PROTEIN GENES

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Summary. – Cucumber mosaic virus (CMV) A strain (CMV-A) isolated from *Amaranthus tricolor* was partially characterized at molecular level. Complete coat protein (CP) and movement protein (MP) ORFs were cloned and sequenced. The 657 bp region of CP gene and the 840 bp region of MP gene encode 218 and 276 amino acids, respectively. CP, at nucleotide level, showed 90–98% sequence identity with the CMV subgroup I and less than 80% with the CMV subgroup II, it showed at amino acid level 92–96% identity with the subgroup I and 74–87% with the subgroup II. The nucleotide and amino acid sequence identities of MP ranged in 91–94% and 92–96%, respectively with the subgroup I but in 81–83% with the subgroup II. Phylogenetic trees generated from nucleotide and amino acid sequences of both CP and MP genes identified the virus strain as a member of the subgroup IB. CMV-A CP also displayed a remarkably higher homology with Indian strains of CMV than with other CMV strains and formed a separate cluster within the subgroup IB.

Key words: Cucumber mosaic virus; coat protein; movement protein; genes; sequence identity; phylogenetic analysis

Introduction

CMV, the type species of the genus *Cucumovirus,* belongs to the family *Bromoviridae.* It is economically important due to its largest host range among known plant viruses and infects approximately 1000 plant species representing 86 families including both monocots and dicots (Palukaitis *et al*., 1992). A tripartite genome of CMV consists of three single-stranded positive-sense RNA species designated as RNA 1, RNA 2 and RNA 3. RNA 1 and RNA 2 encode the proteins 1a and 2a, respectively, essential for replication (Hayes and Buck, 1990). RNA 2 also codes for a second protein called 2b that affects the long distance movement and symptomatology of the virus (Ding *et al*., 1995). RNA 3 is dicistronic and encodes two proteins, MP and CP. MP encoded by an ORF located within the 5'-half of RNA 3 is involved in cell-to-cell movement of the virus (Canto *et al*., 1997; Suzuki *et al*., 1991). The second ORF located within the 3'-half encodes the 24 K CP, which is expressed through a sub-genomic RNA 4 (Palukaitis *et al*., 1992). CP has been demonstrated to have roles in encapsidation (Suzuki *et al*., 1991), systemic movement within infected plants (Schmitz and Rao, 1998; Taliansky and Garcia-Arenal, 1995; Wong *et al*., 1999), host range (Suzuki *et al*., 1991), and aphid transmission (Ng *et al*., 2000; Perry *et al*., 1998).

Strains of CMV have been divided into two subgroups, I and II on the basis of serological properties (Anonymous, 1998; Hu *et al*., 1995; Ilardi *et al*., 1995; Wahyuni *et al*., 1992), nucleic acid hybridization data (Gonda and Symons, 1978; Owen and Palukaitis, 1988), nucleic acid and/or protein sequence composition (Edwards and Gonsalves, 1983; Owens *et al*., 1990; Szilassy *et al*., 1999), RNase protection assay data (Fraile *et al*., 1997), and RT-PCR RFLP data (Anonymous, 1998; Rizos *et al*., 1992; Sialer *et al*., 1999. Further splitting of subgroup I into IA and IB has

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Abbreviations: CMV = Cucumber mosaic virus; CMV-A = CMV A strain; PSV = Peanut stunt virus; CP = coat protein; MP = movement protein

been proposed on the basis of sequence data (Palukaitis and Zaitlin, 1997), analysis of 5'-non-translated region of RNA 3 of several strains from subgroup I, and phylogenetic analysis of CP genes (Roossinck *et al*., 1999; Roossinck, 2002). Accordingly, Asian strains of CMV have been grouped into the subgroup IB (Palukaitis and Zaitlin, 1997).

The natural occurrence, biological and serological characteristics of CMV-A isolated from *Amaranthus tricolor* has been investigated earlier by Raj *et al*. (1997). Here, we report partial molecular characterization of CMV-A based on the cloning and sequencing of complete CP and MP ORFs located in RNA 3. Its relationship with other CMV strains was also investigated with the aim to establish its taxonomic position among the CMV strains reported from India as well as other parts of the world.

Materials and Methods

Viral RNA. CMV-A was propagated on *Nicotiana tabacum* cv. Samsun NN plants (Raj *et al*., 1997) and purified according to Lot *et al*. (1972). The identity of the virus strain was checked by back inoculation tests on *Amaranthus tricolor* plants. Viral RNA was isolated from the virus purificate (~100 µg) by disruption of virions with 1% SDS followed by extraction with phenol-chloroform, ethanol precipitation and centrifugation at 10,000 rpm for 15 mins at 4°C. The pelleted RNA was washed with 70% ethanol, dried and resuspended in RNase-free sterile water.

RT-PCR. To amplify the complete ORFs of CP and MP genes of the CMV strain, RT-PCR was performed using viral RNA as template and two primer pairs specific for CP and MP, respectively. The primers were designed on the basis of sequences of several strains available in GenBank including CMV-Fny (Palukaitis *et al*., 1992). The primers for complete ORF of CMV CP yielding an amplicon of 660 bp were as follows: the downstream primer 5'-GCATGGTACCTCAAACTGGGAGCAC-3' (25-mer) and the upstream primer 5'-GCATTCTAGATGGACAAATCTGAATC-3' (26-mer). The underlined sequences are the *Kpn*I and *Xba*I sites, respectively. The primers for complete ORF of CMV MP yielding an amplicon of 850 bp were as follows: the downstream primer 5'-CTAAAGACCGTTAACCTCC-3' (19-mer) and the upstream primer 5'-ATGGCTTTCCAAGGTACCA-3' (19-mer).

First-strand cDNA synthesis of CP and MP genes was performed separately using viral RNA (~1 µg) as template and AMV reverse transcriptase (Pharmacia Biotech Ltd) in a 20 µl reaction mixture containing the downstream primer (25 pmoles), dNTPs (20 mmoles each), RNA guard (20 U, Pharmacia Biotech Ltd) and AMV reverse transcriptase (10 U). The mixture was incubated at 42°C for 90 mins. For PCR (50 µl) 3 µl of cDNA, the upstream and downstream primers (25 pmoles each), dNTPs (10 mmoles each), and Taq DNA polymerase (3 U, Bangalore Genei India Ltd.) in a PCR buffer containing 15 mmol/l $MgCl_2$were employed. The PCR conditions for both reactions were as follows: initial template denaturation at 94°C for 5 mins was followed by 30 cycles consisting of 94°C/60 secs (denaturation), 52°C/45 secs (annealing), and 72°C/90 secs (primer extension), and final extension at 72°C for 5 mins. The amplified products were electrophoresed on 1% agarose gel.

Cloning and sequencing of RT-PCR amplicons. The product of the RT-PCR for CP was cloned at *Xba*I and *Kpn*I sites of the vector pBluescript II SK+ (Stratagene) through directional cloning techniques (Sambrook *et al*., 1989). Similarly, the product of the RT-PCR for MP was cloned in the vector pGEM-T Easy (Promega). Competent cells of *Escherichia coli* (strain XL1-Blue with resistance to tetracycline) were transformed and transformed colonies were selected on LBA plates containing ampicillin (100 µg/ml) and tetracycline (12.5 µg/ml). The resistant clones were verified by restriction digestions and PCR analysis for the presence of CP and MP genes, respectively. Positive clones were sequenced. For sequencing, T3 or T7 primers (5 pmoles) were mixed with plasmid DNA (500 ng) and the reaction was done using the FS-Dye Termination Cycle Sequencing Ready Reaction Kit (Perkin Elmer) according to the manufacturer's instructions. The reaction products were loaded on an automated DNA sequencer (Applied Biosystems). At least two independent clones of each gene were sequenced and corrected by comparing with corresponding electropherograms.

Sequence analysis. The sequence data of CP and MP obtained were submitted to GenBank and compared with those of various CMV strains by the Entrez program using BLAST from NCBI, Bethesda, MD, USA. The matrix for pairwise alignments of selected CMV strains with A strain for nucleotide and amino acid sequences of CP and MP was obtained using the DiAlign 2 Program (Morgenstern, 1999). Multiple sequence alignments of nucleotide and amino acid sequences of CP and MP genes of selected CMV strains with A strain were performed using Clustal W provided in the Bio-Edit Program Version 5.0.9. The alignment files created by Clustal W were bootstrapped 100 times using the TreeCon Program Version 1.3b (Van de Peer and De Wachter, 1993) to generate 100 replicate sets for each data and used for generating neighbor-joining phylogenetic trees. All trees were rooted on Peanut stunt virus (PSV, U15730), another member of the genus *Cucumovirus* taken as outgroup. GenBank accession numbers of the CMV strains of subgroups IA, IB and II along with their place of origin, considered for phylogenetic analysis are described in Table 1. In order to analyze molecular variability among Indian CMV strains, CP gene sequences of CMV strain Phym (Haq *et al*., 1996), CMV-D (Raj *et al*., 1999) and CMV-H (Samad *et al*., 2000), deposited in GenBank under Acc. Nos. X89652, AF281864 and AF350450, respectively, were considered.

Results and Discussion

The virus purificate from experimentally infected *N. tabacum* cv. Samsun NN, when back inoculated on *A. tricolor,* produced original characteristic symptoms as described earlier (Raj *et al*., 1997). The RNA isolated from the purified virus, when used as template for RT-PCR with CP- and MP-specific primers, gave ~660 bp and ~850 bp products, respectively. The amplicons of CP and MP were successfully cloned in the pBluescript II and pGEM-T Easy vectors. Restriction analysis and PCR screening of the minipreparations of DNA obtained from antibiotic-resistant

Table 1. CMV strains considered for phylogenetic analysis

Ser. No.	CMV strain	Acc. No.	Origin	Subgroup
1	Fny	D10538	USA (NY)	IA
2	Y	D12499	Japan	IA
3	Leg	D16405	Japan	IA
4	O	D00385	Japan	IA
5	Cs	D28489	Japan	IA
6	D8	AB004780	Japan	IA
7	N	D28476	Japan	IA
8	C	D00462	USA (NY)	IA
9	117E	X16386	France	IA
10	Plan	U32858	(Columbia)	IA
11	Kor	L36251	Korea	IA
12	E5	D42080	Japan	IA
13	Pepo	D43800	Japan	IA
14	As	AF013291	Korea	IB
15	K	AF127977	China	IB
16	B2*	AB046951	Japan	IB
17	Tfn	Y16926	USA	IB
18	Ix	U20219	Philippines	IB
19	IA-CP/IA-3a	AB042294	Philippines	IB
20	2AI-A*	AJ271416	USA	IB
21	Nt9	D28780	Taiwan	IB
22	Sd	AB008777	(China)	IB
23	C7-2	D42079	Japan	IB
24	K	AF127977	China	IB
25	Phym	X89652	India	IB
26	S	AF063610	South Africa	II
27	WL	D00463	USA	II
28	Trk-7	L15336	Hungary	II
29	Q	M21464	Australia	II
30	Kin	Z12818	Scotland	II
31	LS	AF127976	USA	II
32	H*	AF350450	India	IB
33	D*	AF281864	India	IB

CMV strains reported from India are shown in reverse contrast.
*CMV strains not included by Roossinck *et al.* (1999) for subgroup differentiation.

colonies confirmed the presence of inserts of expected size in several clones. Positive clones of CP and MP were sequenced and the sequence data were deposited in GenBank under Acc. Nos. AF198622 and AF414078, respectively. These data indicated presence of complete ORF of CP (657 bp, 218 aa) and MP (840 bp, 279 aa) genes including their initiation and termination codons.

Sequence analysis of CP revealed the presence of highly conserved regions in amino acid sequences of CMV-A e.g. amino acids P129 and A162 (Fig. 1). These conserved regions have been reported earlier to be associated with mosaic symptoms (Shintaku *et al*., 1992) and essential for aphid transmission (Ng *et al*., 2000). However, three amino acid substitutions were found to be unique in the A strain and other Indian strains (D, H and Phym) but not present in any of the CMV strains reported from other parts of the world. These unique substitutions were N31T, A137S (except Ix, IA-CP and 2AI-A where T was present) and A172V (except the subgroup II where A was replaced by E). Substitutions H and S (instead of R and P) at the positions 16 and 17 located at the N'-terminus were also found in CMV-A although this region has been found to be highly conserved among all CMV strains and considered to be important for viral RNA and CP interactions (Schmitz and Rao, 1998; Perry *et al*., 1998).

The sequence analysis of MP identified the nucleotide positions 596–633 (38 nt) as identical among all CMV strains (data not shown). Alignment of its deduced amino acid sequence identified the position 88–154 (67 aa) as conserved among all the strains studied. Four amino acid positions, namely N20, I51, G207 and E275 were found to be unique in the CMV-A, whereas in other strains of CMV, they were D, N/S, R and V, respectively. The changes in these positions are, however, not conservative, presumably they could have arisen due to some mutations in the gene. Hence, whether alterations at these positions affect the function of individual subunit during host infection and/ or whether they provide some functional importance to the virus for its survival needs to be determined.

Sequence identities of the A strain with other CMV strains at nucleotide and deduced amino acid levels of CP and MP were over 90% with the subgroup I and below 80% with the subgroup II (Tables 2 and 3) which clearly identified the A strain as a member of the subgroup I instead of the subgroup II. Moreover, the overall nucleotide and amino acid percent identities of the strain obtained within the subgroup I were higher with the subgroup IB than with the subgroup IA. A noticeable observation was that the percent sequence identity obtained in CP among the Indian strains (A, D, H and Phym) was found to be still higher as compare to rest of the CMV strains whether belonging to the subgroup IA, IB or II (Table 2). MP gene of A strain revealed a higher sequence identity at nucleotide level with the subgroup IB than with the subgroup IA, but its corresponding deduced amino acid sequence revealed higher identity with the subgroup IA than with the subgroup IB (Table 3). However, an overall higher sequence identity exhibited at amino acid level than at nucleotide level between A strain and other strains might indicate a constraint imposed on virus for variation in CP to maintain its structural and functional role presumably for virion stability, transmission by aphids and virus movement within host plants as suggested by Ng *et al*. (2000), Perry *et al*. (1998), and Wikoff *et al*. (1997).

The phylogenetic relationship of A strain with other CMV strains, based on nucleotide and amino acid sequence alignments of CP and MP, supported the results of sequence identities which placed A strain into subgroup I instead of subgroup II (Figs. 2 and 3, respectively). Dendrograms of nucleotide (Fig. 2a) and amino acid (Fig. 2b) sequences of CP grouped A strain with IB strains rather than IA strains. However, a separate cluster formed by A strain along with

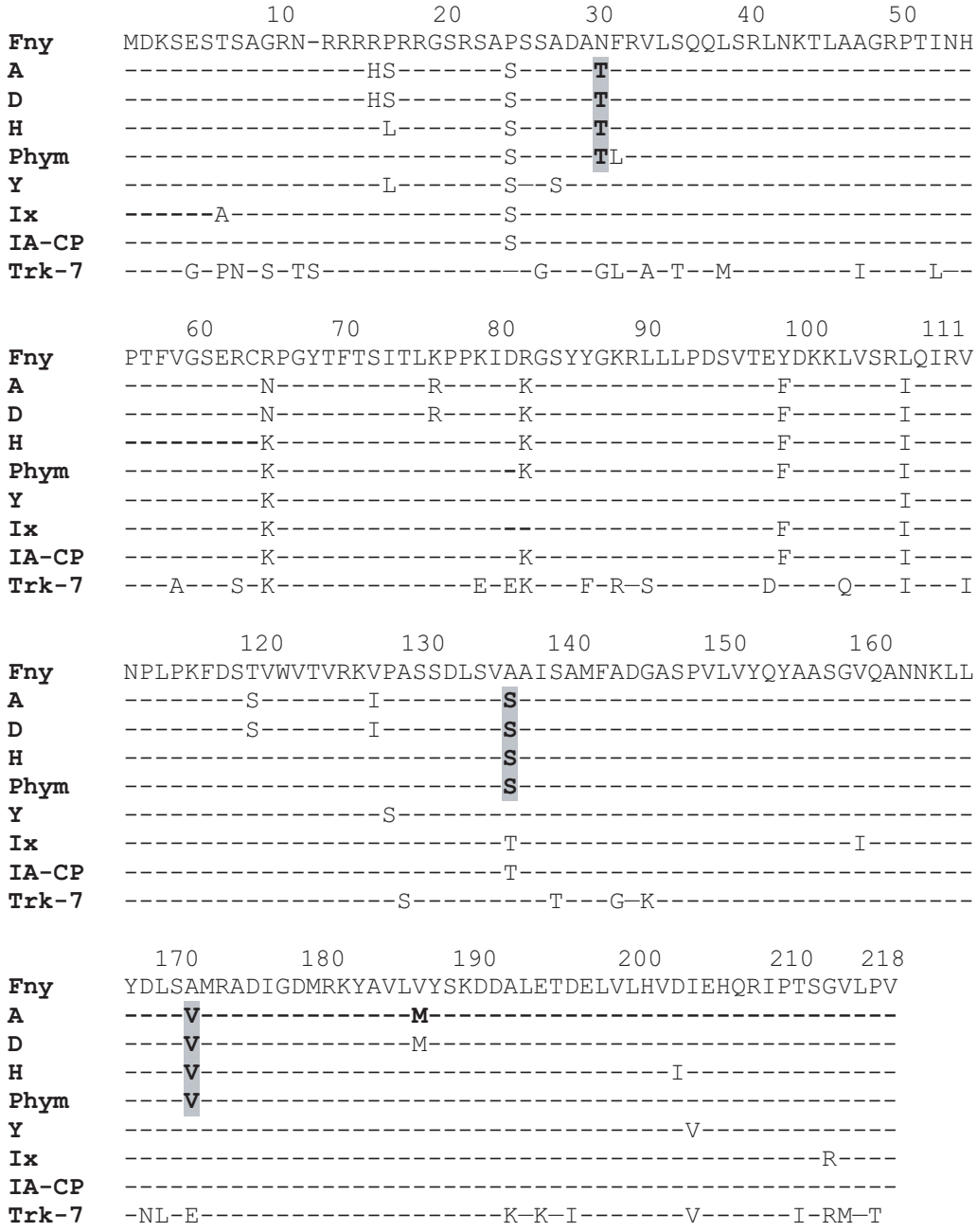

Fig. 1

Amino acid sequence alignment of CP of CMV-A with other CMV strains of subgroups IA, IB, and II and Indian strains (D, H and Phym)

Broken lines indicate identical sequences. Sequences unique to Indian strains are indicated in bold and reverse contrast.

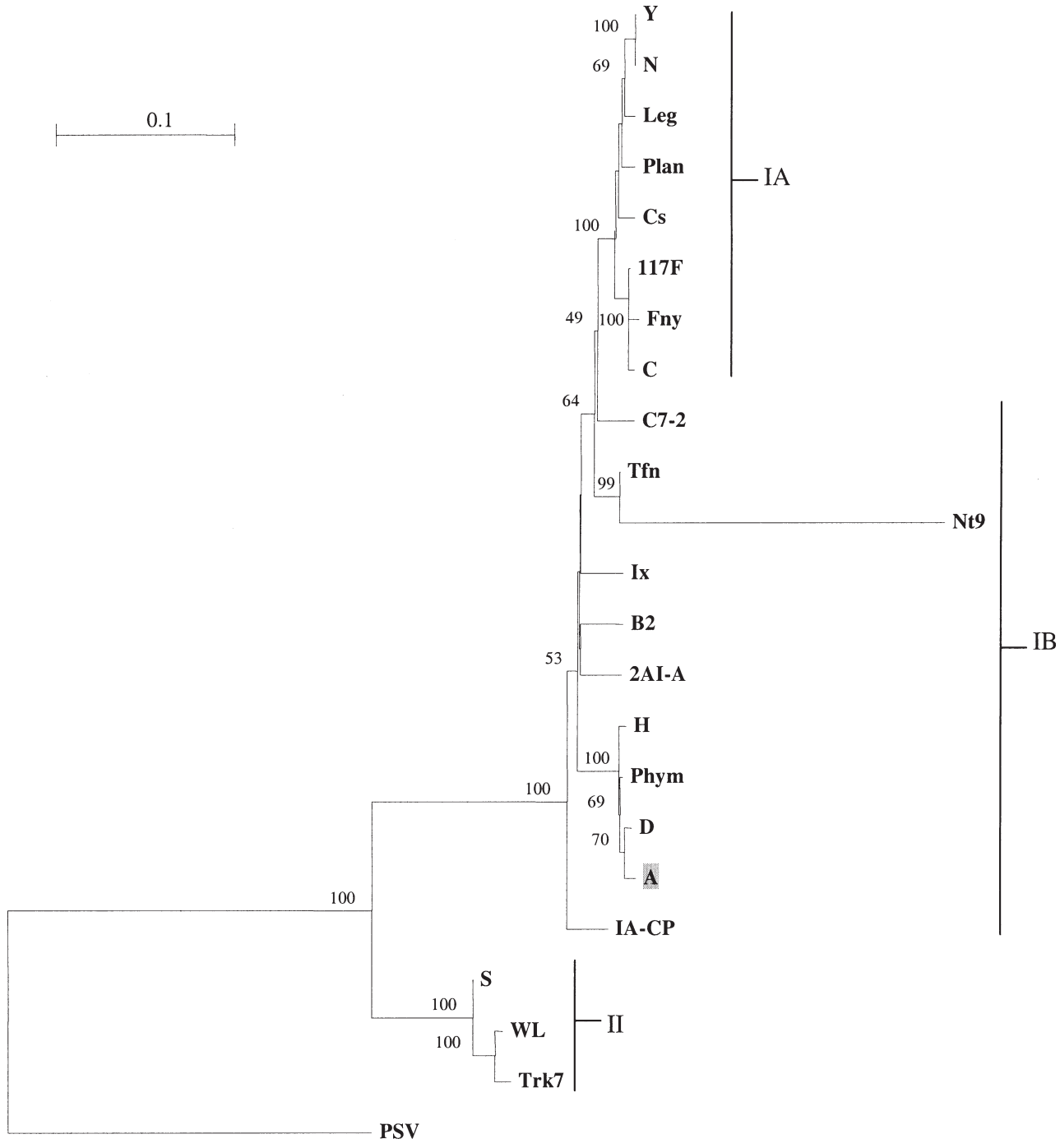
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Fig. 2a

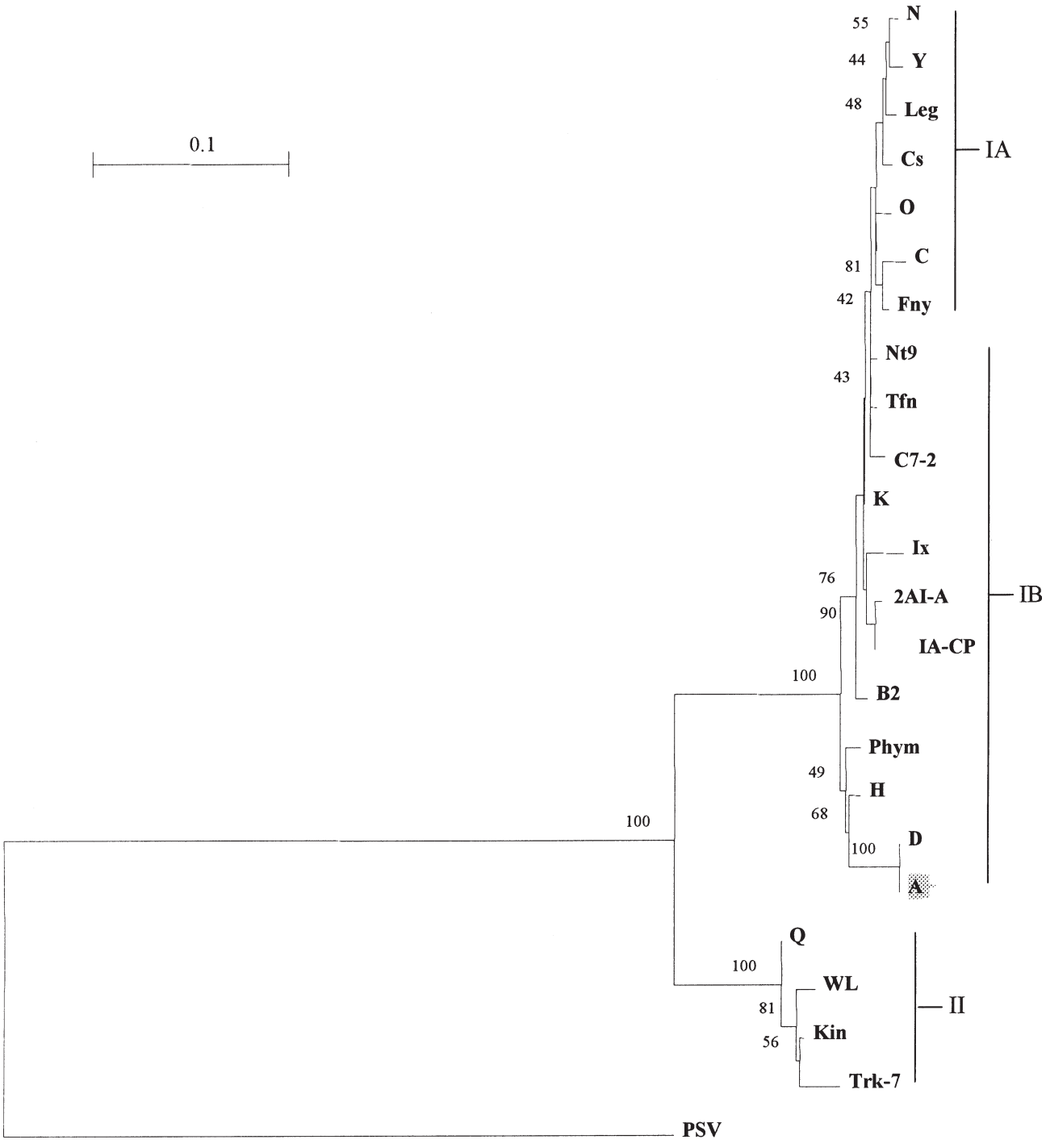


Fig. 2b

Phylogenetic relationship between CMV-A strain with other CMV strains including those from India (D, H and Phym), based on the alignment of nucleotide (a) and amino acid (b) sequences of CP

The NJ trees were constructed using Tree Con Program Version 1.3b and rooted on CP sequence of PSV. Whereas the numbers on the nodes indicate the bootstrap values obtained from the corresponding cladograms; vertical distances are arbitrary and horizontal distances reflect sequence divergence. Only bootstrap values higher than 40 are shown.

Table 2. Comparison of percent identities of CMV-A for CP with CMV strains of subgroups IA, IB, and II and other strains from India at nucleotide (below diagonal) and deduced amino acid (above diagonal, bold) levels

Strain	Indian strains				Subgroup IA			Subgroup IB			Subgroup II	
	A	D	H	Phym	Fny	Y	Nt9	IACP	Ix	2aIA	Trk7	WL
A	–	**100**	**95**	**94**	**92**	**92**	**93**	**94**	**91**	**94**	**75**	**77**
D	97	–	**95**	**94**	**92**	**92**	**93**	**94**	**91**	**94**	**75**	**77**
H	97	98	–	**97**	**94**	**95**	**95**	**97**	**93**	**96**	**77**	**79**
Phym	97	98	98	–	**94**	**94**	**95**	**97**	**94**	**96**	**77**	**79**
Fny	80	80	80	81	–	**96**	**98**	**96**	**94**	**96**	**77**	**79**
Y	88	88	88	88	88	–	**97**	**95**	**94**	**94**	**78**	**80**
Nt9	78	78	78	79	72	81	–	**97**	**95**	**97**	**77**	**79**
IA-CP	89	89	89	90	81	89	79	–	**97**	**99**	**77**	**79**
Ix	89	89	91	90	82	90	78	90	–	**96**	**77**	**78**
2AI-A	89	90	90	90	81	89	79	79	90	–	**77**	**79**
Trk7	66	67	68	69	65	69	59	68	65	70	–	**95**
WL	66	66	67	67	61	66	59	68	67	67	96	–

Percent values for CMV-A strain are shown in reverse contrast.

Table 3. Comparison of percent identities of CMV-A for MP with CMV strains of subgroups IA, IB, and II and other strains from India at nucleotide (below diagonal) and deduced amino acid (above diagonal, bold) levels

Strain	Indian	Subgroup IA		Subgroup IB			Subgroup II	
	A	Fny	Y	Nt9	IA-3A	Ix	Trk7	Q
A	**–**	**95**	**96**	**94**	**94**	**92**	**80**	**78**
Fny	89	–	**99**	**95**	**96**	**92**	**83**	**80**
Y	89	97	–	**96**	**96**	**94**	**82**	**80**
Nt9	91	92	92	–	**92**	**92**	**82**	**80**
IA-3A	92	91	91	94	–	**88**	**81**	**79**
Ix	87	88	90	96	92	–	**81**	**79**
Trk7	77	74	74	78	75	74	–	**96**
Q	77	75	75	78	75	74	97	–

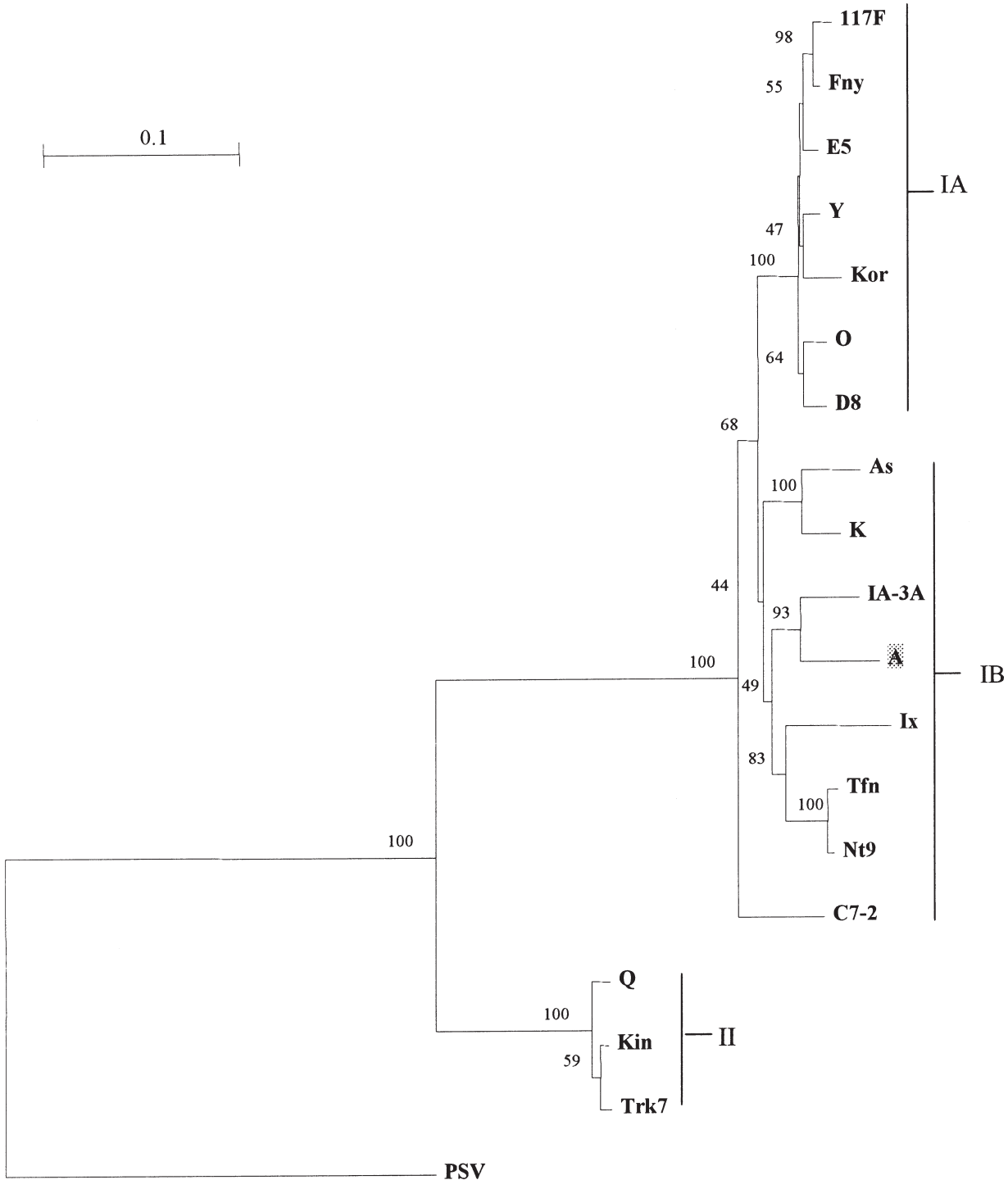
Percent values for CMV-A strain are shown in reverse contrast.

other Indian CMV strains (D, H and Phym) within the subgroup IB in both CP nucleotide and amino acid trees (Figs. 2a and 2b) further supports the sequence data indicating higher homology among Indian strains as compared to other CMV strains. Such a high homology suggests a common origin of Indian strains belonging to this region.

The phylogenetic tree generated on the basis of nucleotide and amino acid sequences of MP (Figs. 3a and 3b, respectively) placed more consistently A strain into subgroup IB than did the CP alignment. The MP nucleotide alignment depicted a maximum relationship of A strain with the Indonesian strain IA-3a, while the MP amino acid alignment clustered A strain with IA-3a and B2 strains (Fig. 3b).

The phylogenetic analysis identified CMV-A as a new member of the subgroup IB containing CMV strains of Indian origin. Earlier Roossinck *et al.* (1999) had placed the Phym strain of CMV (at that time the only Indian strain available, considered for analysis) into the subgroup IB on the basis of sequence analysis of the untranslated 5'-end of CMV RNA3 and CP.

CMV is economically important because of its extreme broad host range and its successful adaptability to new hosts and environments as it is reported from all parts of the world (Roossinck, 2002). Although there are reports on biological and serological characterization of various Indian strains of CMV, their differentiation and molecular characterization based on complete sequence data have not yet been done except for some preliminary findings (Raj *et al.*, 1995; Haq *et al.*, 1996). Therefore, our study was aimed at understanding the correct taxonomic position of CMV-A and its phylogenetic relationship with other CMV strains reported from India as well as from other parts of the world. This study might help in (i) furthering the knowledge on the origin and relatedness of CMV-A to other Indian CMV strains and cucumoviruses in general and (ii) design of strategies for efficient control of CMV.


0.1
117F
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C7-2
IB
Q
Kin
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Fig. 3a

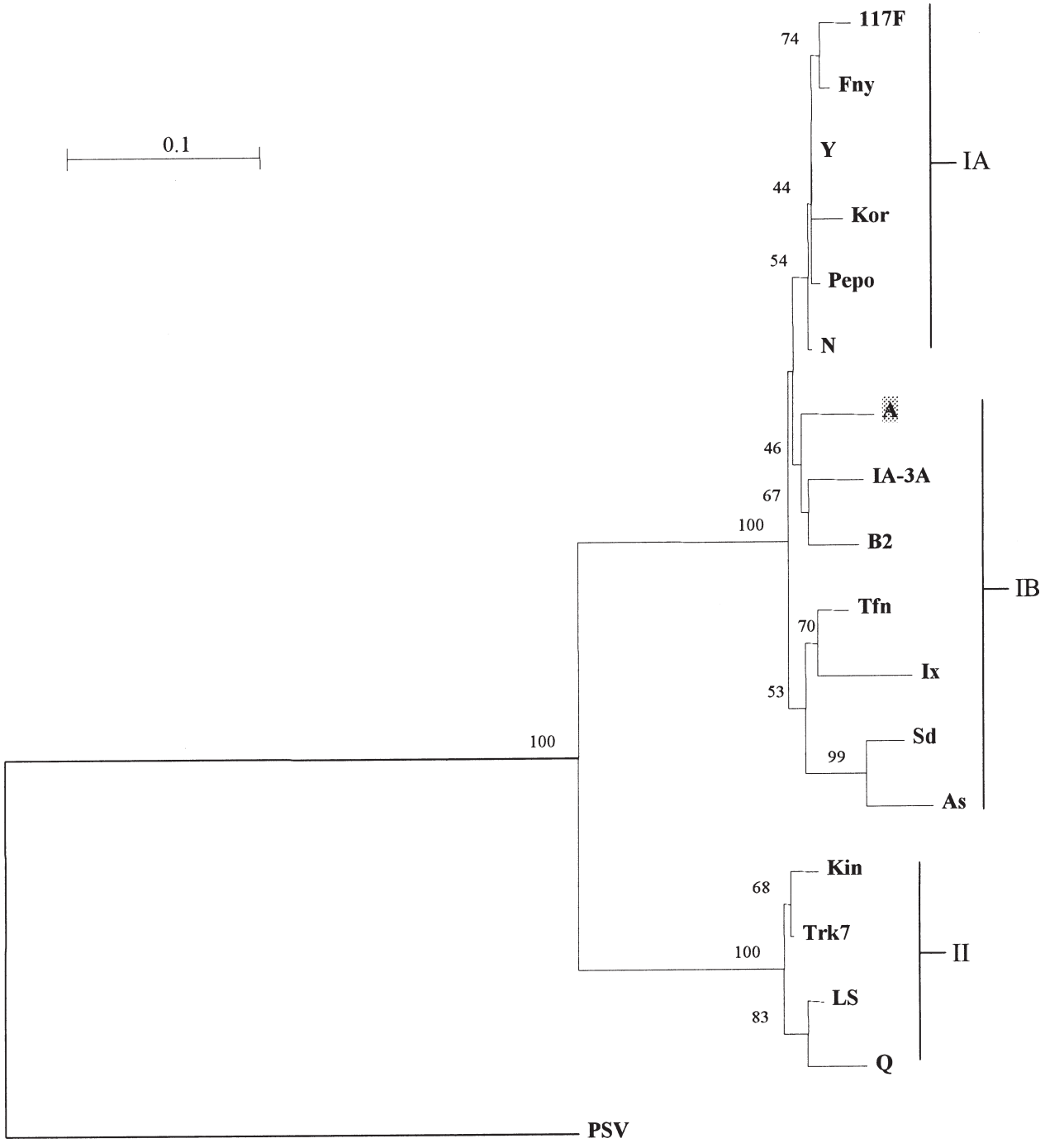

Fig. 3b

Phylogenetic relationship between CMV-A strain with other CMV strains including those from India (D, H and Phym), based on the alignment of nucleotide (a) and amino acid (b) sequences of MP

The NJ trees were constructed using Tree Con Program Version 1.3b and rooted on MP sequence of PSV. Whereas the numbers on the nodes indicate the bootstrap values obtained from the corresponding cladograms, vertical distances are arbitrary and horizontal distances reflect sequence divergence. Bootstrap values higher than 40 are shown.

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References

Anonymous (1998): Detection and biodiversity of cucumber mosaic cucumovirus. Conclusions from a ring test of European Union cost 823 (New technologies to improve phytodiagnosis). *J. Plant Pathol.* **80**, 133–149.

Canto T, Prior DAM, Hellwald KH, Oparka, Kaper J, Palukaitis P (1997): Characterization of cucumber mosaic virus. IV. Movement protein and coat protein are both essential for cell-to-cell movement of cucumber mosaic virus. *Virology* **237**, 237–248.

Ding S, Li W, Symons RS (1995): A novel naturally occurring hybrid gene encoded by a plant RNA virus facilitates long distance movement. *EMBO J*. **14**, 5762–5772.

Edwards M, Gonsalves D (1983): Grouping seven biologically defined isolates of cucumber mosaic virus by peptide mapping. *Phytopathology* **73**, 1117–1120.

Fraile A, Alonso Prados JL, Aranda MA, Bernal JJ, Malpica JM, Garcia-Arenal F (1997): Genetic exchange by recombination or reassortment is infrequent in natural populations of a tripartite RNA plant virus. *J. Virol.* **71**, 934–940.

Gonda AR, Symons RH (1978): The use of hybridization analysis with complementary DNA to determine the RNA sequence homology between strains of plant viruses, its application to several strains of cucumoviruses. *Virology* **8**, 361–370.

Haq QMR, Singh BP, Srivastava KM (1996): Biological, serological and molecular characterization of a cucumber mosaic virus from India. *Plant Pathol.* **45**, 823–828.

Hayes RJ, Buck KW (1990): Complete replication of a eukaryotic virus RNA *in vitro* by a purified RNA-dependent RNA polymerase. *Cell* **63**, 363–368.

Hu JS, Li HP, Barry K, Wang M, Jordan R (1995): Comparison of dot blot ELISA and RT-PCR assays for detection of two cucumber mosaic virus isolates infecting banana in Hawaii. *Plant Dis.* **79**, 902–906.

Ilardi V, Mazzei M, Loreti S, Tomassoli L, Barba M (1995): Biomolecular and serological methods to identify strains of cucumber mosaic cucumovirus on tomato. *EPPO Bulletin* **25**, 321–327.

Lot H, Marrou J, Quiot JB, Esvan, C (1972): Contribution a l'etude du virus de la mosaique du concombre (CMV) II. Methodede purification rapide du virus. *Ann. Phytopathol.* **4**, 25–38.

Morgenstern B (1999): DIALIGN 2, improvement of the segment-to-segment approach to multiple sequence alignment. *Bioinformatics* **15**, 203–210.

Ng JCK, Liu S, Perry KL (2000): Cucumber mosaic virus mutants with altered physical properties and defective in aphid vector transmission. *Virology* **276**, 395–403.

Owens J, Palukaitis P (1988): Characterization of Cucumber mosaic virus. I. Molecular heterogeity mapping of RNA3 in eight CMV strains. *Virology* **69**, 496–502.

Owens J, Shintaku M, Aeschleman P, Tahar SF, Palukaitis P (1990): Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains, CMV RNA3. *J. Gen. Virol.* **71**, 2243–249.

Palukaitis P, Zaitlin M (1997): Replicase-mediated resistance to plant virus diseases. *Adv. Virus Res.* **48**, 349–377.

Palukaitis P, Roossinck M, Dietzgen RG, Francki RIB (1992): Cucumber mosaic virus. *Adv. Virus Res.* **41**, 281–348.

Perry KL, Zhang L, Palukaitis P (1998): Amino acid changes in the coat protein of cucumber mosaic virus differentially affect transmission by the aphids *Myzus persicae* and *Aphis gossypii*. *Virology* **242**, 204–210.

Raj SK, Aminuddin, Singh BP, Pal M (1997): Characterization of a cucumber mosaic virus isolate causing leaf crinkle and severe mosaic of *Amaranthus* in India. *Canadian J. Plant Pathol.* **19**, 97–100.

Raj SK, Haq QMR, Srivastava KM, Singh BP (1995): Sequence homology of cucumber mosaic virus at N terminal amino acid sequences of the coat protein gene with CMV strains. *J. Plant Biochem. Biotechnol.* **4**, 77–80.

Raj SK, Srivastava A, Chandra G, Singh BP (1999): Natural occurrence of cucumber mosaic cucumovirus in *Datura innoxia* in India. *EPPO Bulletin* **29**, 455–457.

Rizos H, Gunn LV, Pares RD, Gillings MR (1992): Differentiation of cucumber mosaic virus isolates using the polymerase chain reaction *J. Gen. Virol.* **73**, 2099–2103.

Roossinck MJ (2002): Evolutionary history of cucumber mosaic virus deduced by phylogenetic analyses. *J. Virol.* **76**, 3382–3387.

Roossinck MJ, Zhang L, Hellward K (1999): Rearrangements in the 5' nontranslated region and phylogenetic analyses of cucumber mosaic virus RNA3 indicate radial evolution of three subgroups. *J. Virol.* **73**, 6752–6758.

Samad A, Raj SK, Srivastava A, Chandra G, Ajaikumar PV, Zaim M, Singh BP (2000): Characterization of an Indian isolate of cucumber mosaic virus infecting Egyptian Henbane (*Hyoscyamus muticus* L.). *Acta Virol.* **44**, 131–135.

Sambrook J, Fritsch EF, Maniatis T (1989): *Molecular Cloning. A Laboratory Manual*. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schmitz I, Rao ALN (1998): Deletions in the amino conserved basic arm of cucumber mosaic virus coat protein disrupt virion assembly but do not abolish infectivity and cell-to-cell movement. *Virology* **248**, 323–331.

Shintaku MH, Zhang L, Palukaitis P (1992): Single amino acid substitution in the coat protein of cucumber mosaic virus induces chlorosis in tobacco. *Plant Cell.* **4**, 751–757.

Sialer MM, Cillo F, Barbarossa L, Gallitelli D (1999): Differentiation of Cucumber mosaic virus subgroups by RT-PCR RFLP. *J. Plant Pathol.* **81**, 145–148.

Suzuki M, Kuwata S, Kataoka J, Masuta C, Nitta N, Takanami Y (1991): Functional analysis of deletion mutants of cucumber mosaic virus RNA3 using an *in vitro* transcription system. *Virology* **183**, 106–113.

Szilassy, D, Salanki, K and Balazs, E. (1999): Molecular evidence for the existence of two distinct subgroups in cucumber mosaic cucumovirus. *Virus Genes* **18**, 221–227.

Taliansky ME, Garcia-Arenal (1995): Role of cucumovirus capsid protein in long distance movement within the infected plant. *J. Virol.* **69**, 916–922.

Van de Peer Y, De Wachter R (1993): TREECON a software package for the construction and drawing of evolutionary trees. *Comput. Appl. Biosci.* **9**, 177–182.

Wahyuni WS, Dietzgen RG, Hanada K, Francki RIB (1992): Serological and biological variation between and within subgroup I and II strains of cucumber mosaic virus. *Plant Pathol.* **41**, 282–297.

Wikoff WR, Tsai CJ, Wang G, Baker TS, Johnson JE (1997): The structure of cucumber mosaic virus-cryoelectron microscopy, X-ray crystallography and sequence analysis. *Virology* **232**, 91–97.

Wong S, Thio SS, Shintaku MH, Palukaitis P (1999): The rate of cell-to-cell movement in squash of cucumber mosaic virus is affected by the sequences of the capsid protein. *Mol. Plant-Microbe Interact.* **12**, 628–632.