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Molecular Analysis of Korean Isolate of *Barley mild mosaic virus* (Iks Isolate)

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The complete nucleotide sequences of both RNA of an isolated *Barley mild mosaic virus* (BaMMV) from Iksan, Korea, have been determined. RNA1 was 7273 nucleotides long and encodes for a polyprotein of 2261 amino acids, which contains the eight putative functional proteins. RNA2 was 3520 nucleotides long and encodes for a polyprotein of 894 amino acids, which contains two functional proteins. Results of multiple sequence alignment showed 92.9% similarity with Na1 isolate, followed by Sil, UK(F), Asl1, Remis M and UK (M) isolates, respectively. Comparison of the BaMMV-Iks polyproteins with the corresponding proteins of BaMMV-Na1 isolates showed 95% amino acid sequence identity. The phylogenetic analysis revealed that Iks isolate was closely related to Na1 strain and have a common origin.

Keywords **:** *Barley mild mosaic virus*, capsid protein, fungus transmission, *Hordeum vulgare*, *Potyvirus*

Barley mild mosaic virus (BaMMV) and *Barley yellow mosaic virus* (BaYMV) has been identified as important causative agents for an economically important yellow mosaic disease of winter barley in East Asia and European countries (Peerenboom et al., 1997). These soil borne filamentous viruses belong to genus *Bymovirus* and family *Potyviridae*. These viruses are transmitted to plants exclusively by a vector, *Polymyxa graminis*, which is a characterized eukaryotic fungus-like microorganism. It survives in the soil as resting spores containing the viruses within the protective 'shells'. With the availability of host plant and the presence of favorable environmental conditions, *P. graminis* release a swimming spore called zoospores that invade and develop in the roots of barley and other cereal crops and cause the infection (Lee et al., 2006).

BaMMV has been isolated as one of the causative agent responsible for malting barley in Korea. Generally it infects the barley in association with BaYMV, so mild mosaic symptoms are similar to BaYMV. The virus has a single-stranded, positive-sense RNA genome with two components of approximately 7.2 and 3.5 kb. Each of the RNA encodes a polyprotein, which is subsequently processed into several putative smaller proteins, and the virus coat protein is located at the C-terminal region of the RNA1 (Kashiwazaki, 1996).

The presence of BaMMV has also been reported in the Japan, France, China, U.K and Germany. The complete genome sequence of these isolates including BaMMV-Na1 (Accession Numbers RNA1: D83408, RNA2: D83409), BaMMV-UK(F) (Accession Numbers RNA1: Y10973, RNA2: X90904), BaMMV-UK(M) (Accession Numbers RNA1: Y10974, RNA2: X84802), BaMMV-Asl1 (Accession Numbers RNA1: AJ242725, RNA2: X75933), BaMMV-Reims-M (Accession Numbers RNA1: L49381, RNA2: X82625), BaMMV-China (Accession Number RNA1: AJ224872), and BaMMV-Sil (Accession Numbers RNA1: AJ544267, RNA2: AJ544271) show similar genomic organization with potyviruses (Hariri et al., 2003; Kashiwazaki, 1996; Peerenboom et al., 1997; Timpe and Kühne, 1994; Zheng et al., 1999). However, only limited information is available about BaMMV Korean isolates (Jonson et al., 2006). Hence the present study has been carried out to determine the complete sequence of a Korean isolate of BaMMV-Iks and to establish the relationship to previously studied isolates.

Materials and Methods

Virus isolation and RNA extraction. BaMMV were collected from the infected barley fields from Iksan, South Korea and propagated in barley plants (*Hordeum vulgare* L.) by mechanical inoculation. Virus particles were purified

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Table 1. Oligonucleotide primers used to amplify the RNA 1 and RNA 2 of BaMMV

Primers	Sequences	Nucleotide positions	Restriction enzymes
S48(+)	CCCCGGTACCATCGATG-TAATACGACTCACTATA-GAAAAATAAAACAAAACTA CAACC	RNA 1, 1~23	*Kpn*I, *Cla*I
S49(−)	CCCACTCCCATGCGTC-CATCC	RNA 1, 5387~5408	
S67(−)	CTGCGTTATATGCTAAGGCT-GCCCGAGATCTAAA	RNA 1, 1319~1343	*Xba*I
S68(−)	TGGATACCGGATTGACGTCT-GTTA	RNA 1, 1427-1450	
S50(−)	AAAGGATCCGGTGCAGCGC-CAACGGGATA	RNA 2, 817~836	*Bam*HI
S51(−)	GTAATCCTGTTCAAGCGCAC-TAGC	RNA 2, 919~942	

from the infected leaves according to Usugi and Saito (1976).

RT-PCR. The purified RNA was used as a template for oligo (dT)-primed cDNA synthesis using a cDNA syntheisis system plus kit (Amersham) according to the manufacturer's protocol. Different parts of the BaMMV RNA1 and RNA2 were amplified using different primer pairs (S48, S49, S67, S68, S50, and S51) scanning virtually all of RNA1 and RNA2 designed using the published and unpublished sequence data (Table 1; Fig. 1). PCR reactions were carried out in a volume of 50 μl containing 200 ng of template, 20 pmol of each primer, 10 mM of deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, and 2.5 U *Taq* DNA polymerase (GIBCO). PCR reaction conditions for amplification was 1 cycle at 94°C for 3 minutes, 35 cycles for 1 minute at 94°C, 50°C for 1 minute and 72°C for 2 minutes and the final extension step of 72°C for 10 minutes. DNA complementary to the extreme 5' termini of RNA1 and RNA2 were amplified using the 5'-full RACE core kit (Takara Bio Inc) according to the manufacturer protocol.

PCR product and DNA sequencing. The PCR products were electrophoresed on 0.8% agarose gel, and the bands corresponding to the full lengths of RNA1 (7.2 kb) and RNA2 (3.5 kb) were excised from the gel. Eluted amplicons were ligated in a dephosphorylated, *Sma*I-cut pBluescriptKS(+) (Stratagene) vector and used to transform *Escherichia coli* JM109 cells according to the manufacturer protocol (Qiagen, USA). Recombinant plasmids were isolated by modified alkali method and the sizes of the cDNA inserts were measured electrophoretically.

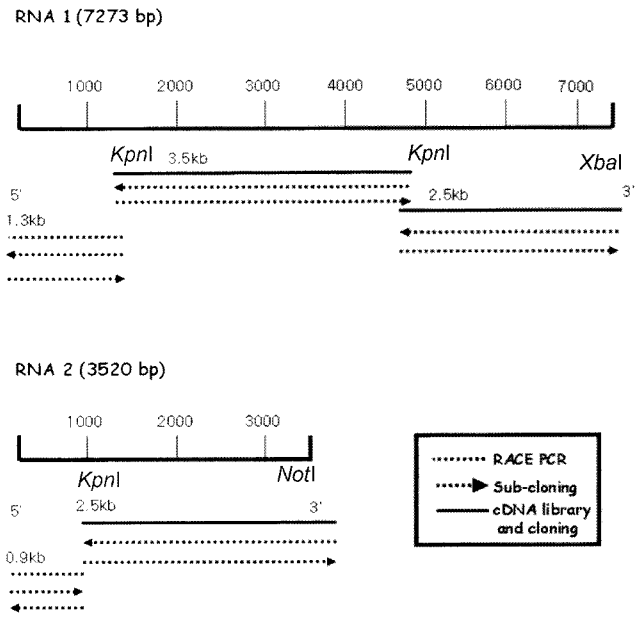

Fig. 1. Schematic sequencing strategy of the complete genome sequencing and sequence directions for BaMMV-Iks isolate.

The large clones (RNA1: 3.5 and 2.5kb, RNA2: 2.5 kb) were selected by restriction mapping and revealed to be from RNA1 and 2, respectively by partial sequencing and comparison of genomic organization with those of other bymoviruses. Later, cDNA inserts were subcloned using restriction enzyme fragments or exonuclease III-generated nested deletions. The DNA sequences were determined by the dideoxynucleotide chain termination method in a DNA sequencer (Model 377A, Perkin Elmer). All parts of the cDNA were sequenced in both orientations and the sequences were deposited in the GenBank under accession numbers AF536942 and AF536943 for RNA1 and RNA2, respectively.

Sequence Analysis. The nucleotide and amino acid sequences were analyzed using DNASIS software (Hitachi Software Engineering Co., Japan). Nucleotide and amino acid sequence alignment were carried out using GENETYX-WIN 4.0. (Software Development Co., Ltd) Phylogenetic analysis was performed employing Neighbor-joining method with Kimura 2-parameter as a model of nucleotide substitution in GENETYX-WIN 4.0. Bootstrap analysis was performed by using 100 pseudo replications.

Results and Discussion

The present study reports the molecular analysis of BaMMV isolated from Iksan, South Korea. BaMMV-Iks RNA1 consists of 7273 nucleotides, excluding the 3'poly (A) tail. Sequence analysis revealed a single large open

a) K G P I L **Q**^{294}↓ ^{295}**A** S Q K D

b) F T T I L **Q**^{361} ↓ ^{362}**S** G T S S

c) E I V E L **Q**^{1019} ↓ ^{1020}**S** K N S L

d) D D I T I **E**^{1084} ↓ ^{1085}**G** K G R A

e) S A A T L **E**^{1263} ↓ ^{1264}**G** M T M

f) K P R N L **Q**^{1476} ↓ ^{1477}**S** A P N T

g) E E T Y L **Q**^{2010} ↓ ^{2011}**S** G K D E

Fig. 2. Deduced amino acid sequences around the cleavage sites for the NIa Protease. The cleavage sites were marked as bold letters.

reading frame (ORF) starting with an AUG codon at position 149-151 and terminating with an UAG codon at position 6932-6934. The large ORF encodes for a polypeptide of 2261 amino acids with the calculated molecular weight of 256 kDa. Analysis of the amino acid sequences revealed a seven cleavage sites, leading to a putative division of polyprotein into eight mature proteins corresponding to the potyvirus proteins P3, 6K1, CI, 6K2 NIa-VPg, NIa-Pro, NIb and CP (Shukla et al., 1994). The first cleavage site glutamine-alanine (Q//A) was located at the amino acid position 294-295 and which cleaves the N-terminal P3 and 6K1 proteins. The protease cleavage site glutamine-serine (Q//S) was located at the amino acid positions 361-362 and 1019-1020 and which separates the smaller protein 6K1, CI and 6K2 proteins. The glutamic acid-glycine (E//G) cleavage site between the 6K2 and NIa-VPg protein was located at the position 1084-1085, whereas the site between the NIa-VPg and NIa-Pro protein was located at the position 1263-1264 (E//G) (Fig. 2). In most of the potyviruses, a cleavage site between NIa-VPg and NIa-Pro has glutamic acid at position 1, instead of glutamine which is the most common in other cleavage sites (Dougherty and Parks 1991). Similarly BaMMV-Iks isolate also has glutamic acid (1) at the cleavage site between NIa-VPg and NIa-Pro, as well as at the cleavage site between the 6K2 and NIa-VPg (Fig.2). The sixth and seventh putative cleavage sites (Q//S) was located at the position 1476-1477 and 2010-2011 respectively, which separates the proteins NIa-Pro, NIb and CP (Fig. 2). The protein responsible for these cleavage was NIa protease, hence it cleaves the protein at the Q//S, Q//A and E//G dipeptide sequences. The results are in consistent with the other researchers reported that the cleavage sites were similar in BaMMV isolates and other potyviruses (Kashiwazaki, 1996; Peerenboom et al., 1997; Shukla et al., 1994). Interestingly in BaMMV-Iks isolate, six of the NIa-Protease cleavage sites share a leucine amino acid at a position –2, whereas, in other reported BaMMV isolates only five of the cleavage site shares leucine amino acid at position –2.

A conserved motif (EPYx$_7$SPx$_2$LxAx$_2$NxGx$_2$Ex$_5$W) of the potyvirus P3 protein was also observed in BaMMV-Iks P3 protein and which shares amino acids sequence with the BaMMV-Na1 isolate. The nucleotide binding motif in the CI protein of BaMMV-Iks was observed at the amino acid position 454 to 461 and which showed similarity to the other BaMMV and potyviruses (Kashiwazaki et al., 1990; Shukla et al., 1994). The two smaller proteins (6K1 and 6K2) of BaMMV-Iks were located in the analogous position and showed similarity with other BaMMV isolates. The NIa-VPg protein of potyviruses has a consensus motif (NMY) with a tyrosine residue, which is necessary for the linkage of NIa-VPg protein to the viral RNA. Similarly NIa-VPg protein of BaMMV-Iks isolate also have a consensus motif (NMY) with tyrosine residue and which was located at the amino acid position 1144. The NIb protein of potyviruses contains the conserved stretches SGXXXTXXXNT and GDD; such stretches are thought to form the core of RNA-dependent RNA polymerase (Kamer and Argos, 1984). BaMMV-Iks Nib protein also have the consensus motif SGXXXTXXXNT-(32 aa)-GDD and which was located at the amino acid positions 1814-1824 and 1857-1859 respectively.

The complete nucleotide sequence of BaMMV-Iks RNA2 consists of 3520 nucleotides excluding the 3' poly (A) tail. The sequence predicts a single large ORF starting with a sequence of three AUG codon at positions 142-150 and terminating with the UAA stop codon at position 2824-2826. The large ORF (2685 nucleotides) encodes for the putative polyprotein comprises 894 amino acids excluding the stop codon. The polyprotein has a predicted molecular weight of 98 kDa. Analysis of amino acid sequences revealed a putative cysteine-histidine protease domain (GFCY and HV motif) at positions 115-118 and 189-190 respectively. The putative glycine-alanine (G//A) cleavage site was located at the amino acid position 229-230, hence upon cleavage, the polyprotein could produce a 25 kDa N-terminal (putative proteinase) and 73 kDa C-terminal protein. The results are in agreement with the other researchers reported a putative cysteine-histidine protease domain and G//A cleavage sites within the ORF of BaMMV and other potyviruses (Dessens et al., 1995; Kashiwazaki, 1996; Peerenboom et al., 1996; Timpe and Kühne, 1994).

The 5' untranslated region (UTR) of BaMMV-Iks RNA1 (148 nt) and RNA2 (141 nt) are similar in length and shares extensive sequence similarity, interestingly the first 36 nucleotide sequence are completely identical between the two strands. The 5' UTR of both RNAs contains relatively few G residues (6.7 and 9%), which was consistent with the sequence of several other plant viruses (Gallie et al., 1987). The 3' UTR of RNA1 (339 nt) and RNA2 (700 nt) are greatly differ in length and have no sequence similarity, but the strands share a polyadenylation signal UAUGU at nucleotide position 7188-7192 (RNA1) and 3501-3505 (RNA2) respectively. The results are in agreement with the earlier workers reported the ployadenylation signal in the 3' UTR of potyviruses (Peerenboom et al., 1996).

Table 2. Percentage of nucleotides and amino acids sequence identity of RNA1 and RNA2 among BaMMV strains

Strain	BaMMV-Iks	BaMMV-Remis M	BaMMV-Sil	BaMMV-Asl1	BaMMV-Na1	BaMMV-UK(F)	BaMMV-UK(M)	BaMMV-China
a. P3 Protein region of RNA1								
BaMMV-Iks	...	86.4*	80.0	86.0	93.2	86.4	86.7	
BaMMV-Remis M	89.2*	...	97.9	99.0	87.8	99.3	99.7	
BaMMV-Sil	89.9	94.8	...	99.0	86.4	98.6	98.3	
BaMMV-Asl1	90.1	95.1	98.9	...	87.4	99.7	99.3	
BaMMV-Na1	93.7	89.7	90.0	90.2	...	87.8	88.1	
BaMMV-UK(F)	89.7	99.0	95.8	96.0	90.1	...	99.7	
BaMMV-UK(M)	89.7	99.3	95.5	95.8	90.1	99.8	...	
b. CI Protein region of RNA1								
BaMMV-Iks	...	96.2	96.3	96.8	98.9	96.8	96.7	
BaMMV-Remis M	89.5	...	95.6	96.0	95.9	98.8	99.2	
BaMMV-Sil	88.4	88.7	...	99.2	96.0	96.5	96.4	
BaMMV-Asl1	88.5	88.6	98.0	...	96.5	97.0	96.8	
BaMMV-Na1	93.5	98.1	87.7	87.9	...	96.5	96.4	
BaMMV-UK(F)	89.9	99.0	89.4	89.2	89.4	...	99.5	
BaMMV-UK(M)	89.9	99.4	89.2	89.0	89.4	99.5	...	
c. NIa-VPg protein region of RNA1								
BaMMV-Iks	...	93.3	92.7	95.0	94.4	93.9	93.3	
BaMMV-Remis M	87.3	...	93.3	96.1	92.7	99.4	100	
BaMMV-Sil	85.3	89.6	...	97.2	92.7	93.9	93.3	
BaMMV-Asl1	86.9	89.8	97.0	...	95.0	96.6	96.1	
BaMMV-Na1	88.8	87.0	87.3	88.3	...	93.3	92.7	
BaMMV-UK(F)	87.7	99.1	89.6	89.8	87.2	...	99.4	
BaMMV-UK(M)	87.3	99.6	89.2	89.4	86.6	99.1	...	
d. NIa-Pro protein region of RNA1								
BaMMV-Iks	...	97.7	98.1	98.6	98.1	97.7	97.7	
BaMMV-Remis M	88.8	...	98.1	98.6	97.7	100.0	100.0	
BaMMV-Sil	89.3	90.1	...	99.5	98.1	98.1	98.1	
BaMMV-Asl1	89.8	91.4	98.3	...	98.6	98.6	98.6	
BaMMV-Na1	88.6	87.8	88.7	88.6	...	97.7	97.7	
BaMMV-UK(F)	88.6	98.1	90.6	91.1	87.8	...	100	
BaMMV-UK(M)	88.2	98.1	90.3	90.8	87.5	99.7	...	
e. NIb protein region of RNA1								
BaMMV-Iks	...	95.7	97.0	97.2	97.8	95.5	95.7	
BaMMV-Remis M	89.6	...	96.8	97.0	96.4	98.9	99.6	
BaMMV-Sil	88.9	90.8	...	99.4	96.4	96.8	96.8	
BaMMV-Asl1	89.1	90.5	97.3	...	96.6	97.0	97.0	
BaMMV-Na1	94.3	89.3	88.0	87.9	...	96.3	96.4	
BaMMV-UK(F)	90.1	98.9	90.7	90.5	89.5	...	98.9	
BaMMV-UK(M)	89.9	99.2	90.8	90.4	89.7	98.9	...	

Table 2. Continued

Strain	BaMMV-Iks	BaMMV-Remis M	BaMMV-Sil	BaMMV-Asl1	BaMMV-Na1	BaMMV-UK(F)	BaMMV-UK(M)	BaMMV-China
f. Coat protein region of RNA1								
BaMMV-Iks	...	92.8	92.8	92.8	97.2	93.2	92.8	94.5
BaMMV-Remis M	87.8	...	96.0	96.0	94.0	99.6	99.6	89.3
BaMMV-Sil	87.0	89.9	...	99.2	94.0	96.4	95.6	89.7
BaMMV-Asl1	87.5	89.9	97.3	...	94.0	96.4	95.6	90.1
BaMMV-Na1	95.8	89.4	88.2	88.7	...	94.4	93.6	93.3
BaMMV-UK(F)	87.8	99.2	89.9	89.9	89.4	...	99.2	89.7
BaMMV-UK(M)	88.0	99.6	90.0	90.0	89.5	99.1	...	89.3
BaMMV-China	94.9	88.1	87.0	87.1	94.3	88.1	88.4	...
g. 5' and 3' non coding region of RNA1								
BaMMV-Iks	...	92.9‡	91.2	91.8	95.3	92.6	92.9	
BaMMV-Remis M	81.6§	...	95.6	95.6	90.3	99.4	99.7	
BaMMV-Sil	83.0	89.7	...	98.5	90.3	95.3	95.6	
BaMMV-Asl1	84.8	89.7	95.9	...	91.2	95.3	95.6	
BaMMV-Na1	95.3	83.7	85.0	86.8	...	90.0	90.3	
BaMMV-UK(F)	81.1	96.6	91.8	89.1	82.4	...	99.7	
BaMMV-UK(M)	80.4	97.3	90.5	89.1	82.4	97.3	...	
h. P1 protein region of RNA2								
BaMMV-Iks	...	87.3*	89.0	89.0	96.1	89.9	87.7	
BaMMV-Remis M	85.3†	...	94.3	94.3	89.9	96.9	98.7	
BaMMV-Sil	85.1	85.4	...	99.1	91.7	97.4	94.7	
BaMMV-Asl1	85.0	85.3	98.4	...	91.7	97.4	94.7	
BaMMV-Na1	94.0	84.8	85.0	85.0	...	92.5	90.4	
BaMMV-UK(F)	86.0	97.8	86.0	85.9	85.9	...	97.4	
BaMMV-UK(M)	85.0	98.4	85.6	85.4	84.8	97.4	...	
i. P2 protein region of RNA2								
BaMMV-Iks	...	87.5	90.3	91.0	93.7	91.3	87.1	
BaMMV-Remis M	84.8	...	87.0	87.8	88.8	95.6	96.0	
BaMMV-Sil	83.0	81.3	...	99.0	89.9	91.6	86.3	
BaMMV-Asl1	83.3	82.7	98.7	...	90.5	92.5	87.5	
BaMMV-Na1	91.0	84.4	82.8	83.0	...	91.3	88.5	
BaMMV-UK(F)	84.5	97.6	83.6	84.1	83.6	...	94.9	
BaMMV-UK(M)	84.5	98.2	80.8	82.4	84.0	97.6	...	
j. 5' and 3' non coding region of RNA2								
BaMMV-Iks	...	94.3‡	93.1	93.8	96.8	93.7	93.8	
BaMMV-Remis M	71.4§	...	93.2	94.0	92.9	98.9	98	
BaMMV-Sil			...	98.7	92.8	93.1	92.1	
BaMMV-Asl1	75.2	77.9		...	93.3	93.6	92.8	
BaMMV-Na1	92.1	72.0		75.2	...	92.3	92.5	
BaMMV-UK(F)	74.4	96.4		80.9	74.4	...	97.8	
BaMMV-UK(M)	71.4	97.1		77.1	71.2	95.0	...	

*Each value above diagonal is amino acid sequence similarity and †below diagonal is nucleotide sequence similarity.
‡Each value above diagonal is the nucleotide sequence similarity in 3' UTRs and §below diagonal is the nucleotide sequence similarity in 5' UTRs.
Percent identity was calculated with the Genetyx-win Homology Search Program which made an optimal alignment of sequences.

The nucleotide and amino acid sequences of the BaMMV-Iks isolate were compared with the other reported BaMMV isolates (BaMMV-Remis M, BaMMV-Sil, BaMMV-Asl1, BaMMV-Na1, BaMMV-UK (F), BaMMV-UK (M) and BaMMV-China) and the results were presented in the Table 2. The complete nucleotide and deduced amino acid sequence of BaMMV-Iks RNA1 exhibited a high identity with Na1 (92.9% nt and 96.4% aa) isolate followed by Asl1

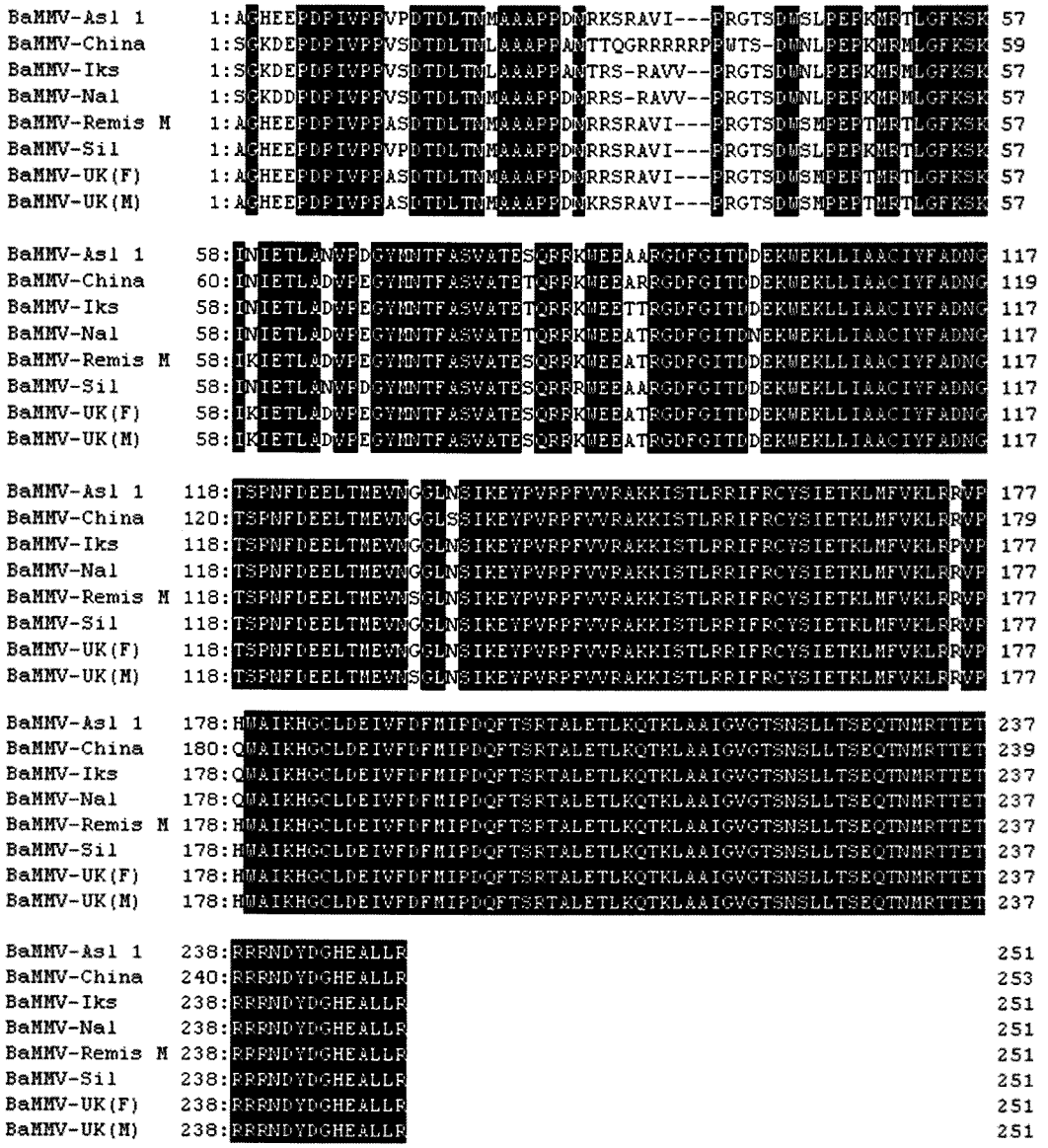

Fig. 3. Deduced amino acid sequences alignment of capsid proteins of BaMMV-Iks and other BaMMV isolates.

(88.1% nt and 94% aa), UK-F (87.8% nt and 93.7% aa), UK-M (87.6% nt and 93.6% aa), Remis M (87.6% nt and 93.5% aa), and Sil (87.4% nt and 92.5 aa) isolates respectively (Table 2a-g). The degree of the identity was extremely high while considering the variable nature of ssRNA. The BaMMV-China isolate had not been included in the entire RNA1 sequence alignment because only the CP sequence of the isolate had reported in the database. Hence the available CP region was compared with the Iks isolate, which exhibited 94.9% nucleotide 94.5% amino acid similarity (Table 2f).

The alignment of the entire CP deduced amino acid sequences of the isolates showed some differences which are scattered in all the regions of sequence (Fig. 3). These differences can be due to the high natural variability of RNA viruses, which lack an RNA repair system (Kashiwazaki *et al.* 1990). Numerous studies have reported that the capsid protein sequence identity was successfully used a parameter for the taxonomy of potyviruses, the average identity (93.7%) between the capsid proteins of BaMMV-Iks and other BaMMV isolates corroborates to the average identity (95%) reported for different isolates of potyviruses (Shukla and Ward 1988; Shukla et al., 1994).

The complete nucleotide and amino acid sequence of the BaMMV-Iks RNA2 was also compared with the other isolates and the results were presented in the Table 2h-j. Like RNA1, the nucleotide and amino acid sequences of RNA2 also exhibited high degree of similarity with Na1 (92.3% nt and 95.5% aa) isolate followed by UK-F (81.6% nt and 91.6% aa), Asl1 (81.1% nt and 91.2% aa), Remis M

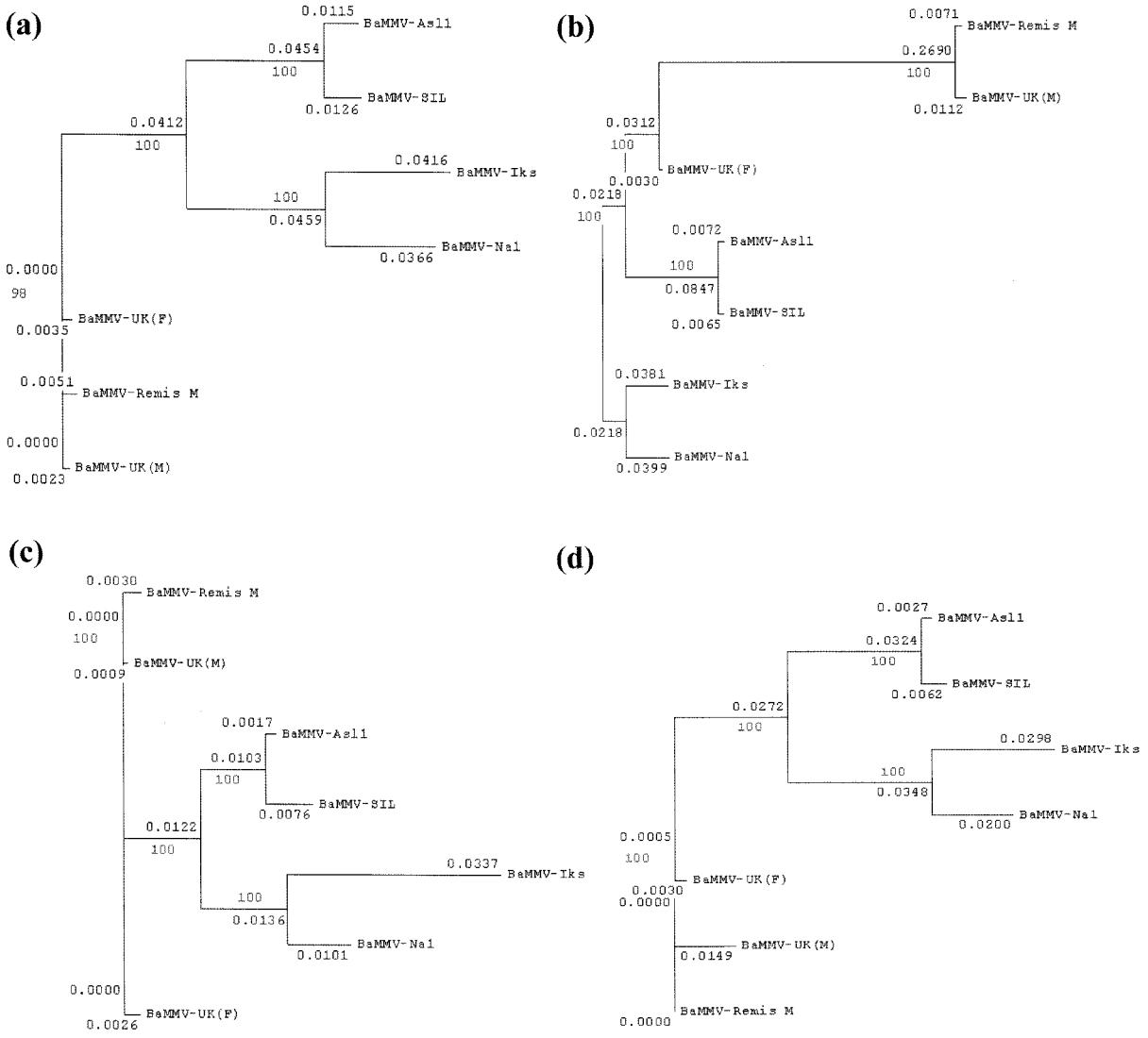

Fig. 4. Neighbor-joining Bootstrap method in GENETYX-WIN 4.0, illustrating phylogenetic relationship based on the multiple alignments of nucleotide and amino acid sequences of RNA1 and RNA2 from 7 distinct isolates of BaMMV. The bootstrap values are shown at the Individual nodes. The number near the branch nodes are branch lengths. (a) Complete nucleotide sequence of RNA1. (b) Complete nucleotide sequence of RNA2. (c) Deduced amino acid sequence of polyprotein of RNA1. (d) Deduced amino acid sequence of polyprotein of RNA2.

(80.5% nt and 89.7% aa) and UK-M (80.3% nt and 89.5% aa) isolates respectively. The complete nucleotide sequence of BaMMV-Sil RNA2 has not been reported in the database hence the available P1, P2 and 3' UTR part of the sequences were compared with the Iks isolate and which also showed high degree of similarity Table 2h-j. Comparison of sequence differences between isolates in the CP and P2 region shows that variation was greater in the P2 region than in the CP region. The variation in the P2 region might associated with the differences in the virulence of the isolate.

Comparison of the 3' non coding regions of both RNA1 and RNA2 with BaMMV Nal isolate exhibited a high sequence similarity (95.3 and 96.8%) and, these values were within the range (83-99%) as reported for potyvirus strains. Similarly, the 5' UTR of RNA1 and RNA2 of BaMMV-Iks exhibits high similarity (95.3 and 92.1%) with BaMMV Nal isolate. Phylogenetic tree derived from nucleotide and amino acid sequences also revealed that BaMMV-Iks isolate was closely related to BaMMV Nal isolates (Fig. 4).

Numerous studies have reported that after serial mechanical transmission, the BaMMV had lost the ability to be transmitted by fungus because of some nucleotide deletions in RNA2 (Dessens et al., 1995). Such deletions in the C-

proximal part of the RNA2 suggested the possible role of P2 protein in fungal transmission. Timpe and Kühne (1995) reported that the deleted form of RNA2 appeared after 10 mechanical passages and become predominant in subsequent passage. Similarly such deletions were also observed in BaMMV-Iks isolate and the possible deletions were located in the P2 regions of the mechanically transmitted isolates (data not shown). However, the effect of this possible deletion on the fungal transmission remains to be examined.

To conclude, the nucleotide and amino acid sequence similarities between the BaMMV-Iks and other BaMMV isolates in their capsid protein, non structural protein, 5' and 3' non coding regions and phylogenetic analysis supports the classification of BaMMV-Iks isolate as a strain of the same virus.

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