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COMPLETE NUCLEOTIDE SEQUENCE ANALYSIS OF RNA2 AND PHYLOGENETIC ANALYSES OF A PAPRIKA ISOLATE OF BROAD BEAN WILT VIRUS IN KOREA

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A B S T R A C T

An isolate of *Broad bean wilt virus 2* (BBWV 2), which was isolated from paprika showed spotty fruits. Host range of BBWV 2 was nine species including *Chenopodium amaranticolor*, and could be characterized by the symptoms include chlorotic local and distortion on upper leaves of *C. amaranticolor. Datura stramonium* induced chlorotic spot on uninoculated upper leaves. The purified virus particles were isometric about 25 nm in diameter. The genomic RNA of this virus consisted of two components of about 6.3 kb and 4.5 kb molecules and coat protein contained two polypeptide species which calculated with about 42 kDa and 22 kDa. We determined the complete sequence of RNA2 from *Broad bean wilt virus* 2 isolate Pa. The complete nucleotide (nt) sequence of RNA 2 for BBWV 2-Pa was 3,597 nt in length. encoding a pupative movement protein (MP) and coat protein (CP). Between BBWV 2-Pa and other serotype II favaviruses, the overall amino acid similarities in the polyprotein coding regions was and 84%-93% for RNA2. In the 5′UTR, the nucleotide similarity was 65%-92%. For the 3′UTRs, they are 38%-60%, respectively. For the Genome organization of BBWV 2-Pa was similar to other comoviruses in the *Comoviridae*.

Keywords: *Comoviridae, broad bean wilt virus 2, complete nucleotide sequence, paprika*.

INTRODUCTION

Broad bean wilt virus (BBWV) has isometric virions of about 30nm in diameter. The virus was first isolated from broad bean (*Vicia faba* L.) in Australia (Stubbs 1947). It is designated as the type member of the genus Favavirus (Murphy *et al.,* 1995). Two viruses, BBWV and *Nasturtium ringspot virus* (NRSV) have been reported from pepper in Morocco, Italy and Argentina (Boccardo *et al.,* 1973; Conti *et al.,* 1972). BBWV causes mild leaf mottle and concentric rings of the fruits similar to symptoms caused by CMV in peppers, but more severely (Lockhart and Fisher 1977; Yankulova and Kaitazova 1979). NRSV is considered to be a strain of BBWV, which was isolated from pepper showing mosaic and ring spot with mosaic symptom in Italy (Conti *et al.,* 1972). The pepper isolate of BBWV does not infect *Tropaeolum majus,* whereas the pepper isolate of NRSV produces ring spot type mosaic on *T. majus* (Boccardo and conti 1973; Lockhart and Fisher 1977). These two viruses resemble *Cucumber mosaic virus* and *Cowpea mosaic virus* in host plant reactions, and morphological and chemical properties. However, BBWV produces systemic mottle on *Vigna unguiculata* in addition to the typical local lesions produced by CMV. BBWV induces a specific tube (Vega *et al.,* 1980; Choi *et al.,* 2001) and comb structures (Choi *et al.,* 2001).

BBWV, which is transmitted by aphids in a nonpersistent manner, is one of the causal agents of economically important diseases of vegetables and ornamental plants. With double immunodiffusion tests (Uyemoto and Provvidenti 1974), isolates of BBWV could be separated into two serotype, I and II. They were subsequently reclassified with BBWV 1 and BBWV 2 because of distinct sequences (Goldbach *et al.,* 1995).

The genome of BBWV consist of two species of positive sense single-strand RNAs, RNA 1 and RNA 2. These are 5,951 to 5,989 nt and 3,569 to 3,607 nt in length respectively, excluding the 3′ terminal poly (A) tail. The 5′UTR of RNA 1 shares 56.6% nucleotide sequence identity with that of the 3′UTR, with the highest identities being within the first 95 nucleotides (89%)

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and nucleotides 149 to 192 (97%); no significant homology was observed between the 3′NCRs of RNA 1 and RNA 2 (Qi *et al.,* 2000). RNA 1 encodes a 210 kDa polyprotein, that may be proteolytically cleaved to yield a putative protease cofactor (Co-Pro, 38 kDa), a nucleotide triphosphate (NTP)-binding protein (NTBM, 67 kDa), a viral genome-linked protein (Vpg, 3 kDa), a protease (Pro, 23 kDa) and a RNA-dependent RNA polymerase (RdRp, 79 kDa) (Koh *et al.,* 2001). RNA 2 encodes a 119 kDa or 104 kDa polyprotein, that is cleaved at Q/G residues and Q/A residues, to release three mature proteins: a N-terminal 53 or 37 kDa protein designated as VP53 and VP37, a large coat protein (44 kDa) and a small coat protein (22 kDa). The VP53 and VP37 proteins are C-coterminally overlapping proteins resulting from two potential translation initiation sites on RNA 2.

MATERIALS AND METHODS

Virus source and isolation: In 2003, paprika (*Capsicum annuum* var. grossum) fruits showing irregular black figures with large ringspot were collected from a glass house in Hwasung, Kyounggi province. A virus was isolated from the paprika fruits. After tree repetition of single local lesion transfer on *Chenopodium quinoa*, biologically pure isolate was maintained in *N. occodentalis*.

Host range and symptomatology: The virus infected leaves were homogenized in 0.01M sodium phosphate buffer (pH 7.0) and rubbed to the healthy indicator plants leaves, after dusting 600 mesh carborundum powder. The indicator plants were rinsed with tap water immediately after inoculation, and maintained for visual inspection of virus symptoms in the glasshouse for at least 3 weeks.

Double-strand RNA (dsRNA) analysis: DsRNA was extracted from the virus infected leaves of *N. occidentalis* by the similar procedure of Morris and Dodds (1979) using CF-11 cellulose column chromatography. Eluted dsRNAs were precipated by ethanol and sodium acetate. The pellet was rinsed with 80% ethanol, dried under vacuum, and then dissolved in RNase free water. Total dsRNAs were load on 6% polyarcrylamide (arcylamide : bis-arcrylamide, 30 : 1) slab gel containing 1×TBE buffer (1M Tris, 0.83M boric acid and 10mM EDTA, pH 8.0). The dsRNA bands were visualized by silver staining with silver stain kit (Bio-Rad, USA).

Purification: BBWV 2-Pa isolate was propagated in *N. occidentalis.* The virions were purified using a modification of the method described by Xu *et al.,* (1988). The virus infected leaves were homogenized with three volume of 0.1 M phosphate buffer, pH 7.0 in a chilled blender, and the homogenate was pressed through two layers of dampened Miracloth. The extracts were clarified by centrifugation at 15,000 g for 20 min. After centrifugation, the supernatant was stirred for 10 min with 0.7 volumes of chloroform and n-butanol (1:1, v/v). The clear aqueous phage after centrifugation at 10,000 g for 20 min was stirred with 4% polyethylene glycol (mol. wt 8,000) and 0.2 M NaCl, respectively. The mixture was centrifuged at 12,000 g for 15 min, and the pellets were resuspended in 0.01 M phosphate buffer, pH 7.0. After clarification of the suspension by centrifugation at 10,000 g for 15 min at 4℃, the suspension was layered on a sucrose cushion. After centrifugation at 150,000 g for 3 hr, the pellets were resuspended in 0.01 M phosphate buffer, pH 7.0. To get a higher purity of virus, further centrifugation was conducted through a linear sucrose gradient. After purification, the particle morphology of BBWV 2-Pa was observed using transmission electron microscope (Carl Zeiss LEO 905, Germany) operating at 80 kV.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE): The molecular weight of coat protein for the purified viruses of BBWV 2-Pa was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The purified virus (1 mg/ml) was mixed with equal volumes of sample buffer (0.125 M Tris-HCl , pH 6.8, 20% glycerol, 4% SDS, 10% ß-mercaptoethanol and 0.002% bromophenol blue) and then heated for 3 min at 100℃. The sample was electrophoresed at 100 V for 1hr using a 4% stacking gel on a 12% discontinuous polyacrylamide slab gel containing 0.1% SDS following the method of Laemmli and Farre (1973). Coat protein bands on the gel were stained with Coomassie brilliant blue R 250.

Extraction of viral RNA from partially purified virions: Viral RNA was extracted from the purified virions by using a modification of the method described by Ryu *et al.,* (1995). The viral RNA precipitated with half volume of 7.5 M ammonium acetate and 2.5 volumes cold absolute ethanol. This mixture was stored at −70℃ for 2 hr. After centrifugation at 14,000 rpm for 20 min, the precipitate of viral RNA was rinsed with 80% ice-cold ethanol and centrifuged at 14,000 rpm for 10 min. The pellets were dried in speedvac for 5 min and then resuspended in 50 µl of DEPC-treated distilled water. It was divided into small aliquots, and the stored at −70℃.

Full-length of RNA2 sequencing: 5′ end cDNA synthesis was performed according to the methods described in the GIBCO BRL Protocols and Applications Guide. For first strand synthesis, approximately 1.5 μg - 3.0 μg (in total volume 20 μl) of each viral RNA of BBWV 2-Pa was denatured for 2 min at 70°C in a reaction volume containing 1 μl of gene specific primer (GSP), SMART II oligo (5′-CGTATCTCACCCTGTCGGCGCTC-3′) and 5 μl of dH_2O. After cooling on ice for 10 min, the following first strand cDNA synthesis mixture [1×first strand reaction buffer, 0.5 mM DTT, 1 mM dNTP, 0.8 units RNase inhibitor, 0.2 unit AMV (Takara)] was added and incubated at 42°C for 1 hr. For amplification of cDNA, 48 μl of the PCR reaction mixture [1×*Taq* polymerase buffer (TaKaRa), 2 mM dNTP, 5 mM $MgCl_2$, 0.5 μM Universal primer mix (UPM; Clontech), 0.1 μM, Gene specific primers (GSPs; BBWV 2-Pa, BR9) and EX-*Taq* DNA polymerase (TaKaRa)**]** was added to the 2 μl of the first strand cDNA solutions. PCR cycling consisted of 94°C for 40 sec, 52°C for 40 sec and 68°C for 10 min (35 cycle). The last cycle was followed by a prolonged extension for 20 min at 68°C to complete synthesis of the cDNA products. The amplified DNAs were purified for nucleotide sequence determination.

Primers for full-length sequencing of BBWV 2-Pa (Table 1) were designed corresponding to BBWV-P137 [AF228423]. RT-PCR was carried out with primer pairs from viral RNA extracted from purified virus particles by SDS-phenol extraction methods. The relative position of the pairs in the BBWV 2 genomes are schematically shown in Fig. 1 and each PCR products were used s a templates for sequencing.

RT-PCR was performed in the same tube of 50 μl reaction mixture containing 500 ng of purified viral RNA, 10 μl 5×reaction buffer, 10 ng of reverse primer and forward primer, 1 mM deoxynucleotide triphosphate (dNTP), 10 unit of RNase inhibitor, 10 unit of AMV reverse transcriptase (Takara) and 5 unit *Ex Taq* polymerase (Takara). The RT-PCR cycle was as follow: 50°C 45 min, 94°C 2 min (one cycle); 94°C 30 sec, 50-55°C 30 sec, 72°C 2 min (40 cycle); 72°C 10 min. The synthesized RT-PCR products were directly analyzed on 1% agarose gel with 1 kb DNA ladder (Promega). Each templates was purified by MicroSpin TM S-400 HR Columns (Amersham Biosciences).

Full-length cDNAs were sequenced by using ABI PRISM BigDye™ Terminator v 3.0 Cycle Sequencing Kits (Roche) with the eight templates directly and designed primers. Sequencing reactions were run on a fluorscent DNA sequencer ABI3100 (Perkin-Elmer). Sequences were analyzed using the Sequencer program (Gene Code Corporation, USA).

Analysis of nucleotide and the deduced amino acid sequences of BBWV 2-Pa were performed by using the DNAStar (Medision, WI, USA) and DNAman Sequence Analysis Program (Version 5.1, Lynnon Biosoft Co., Canada). The nucleotide and amino acid sequences of BBWV 2-Pa and other seven strains of BBWV and those of other viruses of comovirus group were surveyed using Genbank site (http;//www.ncbi.nih.gov).

RESULTS

Host reactions and symptomatology: The host range and symptomatology of BBWV 2-Pa were summarized on Table 2. BBWV 2-Pa systemically infected on eight indicator plants including *C. amaranticolor*. However, no infection was occurred *on N. glutinosa*. In *C. annuum*, BBWV 2-Pa produced chlorotic spot on the inoculated leaves, and vein clearing and distortion on the upper leaves folded by vein necrosis in a week after inoculation.

CP and dsRNA analyses: The purified virus particles of BBWV 2 were isometric with 25 nm diameter (Fig. 2-D). The CP of BBWV 2-Pa had the electrophoretic homogeneity on SDS-PAGE. There were two major protein bands with molecular weights of 42 kDa and 22 kDa estimated by molecular marker proteins (Fig. 3-A). Molecular sizes of BBWV 2-Pa dsRNAs were at approximately 7.0 kb, 6.3 kb and 3.6 kb, respectively (Fig. 3-B).

Sequence analyses: The nucleotide sequence of BBWV 2-Pa RNA 2 was found to consist of 3,596 nucleotides including a single ORF. The base composition of RNA 2 for the virus was 28.3% A, 18.5% C, 23.4% G and 29.8% U. The ORF started at nucleotide 231 and extended to nucleotide 3,422 encoding a polyprotein precursor, which encodes 1,064 amino acid residues with a calculated Mr of 118.6 kDa. The amino acid sequences of the N-terminal region of the 22 kDa small coat protein (SCP) and that of 44 kDa large coat protein (LCP) were Ala-His-Ala-Thr-Pro-Lys and Gly-Leu-Met-Glu-Glu-Asp, respectively. The results of amino acid sequencing indicated that the positions of cleavage sites between the 52 kDa protein and the 44 kDa CP, and between the 44 kDa and 22 kDa CPs are Gln/Gly and Gln/Ala, respectively (Fig. 4). The predicted N-terminal encoding

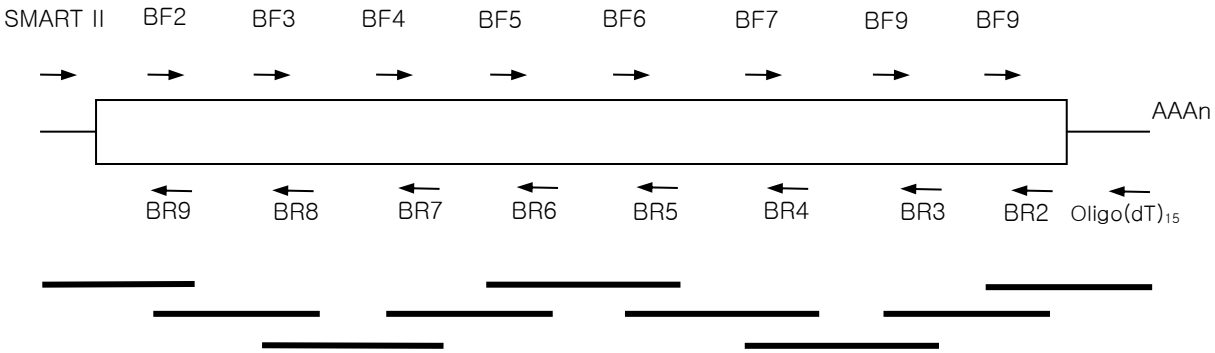
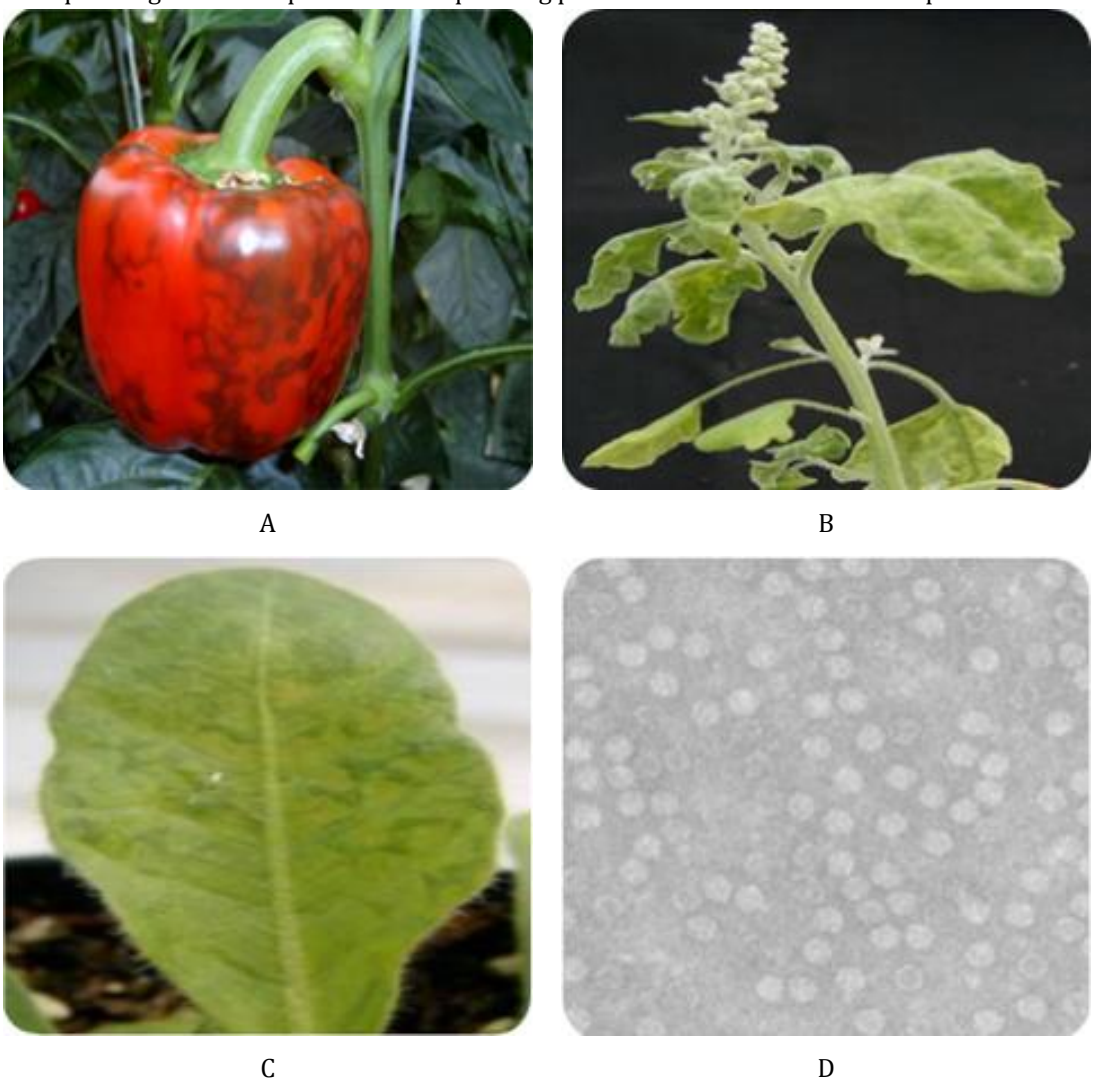


Figure 1. Schematic representation of nucleotide sequencing strategy for BBWV 2-Pa. Horizontal lines represent templates for sequencing. Arrows represent the sequencing position and orientation of each primers.



A B

C D

Figure 2. Symptomatology and purified virus particles of BBWV 2-Pa. Irregular black spot on the paprika fruits (A) and chlorotic spots on the C. quinoa (B). Mosaic and vein clearing of *N. occidentalis* (C). Electron micrograph of purified virus particles with 25 nm diameter (D). Bar represents 100nm.

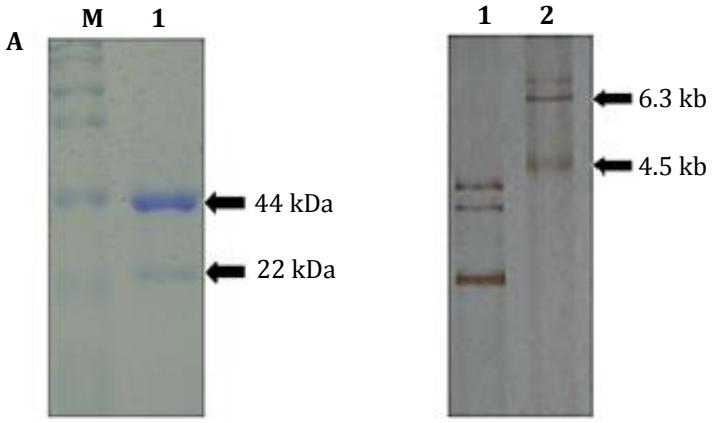


Figure 3. Electrophoresis of coat protein and genomic RNA of BBWV 2-Pa. Molecular weights of coat protein of BBWV 2-Pa were 44 kDa (LCP) and 22 kDa (SCP) Lane (A). M, Standard protein (Novagen); 1, The molecular weight of purified coat protein of BBWV 2. Electrophoresis of genomic RNA of BBWV 2-Pa had two major bands of 6.3 kb and 4.5 kb (B). Lane 1, CMV-Mf; 2, BBWV 2-Pa.

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1       TGTTTTAATAAAATATTAAAACAAACAGCTTTCGTTCCGAGAAAATAGCTTTCAGTTACAAAATAGCTTTCAGTTTCACA
81      ACAGCTTTCAGACACTTTGAAGTTTCAAATTGAACCCGGAAAAAGGGAGTGTGATTCAAAGCGCACCATATATTTTGGAA
                                                          Movement protein (MP)
161     CTTCTTTTTTTTTATTTTCTTTTCTTGCAAGTGCATTCCACATTCAAGATTTGATTTTGAACATGCTGTAATGCGTCCCG
                                                                  M  R  P
241     AACTTGTTGCAGCGCTAGATAGATATTTCTCAGAAATCATAAGTTGCTTCTTTCTGGGCTGGTTACTAAACTTTCTGCTT
      E  L  V  A  A  L  D  R  Y  F  S  E  I  I  S  C  F  F  L  G  W  L  L  N  F  L  L
321     GTTTGGCTTTGTTCCACAAAAAGCACTTTTCTTTTATGGTCAGTATTTTTATACATTTGTTACTACATATTGAGGATTGA
         V  W  L  C  S  T  K  S  T  F  L  L  W  S  V  F  L  Y  I  C  Y  Y  I  L  R  I  E
401     ATTTGCATATATCGTTGCACCCTTCTTAAAAACGATATACACAAATAGTTCTCAGTATCATACTGTAGACTGGGCAAACG
       F  A  Y  I  V  A  P  F  L  K  T  I  Y  T  N  S  S  Q  Y  H  T  V  D  W  A  N
481     CTTATACGGCATTGCCTAAAGGGTTGTGGGAGCAAATCACCGATTACAATTACTGTTACAATTTCCCTCCTCCTTGCGTC
    A  Y  T  A  L  P  K  G  L  W  E  Q  I  T  D  Y  N  Y  C  Y  N  F  P  P  P  C  V
561     GAAGGTTTTGTGTCTGATTTTTCACCGAGATTCACACTTAAAGAACTTGAAATCATGAATGAGGCAAATATCACTCCAGT
     E  G  F  V  S  D  F  S  P  R  F  T  L  K  E  L  E  I  M  N  E  A  N  I  T  P  V
641     GCATACTATTCCAAAAGACACATTGTTTAATTGCGGGGGTGGTTATGGGGTCGCTGTGGAGAGCAAGAAATCCATCTTAC
          H  T  I  P  K  D  T  L  F  N  C  G  G  G  Y  G  V  A  V  E  S  K  K  S  I  L
721     CAAGAGTGCAAGATTTGTATGAAATGGATAATTGGCACAACTTGAGAAGCAAATTGAGCAAAAATGTTCCTAGTTACGTC
      P  R  V  Q  D  L  Y  E  M  D  N  W  H  N  L  R  S  K  L  S  K  N  V  P  S  Y  V
801     ACAACGTCAGAGATTGCTGTTGGTGCAATGTCAGGTGCAGGAAACACCAAATTGGCGATACCAGTCGTGGAGAAGTACAC
         T  T  S  E  I  A  V  G  A  M  S  G  A  G  N  T  K  L  A  I  P  V  V  E  K  Y  T
881     TGAGGAAGTGGCTGATGACAGGTTACCAGACAAGGTTCGTGCCAAAGCTGATCAGATAATGGTCGCAGCTATTGAGTTGG
        E  E  V  A  D  D  R  L  P  D  K  V  R  A  K  A  D  Q  I  M  V  A  A  I  E  L
961     TGGCGGATGGTTTTGCTTCAGTCAATTCAGACGTTACCATGGCTGGTGCACTCTATGACAAGCGCCACAAGACCATTGCA
     V  A  D  G  F  A  S  V  N  S  D  V  T  M  A  G  A  L  Y  D  K  R  H  K  T  I  A
1041    AGTTCTTTCAAGGGAGCTTTTGCATCTCGGGCAAGTGGAGTCCCTTCACATGTAATTTATTTTCCAATGCATAGAGTCCC
         S  S  F  K  G  A  F  A  S  R  A  S  G  V  P  S  H  V  I  Y  F  P  M  H  R  V  P
1121    AGCATGTGATGATCCGAACACTACCTTGGAACTTTCAATGGTAAGTCGAGATTCAGATTTTGATGAGGGTTACACACTGG
            A  C  D  D  P  N  T  T  L  E  L  S  M  V  S  R  D  S  D  F  D  E  G  Y  T  L
1201    CCAACATATCAGCTCGCACCCTTTACGTTCGAGCAAAAGGACCTGAAAAGGTGACTGAAACAAGGCATCTTTTGAAAGCC
     A  N  I  S  A  R  T  L  Y  V  R  A  K  G  P  E  K  V  T  E  T  R  H  L  L  K  A
1281    AAAACTGAGGATGTGGTTAAAGCGCGCCAGTTTGCTAGCGAGGCACAAGTTGTTTTCGCTACGCCACGATTATTTCCTGA
     K  T  E  D  V  V  K  A  R  Q  F  A  S  E  A  Q  V  V  F  A  T  P  R  L  F  P  E
1361    AGTAAATCTGGATAATTACAATTTACCTGGGCCTAGTAATGCGCAACATACAGAGGCTATCACCACTGATAGAGGAATAT
            V  N  L  D  N  Y  N  L  P  G  P  S  N  A  Q  H  T  E  A  I  T  T  D  R  G  I
1441    TGTTTCCAAAGCCAAAATTCAAAGGTAATGAAGTGGTACTCAATTACACAGGTTCAGGGAAAATCAGGAACGTTGGCTCT
     L  F  P  K  P  K  F  K  G  N  E  V  V  L  N  Y  T  G  S  G  K  I  R  N  V  G  S
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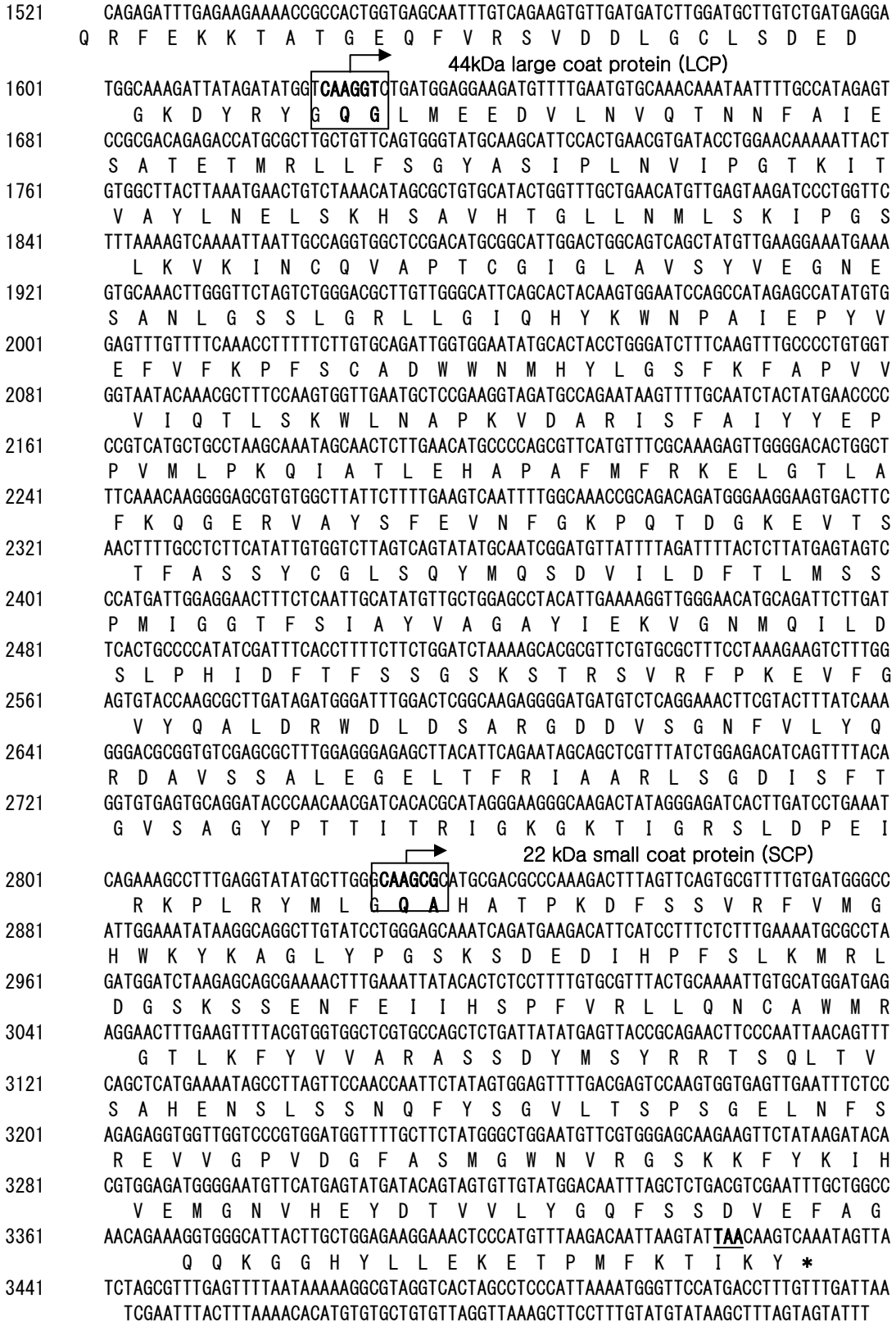

Figure 4. Nucleotide sequences of the coat protein gene of BBWV 2-Pa and their its deduced amino acid residues. Translational start (ATG) and stop codons are underlined with bold character. The black boxes with bold character indicate the cleavage sites of putative Q/G and Q/A cleavage sites are surrounded by solid lines.

52 kDa protein contains a possible rNTP-binding motif ($LxDx_{13}FAx_4GV$), which was suggested to be involved in virus movement (Fig. 5).

The RNA sequence and the deduced amino acid sequence of RNA 2 of the BBWV 2-Pa isolate were compared with other fabaviruses and representatives of comoviruses. As shown in Table 3 for BBWV isolates and Table 4 for comoviruses, the overall similarities in the polyprotein coding regions of BBWV 2-Pa were 84%-93% for RNA 2. In the 5′ and 3′ UTRs, the nt similarities were 65%-92% and 38%-60%, respectively. The results indicated that the coding region of BBWV 2-Pa was more conserved than the UTRs. The 5′ UTR sequences was more conserved than the 3′ UTR sequences.

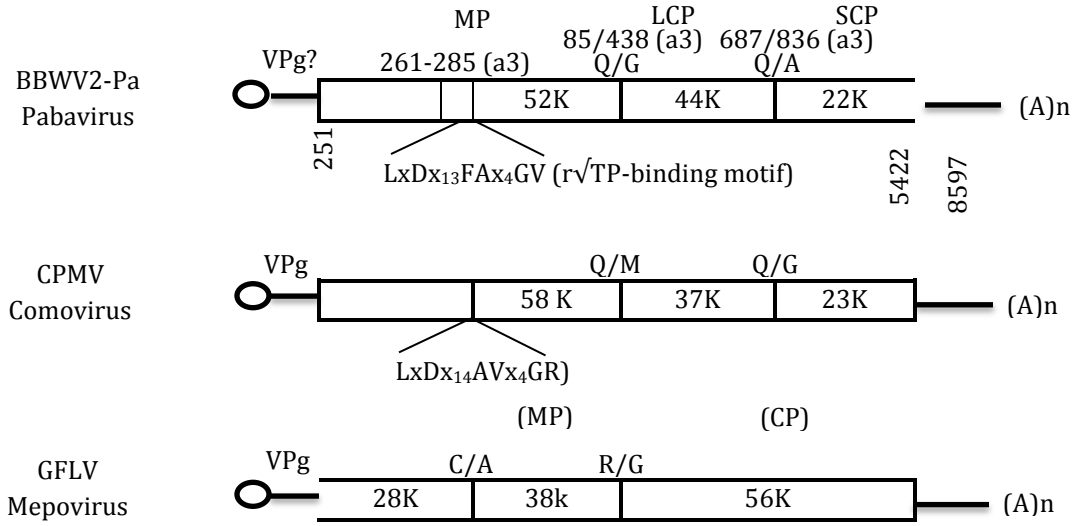

Figure 5. Proposed genome structure of BBWV 2-Pa RNA 2, and comparison with Cowpewa mosaic virus (CPMV) and Grape fanleaf virus (GFLV) genome structure of the family Comoviridae. Lines indicate noncoding sequence and the bars represent the polyprotein sequence encoded by long ORF. Vertical thick lines indicate known and putative protease cleavage sites. Movement protein (MP) included rNTP-binding motif. Question marks represent undetermined but predicted presence of VPg. (A) n represents polyadenylated tail.

Table 1. Oligo-nucleotide sequence for full-length of RNA 2 sequencing of BBWV 2-Pa.

Direction	Primer	Sequence	Position
Forward primers of RNA 2	BF2	5′-TCTTTTGTGGTCAGTATTTTTGTA-3′	349-372
	BF3	5′-TGCATACTATTCCAAAATACACAT-3′	639-662
	BF4	5′-TTTTCCAATGCACAGAGGTCC-3′	1099-1118
	BF5	5′-TCCAAAGCCAAAATTCAAAGGTAA-3′	1444-1467
	BF6	5′-CCAGGTGGCTCCGACAT-3′	1861-1877
	BF7	5′-GCCCAAGCAAATAGCAACTC-3′	2170-2189
	BF8	5′-ACGTTCTGTGCGCTTTCCTA-3′	2527-2851
	BF9	5′-CGCATGTGACACCCGAAGAT-3′	3145-3168
Reverse primers of RNA 2	BR2	5′-GACACTCCACTATAAAACTGATTG-3′	3496-3517
	BR3	5′-ATCTTCGGGTGTCACATGCG-3′	3145-3168
	BR4	5′-TAGGAAAGCGCACAGAACGT-3′	2527-2851
	BR5	5′-GTTCCTCTTTCATACTTTTCTTTT-3′	2170-2189
	BR6	5′-ATGTCGGAGCCACCTGG-3′	1861-1877
	BR7	5′-TTACCTTTGAATTTTGGCTTTGGA-3′	1444-1467
	BR8	5′-GGACTCTGTGCATTGGAAAA-3′	1099-1118
	BR9	5′-ATGTGTCTTTTGGAATAGTATGCA-3′	639-662

Table 2. Reactions of BBWV on the indicator plants by mechanical inoculation.

Indicator plants	*Reaction for the isolate of*		
	P[a]	PE-ASA3[b]	P-K2[b]
Chenopodium amaranticolor	CS/ D,VC,VN[a]	CL/SM, MAL	CS/CS, SM
C. quinoa	CS/ CS,MAL	CL/SM, MAL	CS/CS, SM
Datura stramonium	CS/ CS	NRL/-	CRS/CRS
Gomphrena globosa	-/ M	-/-	NS/M, MAL
Physalis floridana	-/ VC	CL/SM	CS/CS, M
Nicotiana benthamiana	-/ M, MAL	NL/SM	CS/CS, SM
N. occidentalis	-/ VC, M	nt	nt
N. clevarandii	-/ CS	nt	nt
N. glutinosa	-/-	NL/SM, MAL	CRS/CRS

[a]CL, chlorotic local; CRS, chlorotic ring spot; CS, chlorotic spot; M, mosaic; MAL, marfom; N, necrosis; NS, necrotic spot; RS,ring spot; SM, severe mosaic; VC, vein clearing; VN, vein necrosis; -, negative reaction; nt, non-test.

Table 3. Identity of amino acid and nucleic acid for RNA 2 of BBWV 2-Pa with other seven strains of BBWV 2 and BBWV 1.

Region	No. of amino acids or RNA residues (% identity)							
	BBWV 2							BBWV 1
	Pa	IP	MB7	E	L	1-2	PV131	PV132
5′UTR(nt)	230	205 (92)	221 (68)	-	-	-	-	-
MP (aa)	465	465 (92)	465 (81)	-	-	-	-	-
LCP (aa)	402	402 (94)	402 (92)	402 (92)	402 (89)	402 (89)	402(91)	402 (63)
SCP (aa)	197	197 (93)	192 (89)	197 (88)	197 (87)	197 (86)	-	197 (57)
3′UTR(nt)	171	169 (60)	173 (38)	176 (59)	191 (39)	193 (66)	-	158 (37)
Polyprotein (aa)	1064	1064 (93)	1064 (87)	-	-	-	-	-
Geneme RNA2(nt)	3596	3569 (89)	3589 (76)	-	-	-	-	-

The sequences were obtained from NCBI GenBank database. BBWV 2 isolates IP [AB018698], MB7 [AB013616], E [AB018699], L [AB018700], 1-2 [AB018701], PV131 [U65985] and BBWV 1-PV132 [AB018702].

Table 4. Identity of amino acid and nucleic acid for RNA 2 of BBWV 2-Pa with other comovirus.

Region	No. of amino acids or RNA residues (% identity)				
	Pa	PaMMV	BPMV	CPMV	RCMV
5′UTR(nt)	230	220 (65)	-	-	-
MP (aa)	465	465 (92)	465 (81)	-	-
LCP (aa)	402	402 (94)	402 (92)	402 (92)	402 (89)
SCP (aa)	197	197 (93)	192 (89)	197 (88)	197 (87)
3′UTR(nt)	171	169 (60)	173 (38)	176 (59)	191 (39)
Polyprotein (aa)	1064	1064 (93)	1064 (87)	-	-
Geneme RNA 2 (nt)	3596	3569 (89)	3589 (76)	-	-

The sequences were obtained from NCBI GenBank database. PaMMV (Patchouli mild mosaic virus)[E13232], BPMV (Bean pod mottle virus)[NC_003495], CPMV (Cowpea mosaic virus)[NC_ 003550] and RCMV (Red clover mottle virus) [NC_003738].

DISCUSSION

In this study, characterization of new strain of BBWV 2-Pa isolated from diseased paprika was investigated. The virus has some of the main biological and molecular properties characteristic of BBWV 2. However, the host range and symptoms of BBWV 2-Pa differed in some respects from those reported for other BBWV (Taylor and Stubbs 1972; Hong 1998). When BBWV 2-Pa was compared with other Korean isolates, the isolate did not induce symptoms on inoculated leaf in *P. floridana, N. benthamiana* and *N. glutinosa.*

The complete sequence of BBWV 2-Pa consisted of 3,597 nt excluding the 3′ poly(A) tail and contained one large ORF encoding 1,064 amino acids with a predicted Mr of 118.6 kDa. The BBWV 2-Pa RNA 2 encodes the putative MP and the viral CPs as reported for other fabaviruses (Nakamura *et al.,* 1998). The polyprotein was found to share amino acid (aa) homologies with comoviruses, but

not with nepoviruses or other viral proteins. The possible site for the two proteolytic processing of the polyprotein, a Q/G at positions 456-466 aa and a Q/A at positions 867-868 aa, have been deduced from other fabaviruses. These results suggest that the BBWV 2-Pa is translated into a polyprotein and is processed into three mature proteins. The 44 kDa central and 22 kDa C-terminal proteins are predicted to be the coat proteins. The calculated molecular weights agree with the values estimated by SDS-PAGE analysis (Fig. 14), 44 kDa and 22 kDa, respectively. Consensus sequences with comovirus RNA 2 polyproteins could be aligned beginning only at the second in frame AUG. For comoviruses, there is evidence to indicate that initiation from both the first and the second AUG codons of the long ORF of CPMV RNA 2 occurs *in vivo* to yield the 58/48 kDa MP proteins (Margis *et al.,* 1994). It is uncertain if this leaky scanning mechanism also occurs in fabaviruses. The deduced N-terminal protein of BBWV 2-Pa RNA 2 consists of 465 aa with a calculated Mr of 52 kDa. This 52 kDa protein shares 15% amino acid sequence homology with the 58/48 kDa cell-to-cell movement protein of CPMV RNA 2 with a rNTP-binding motif (L-x-D-x14-V-A-x4-G-R) (Holness *et al.,* 1989; Ikegami *et al.,* 1998). The presence of a similar motif (L-x-D-x13-F-A-x4-G-V) in the BBWV 52 kDa protein suggests that this protein might also be involved in virus movement. Between BBWV 2-Pa and other serotype II fabaviruses, the overall amino acid similarities in the polyprotein coding regions was and 84%–93% for RNA 2. In the 5′UTRs, the nucleotide similarity was 65%–92% (RNA 2). For the 3′UTRs, they are 38%–60% (RNA 2), respectively. The results indicated that the coding region of BBWV 2-Pa was more conserved than the UTRs. The 5′UTR sequences was more conserved than the 3′UTR sequences.

The clusters-derived following analysis of the CP sequence suggested distinct evolution lineages. Serotype II fabaviruses are more prevalent in Asia, Australia and North America, whereas serotype I is more prevalent in Europe (Cooper 1994). So far, no fabavirus CP sequence with homology spanning across the two serotypes has been reported. A serotype III fabavirus isolate from *Ajuga reptans*, which reacts with both fabavirus antisera, was detected in Australia (Shukla *et al.,* 1983). PaMMV has also been classified as a possible serotype III fabavirus (Ikegami *et al.,* 1998). PaMMV is similar to other serotype II fabaviruses by molecular phyologenetic analysis except for its lower LCP homology as compared to other members of the cluster. For each serotype group, lineage within the cluster implies divergent from the origin. BBWV 2-Pa appears to share the same origin as the Japan isolate IP. To obtain a more complete picture on the evolutionary relationship of the fabaviruses, more sequence information from other isolates of the virus is required.

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