

Molecular Identification and Sequence Analysis of Coat Protein Gene of *Ornithogalum mosaic virus* Isolated from *Iris* Plant

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A potyvirus was isolated from cultivated *Iris* plants showing leaf streak mosaic symptom. Reverse transcription and polymerase chain reaction (RT-PCR) product of 1 kb long which encoded partial nuclear inclusion B and N-terminal region of viral coat protein (CP) genes for potyviruses was successfully amplified with a set of potyvirus-specific degenerate primers with viral RNA samples from the infected leaves. The RT-PCR product was cloned into the plasmid vector and its nucleotide sequences were determined. The nucleotide sequence of a cDNA clone revealed that the virus was an isolate of *Ornithogalum mosaic virus* (OrMV) based on BLAST search analysis and was denoted as OrMV Korean isolate (OrMV-Kr). To further characterize the CP gene of the virus, a pair of OrMV-specific primers was designed and used for amplification of the entire CP gene of OrMV-Kr. The virus was easily and reliably detected from virus-infected *Iris* leaves by using the RT-PCR with the set of virus-specific primers. The RT-PCR product of the CP gene of the virus was cloned and its sequences were determined from selected recombinant cDNA clones. Sequence analysis revealed that the CP of OrMV-Kr consisted of 762 nucleotides, which encoded 253 amino acid residues. The CP of OrMV-Kr has 94.1-98.0% amino acid sequence identities (20 amino acid alterations) with that of other three isolates of OrMV. Two NT rich potential *N*-glycosylation motif sequences, NGTS and NWTM, and a DAG triple box responsible for aphid transmission were conserved in CPs of all the strains of OrMV. The virus has 58.5-86.2% amino acid sequence identities with that of other 16 potyviruses, indicating OrMV to be a distinct species of the genus. OrMV-Kr was the most related with *Pterostylia virus Y* in the phylogenetic tree analysis of CP at the amino acid level. This is the first report on the occurrence of OrMV in *Iris* plants in Korea. Data in this study indicate that OrMV is found in cultivated *Iris* plants, and may have mixed infection of OrMV and *Iris severe mosaic virus* in Korea.

Keywords : coat protein, *Iris*, *Iris severe mosaic virus*, *Ornithogalum mosaic virus*, *Potyvirus*, sequence.

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To date, more than 15 viruses have been reported in *Iris* plants (Alper et al., 1984; Asjes, 1979; Van der Vlugt and Derks, 1995). There are at least five different *Iris*-infecting potyviruses, namely, *Iris severe mosaic virus* (ISMV), *Iris fulva mosaic virus* (IFMV), *Bean yellow mosaic virus* (BYMV), *Iris mild mosaic virus* (IMMV), and *Ornithogalum mosaic virus* (OrMV) (Alper and Loebenstein, 1981; Barnett, 1985; Brunt, 1988; Brunt et al., 1988; Burger et al., 1990; Van der Vlugt and Derks, 1995). *Iris* (*Iris* sp.) is one of the most popular ornamental cutflowers and a perennial garden plant known for its beautiful colors and various shapes of flowers with a number of varieties worldwide. Marketing of *Iris* plants as a cutflower (mainly violet in color) is ranked 9th in the world and 11th in Korea. It is propagated vegetatively through bulbs or rhizomorphs. This asexual propagation tends to cause virus transmission from infected plants. Viral diseases caused by ISMV in cultivated *Iris* plants have caused serious problems for growers in Korea (Park et al., 2000). The virus, a species of the genus *Potyvirus* in the family *Potyviridae*, is one of the most economically important virus diseases of cultivated *Iris* and crocus in the world (Brunt et al., 1988). Partial sequence information is available for ISMV and OrMV (Burger et al., 1990; Park et al., 2000; Van der Vlugt et al., 1994). There is no known sequence of IFMV, BYMV (for *Iris* isolate) and IMMV, and furthermore, little has been characterized for pathology and genome information of these potyviruses and their interactions.

Members of the genus *Potyvirus* are flexuous filamentous virus particle with 680-900 nm long and 11-13 nm wide (Shukla et al., 1994). These are transmitted by aphids in a non-persistent manner (Shukla et al., 1994). The potyviral genome, about 10 kb long, is a single-stranded, positive sense RNA, and has a poly (A) tract at its 3' termini and a genome-linked protein (VPg) at its 5' termini (Gough and Shukla, 1993; Johansen et al., 1991; Riechmann et al., 1992). The potyviral genome is expressed initially as a large polyprotein precursor that is processed into ten functional proteins by virus-encoded proteases (Allison et al., 1985; Riechmann et al., 1992).

In Korea, a potyvirus was detected for the first time in

1995 from imported cultivated *Iris* plants and was identified as ISMV by serological and molecular detection (Park et al., 2000). Partial nucleotide sequence for ISMV was recently reported (Park et al., 2000). During the last two years of virus disease survey in *Iris* plants in Korea, RT-PCR with ISMV-specific primers was not successful (unpublished results) for some of the diseased samples. Symptoms of the plants showing leaf mosaic pattern were indistinguishable from that of ISMV. This indicated that the causal filamentous virus could be one of the other *Iris*-infecting potyviruses. Therefore, molecular identification for the virus was applied, and the RT-PCR product was successfully amplified with a set of potyvirus-specific degenerate primers with the viral RNA samples from the infected leaves. The nucleotide sequence revealed that the virus was an isolate of OrMV and denoted as OrMV-Kr.

Little information is available on the relationship between the Korean and other known OrMV isolates and potyviruses. As a first step for molecular breeding of virus-resistant transgenic *Iris* plant, the coat protein gene of OrMV was cloned and analyzed in this study. Nucleotide sequence analysis of the complete coat protein gene of a Korean isolate of OrMV (OrMV-Kr) was also carried out, and comparison with other OrMVs and members of the *Potyvirus* genus was discussed.

Materials and Methods

Collection of plants and sources of viruses. Diseased *Iris* plants were obtained from the Yangjae flower wholesale market (Seoul, Korea) and private flower shops from September to October 2001, and were used as sources of virus in this study. OrMV-Kr was originally isolated from leaves of *Iris* plant and used as a source of virus (Fig. 1). Dried leaf samples of the virus were prepared by freeze-drying in a lyophilizer and deposited at the Plant Virus GenBank (Seoul, Korea; <http://www.virusbank.org>). Two viruses, a potyvirus (*Potato virus Y*; PVY-O) and a cucumovirus (*Cucumber mosaic virus*; CMV-Fny), were used as controls for detection of OrMV.

Virus purification, extraction, and isolation of virus genomic RNA. The virus was purified by polyethylene glycol precipitation and differential centrifugation methods with sucrose cushion as previously described (Alper et al., 1984). Viral genomic RNA was extracted from purified virus particles by SDS-proteinase K/phenol extraction (Kim et al., 1998), followed by ethanol precipitation (Sambrook et al., 1989).

Potyvirus-general degenerate primers and reverse transcription and polymerase chain reaction (RT-PCR). A set of degenerate primers specific to the known potyviruses was designed based on nucleotide sequence analysis of some selected potyviruses retrieved from the GenBank database. Figure 2 shows the positions of primers for amplification of target regions virus genome in this study. The first strand cDNA was synthesized from



Fig. 1. Leaf streak mosaic symptoms of *Iris* infected by *Ornithogalum mosaic virus* (OrMV).

total nucleic acids from infected leaves or 10 µg of purified viral genomic RNA using the RNA Core PCR Kit (Perkin Elmer) with oligo (dT) primer. Reverse transcription (RT) was carried out in a total of 20 µl reaction volume at 42°C for 30 minutes in 10 mM Tris-HCl (pH 8.3) containing 5.0 mM MgCl₂, 50 mM KCl, 1.0 mM dNTPs, 50 pmol Oligo d(T) primer, 0.5 unit RNase inhibitor, and 2.5 units *Murine leukemia virus* reverse transcriptase (Yun et al., 2002). The PCR mixture (50 µl) contained 1 X PCR buffer [10 mM Tris-HCl (pH 8.8), 2.5 mM MgCl₂, 50 mM KCl, and 0.1% Triton X-100], 50 pmol of reverse and forward primers, 1.0 mM dNTPs, and 1.25 units of *Ampli-Taq* DNA polymerase (Perkin Elmer). The reaction was denatured for 30 seconds at 94°C, annealed for 50 seconds at 43°C, and then extended for 60 seconds at 72°C for a total of 30 cycles in a PCR thermal cycler (i-cycler, Bio-Rad). Amplified PCR product was separated on a 1.2% agarose gel in 1 X TAE buffer (Sambrook et al., 1989) at 100 V for 30 minutes, and then visualized by staining the gel in ethidium bromide solution and photographed under a UV

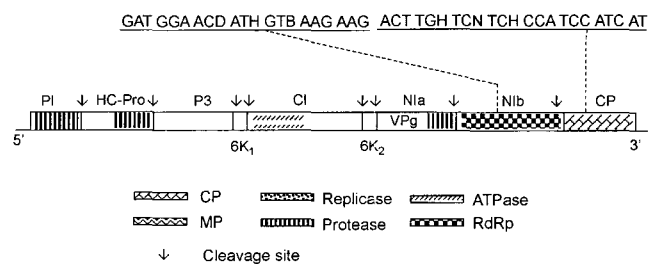


Fig. 2. Genome organization of members of the genus *Potyvirus*, and degenerate primers for the *Potyvirus* used in this study.

transilluminator (Sambrook et al., 1989).

Amplification of CP gene and RT-PCR detection from *Iris* plants. The 3'-primer ORMCP3 (5'-GTCTCTTACACACTATT-GAA-3') and the 5'-primer ORMCP5 (5'-ATGATCGAAGCGTG-GGGTTATCCT-3') were designed to detect and amplify the CP gene of OrMV from cultivated *Iris* plants based on the nucleotide sequences of an Australian isolate of OrMV and partial nucleotide sequences of OrMV-Kr. The RT-PCR was done with the OrMV-specific primers. RT was done at 42°C for 30 minutes. PCR mixture was denatured for 30 seconds at 94°C, annealed for 50 seconds at 43°C, and then extended for 60 seconds at 72°C for a total of 40 cycles in a PCR thermal cycler. The RT-PCR product of CP gene for the OrMV was separated on a 1.0% agarose gel in 1 X TAE buffer (Sambrook et al., 1989) at 100 V for 30 minutes, and then visualized by staining the gel in ethidium bromide solution and photographed under a UV transilluminator (Sambrook et al., 1989).

cDNA cloning of RT-PCR products and restriction enzyme digestion. Double-stranded cDNA molecules of RT-PCR products were cloned into pGEM-T-Easy plasmid vector (Promega) (Sambrook et al., 1989). Recombinant cDNA molecules were transformed into competent cells of *Escherichia coli* strain JM109 (Promega) and selected for nucleotide sequencing (Sambrook et al., 1989). Recombinant clones have been deposited in the Plant Virus GenBank (<http://www.virusbank.org>). The cDNA clones were hydrolyzed with *EcoRV* and *KpnI* restriction endonucleases

(Roche) and separated on the agarose gel electrophoresis. Restriction map was compared with that of other isolates of OrMV.

Nucleotide sequencing and sequence analysis. Nucleotide sequences of some selected recombinant cDNA clones were determined in both directions with T7 primer and M13 primer by the dideoxynucleotide chain termination method (Sanger et al., 1977) using the Model 377 automated DNA Sequencer (ABI). Construction of contigs from the nucleotide sequences of cDNA clones and sequence alignments of the nucleotide and the deduced amino acid sequences were performed with the DNASTAR software package (Madison, WI). Phylogenetic analysis was done between OrMV and other selected members of the genus *Potyvirus*. Accession numbers for sequences and abbreviations of the virus were listed in Table 1.

Results and Discussion

Occurrence and symptoms of OrMV-infected *Iris* plants.

The collected *Iris* plants showed symptoms on the leaves such as mosaic, streak, yellow mosaic, and dwarfing, or were symptomless. Flowers showed no visible symptom. OrMV was found on infected leaves of *Iris* plants showing typical mosaic or irregular streak mosaic symptoms (Fig. 1). Some *Iris* plants showing mosaic or severe mosaic patterns showed ISMV infection (data not shown). Symptoms of OrMV- and ISMV-infected *Iris* plants were indistinguishable. OrMV was purified from diseased *Iris* leaves showing streak mosaic systemic symptom (Fig. 1). Purified virions of OrMV, as observed by using the electron microscope, were filamentous type (data not shown), which is the typical morphology of the members of the genus *Potyvirus* (Shukla et al., 1994). The viral genomic RNA

Table 1. Members of the genus *Potyvirus* used for sequence analysis in this study

Virus name	Abbreviation	GenBank Accession number
<i>Ornithogalum mosaic virus</i>	OrMV-Kr	AJ510250 (this study)
<i>Ornithogalum mosaic virus</i>	OrMV-Au	AF203528
<i>Ornithogalum mosaic virus</i>	OrMV-O	D00615
<i>Ornithogalum mosaic virus</i>	OrMV-Is	AF080587
<i>Pterostylis virus Y</i>	PtVY	AF185964
<i>Lily mottle virus</i>	LMoV	AJ310203
<i>Tulip band-breaking virus</i>	TBBV	AB078007
<i>Leek yellow stripe virus</i>	LYSV	AB005612
<i>Colombian datura virus</i>	CDV	AJ237923
<i>Bean common mosaic virus</i>	BCMV	NC-003397
<i>Peanut stripe virus</i>	BCMV (PStV)	Y11775
<i>Zucchini yellow mosaic virus</i>	ZYMV	D13914
<i>Pepper mottle virus</i>	PepMoV	AF440801
<i>Potato virus Y</i>	PVY	X68226
<i>Papaya ringspot virus</i>	PRSV	AY094985
<i>Iris severe mosaic virus</i>	ISMV-K	AF034839
<i>Plum pox virus</i>	PPV	X58258
<i>Chilli vein-banding mosaic virus</i>	CVbMV	U72193
<i>Bean yellow mosaic virus</i>	BYMV	D00490
<i>Pea speed-borne mosaic virus</i>	PSbMV	AF522162

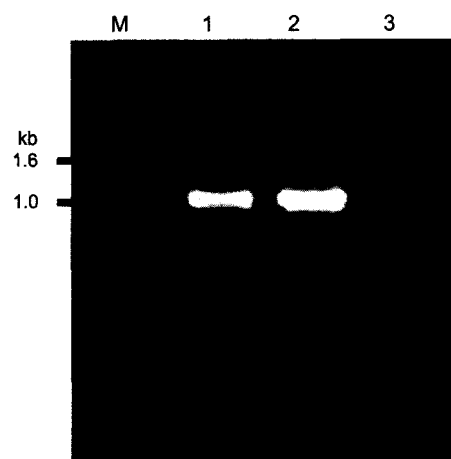


Fig. 3. Agarose gel electrophoresis of RT-PCR products of *Ornithogalum mosaic virus* (OrMV) with a set of potyviral degenerate primers (see Fig. 2). Lane M, 1 kb plus DNA ladder; Lane 1, OrMV; Lane 2, *Potato virus Y*; Lane 3, *Cucumber mosaic virus*.

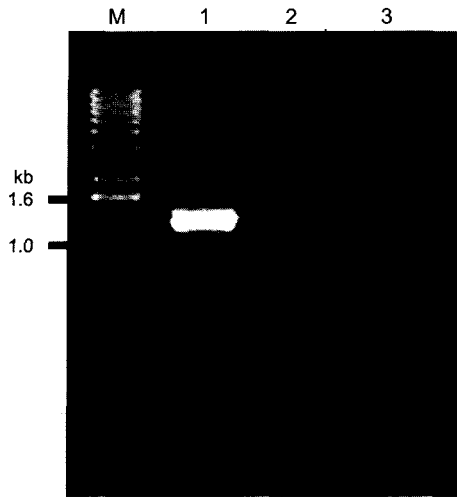


Fig. 4. Agarose gel electrophoresis of RT-PCR product of coat protein gene of *Ornithogalum mosaic virus* (OrMV) with a set of OrMV-specific primers. Lane M, 1 kb plus DNA ladder; Lane 1, OrMV; Lane 2, *Potato virus Y*; Lane 3, *Cucumber mosaic virus*.

was about 10 kb long, and coat protein (CP) for the virus was estimated at 32 kDa in SDS-PAGE (data not shown). **Identification of OrMV by RT-PCR with *Potyvirus*-general degenerate primers.** RT-PCR product of 1 kb long, which encoded partial nuclear inclusion B and N-terminal region of viral CP genes for potyviruses, was successfully amplified with a set of potyvirus-specific degenerate primers (Fig. 2) with the viral RNA samples from the infected leaves (Fig. 3). The RT-PCR product was cloned into the plasmid vector and its nucleotide sequences were determined. The nucleotide sequence of a cDNA clone revealed that the sequences were over 93% identical with the known OrMV in the BLAST search analysis, suggesting that the virus was an isolate of OrMV.

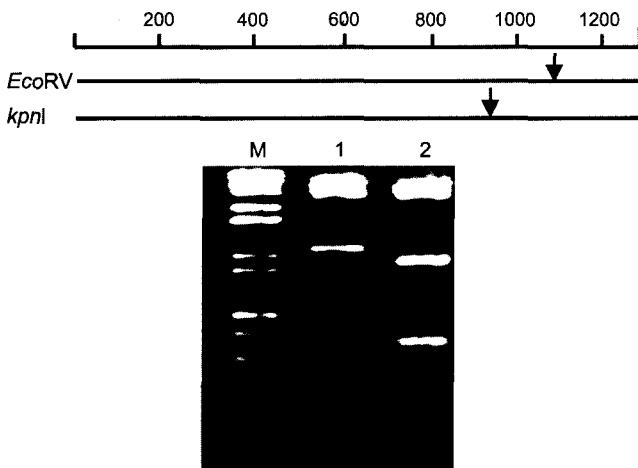


Fig. 5. Restriction pattern of cDNA clone of CP gene of Korean isolate of *Ornithogalum mosaic virus* (OrMV-Kr). Lane M, 1 kb plus DNA ladder; Lane 1, *EcoRV*-digested; Lane 2, *KpnI*-digested.

Therefore, the virus was denoted as OrMV Korean isolate (OrMV-Kr).

Amplification and detection of CP gene of OrMV-Kr.

To further characterize the virus, full-length CP gene was amplified by RT-PCR with OrMV-specific CP primers. A 1.2 kb RT-PCR product was successfully amplified from the preparation of viral RNA (Fig. 4). The RT-PCR product was cloned into the plasmid vector for sequence determination. A cDNA clone of the OrMV-Kr, pORMVCP,

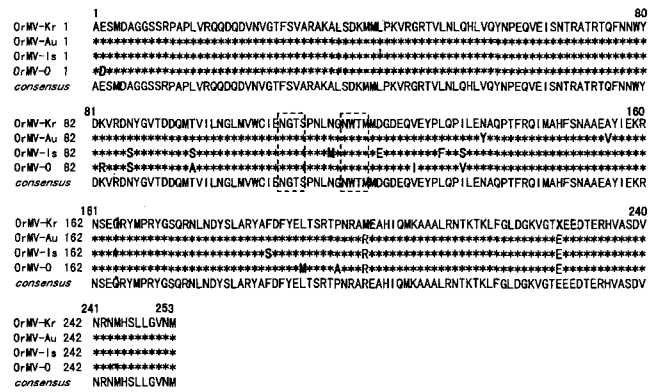


Fig. 6. Multiple alignment of the amino acid sequences of the coat proteins of four isolates of OrMV. Two potential *N*-glycosylation motifs were marked as open boxes. References for virus sequences are listed in Table 1.

Table 2. Percentage sequence identities between the complete coat protein of Korean strain of *Ornithogalum mosaic virus* (OrMV-Kr) and other members in the genus *Potyvirus*^a

Virus	Nucleotide (%)	Amino acid (%)
OrMV-Au	99.3	98.0
OrMV-O	94.5	94.1
OrMV-Is	91.1	94.9
PtVY	48.0	86.2
LMoV	36.7	63.6
TBBV	36.5	63.6
LYSV	36.6	62.8
CDV	37.9	65.2
BCMV	34.3	57.3
BCMV (PStV)	34.8	57.7
ZYMV	34.5	57.3
PepMoV	35.3	58.1
PVY	34.9	59.3
PRSV	37.1	60.5
ISMV-K	33.6	56.7
PPV	39.0	64.0
CVbMV	37.0	63.2
BYMV	37.0	62.1
PSbMV	34.0	56.5

^aReferences for sequences and abbreviations of the viruses are listed in Table 1.

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OrMV-Kr 1 AESMDAGSSRP-----
OrMV-Au 1 *****
OrMV-Is 1 *****
OrMV-O 1 *D*****
PtVY 1 *****GRQ-----
BCMV 1 S-----*TGQPQPPVVDAG-----VDAGKD*RRNRNGKPEPNR-----
BYMV 1 S-----DQEQLNAD-----EKKDKRK*NEENPKNKSEGSQSI-----
CDV 1 *DKV***SNTQAGK-----NQSKERDTSVVT-----
CVBMV 1 *PYI---AETALRCLYMSEHGESDI SPYLRAL I EGAKEEGLDDESEVAHQSGESVDAGRV*GEDSSSKPADKQATEKSK
ISMV-K 1 GD*E**A-----
LMOV 1 *NETLNAGASSST-----QTSRPTRPETAAVDVAPQQSPE-----
LYSV 1 *GDELDA*-----MQTNKKQ*DNTDKS I EQRNPTAPRSS
PepMoV 1 SS*-----RS-----DTLDAG-EE*KKNKEVATVSDGMKKKEV
PPV 1 *DEREDEEVDAGNP I VVTAPAATSP I LQP-----PPARQPAPRTTAPMEN I FTPATTQPATKPVSVQVSGPQLQTFGT
PRRV 1 SR* I *D-----YQPVCNSNTHVFHQSKN-----EAVDAGLNE*RKKEKENGKEKEEKEQKEK
PSbMV 1 *GDETKDEEKRR-----KEEEDR*RREEN I DASQFGSGRDNK
PStV 1 S-----***-TQPVVVDAG-----VDTGKDE*EKSNGKKGSESS-----
PVY 1 *-----N-----DT I DAG-ENSKKD---AKPEQGS I QRNP
TBBV 1 *NETLNA*A*STT-----PSTRPTRPETAAVDVAPQQSPE-----
ZYMV 1 S-----*T---QPTVADAG-----ATKKD*E-DDGKKNKDV-----
consensus A-S-DAGGSS-----K-----

OrMV-Kr 13 -----PAPLVR-Q-QDQDVNVG---TFSVARAKALSDK-MMLPKVGRGRTLNLQHLVQYNPEQVE I SNTRATRTQ
OrMV-Au 13 *****-*****-*****K*****
OrMV-Is 13 -----QTP**-*-----*****K*****
OrMV-O 13 *****-*****-*****K*****
PtVY 13 -----QDSVA*-----*****Q I *****KA*****S***T***** I ***
BCMV 35 -----EGSGNNSRGAGDSTMR*K***A*SKGKV-*P*LQK I TKR-*N**M*K*NV I ***D**LD*K***TDLE*****KM*
BYMV 33 -----RQ I -----VPDRDVNAGTVG*FVSPRL*KTAGK-LN I P* I G*K I ***D**LE**P*DD**V I **QA*
CDV 28 -----GERKD**A-K-*R**A*SSG***P* I ** I PT*-**N** I KNKQ* I S*D**LN*K***HD*****HQ*
CVBMV 79 VSGQAQPQRQSEMEVP*VR*R*****TSG**T I P*L*G I *S*-LT I ** I KTKA*VD*E**LD*A*** I HL*****LQS*
ISMV-K 8 -----R*A*NVTR*R**TAETSG**T*P**M**P**NK-*K*****N*TSNM**D**LV*K***EKL F** I **QQQ
LMOV 36 -----PRI R*R**D**TVG*FQ I P*L***AT*- I S I ** I ** I V**G***N*****TD*****S*QK*
LYSV 35 TQSEKDSGGGSE---LSGK*R*****TTG*F**P* I *Q I PQ*G I S I *MDG*K S I ***D**L**K*S*LN*****A*AA*
PepMoV 33 EST-----R*S***A*TVG*FT*P* I *S I TE*-**RM**QKKKG***A**LE*K*S**D*****S*QA*
PPV 75 YGNEDASPSNSNA**NTN-R*R**DA*S I G*FT*P*L*AMTS*-LS*****K*KA I M**N**AH*S*A**DL****APQSC
PRSV 52 EKDNASDNDVSTSTKTGER*R*N**TSG*FT*P*T*SFT***- I **R I K*K*****N*L*****Q I D*****QS*
PSbMV 38 KNKNGESDTSNKA I *KS---*R**DAGTSG* I T*P*L*E K I *A*- I RM**HK*GVA I S*Q***D***AQ*D*****QS*
PStV 34 -----EGSGNTRGTENQSMR*K***AGSRGK V-*P*LQK I TKR-*N**M*K*NV I ***D**LD*K***TDLF****KM*
PVY 27 NKG-----KEK**AGTSG*HT*P* I ** I TS*-**RM**SK*TA***E**LE*A*Q* I D I *****QS*
TBBV 36 -----PRAR*R**DAGTVG*FQ I P*L**LAT*- I S I ** I R**T I V**G***N*****TD I *****S*QK*
ZYMV 29 -----GSGGSEKTVAAVT-K*K***AGSHGK I -*P*L S K I TKR-*S**R*K*NV I *D I D***E**K*D* I ELY****SHQ*
consensus -----LVR-Q-RDRDVNAGTSGTFSVPRLKA I SDK-MSLPKVKGKTVLNLHLLQYNPEQTD I SNTRATQSQ
    
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Fig. 7. Continued.

was used for restriction mapping and nucleotide sequencing. The pORMVCP contained a single recognition site of *EcoRV* and *KpnI* (Fig. 5). The restriction map of the pORMVCP was identical with that of an Australian isolate of OrMV (Fig. 5).

Sequence characterization of CP gene of OrMV-Kr.

The identified nucleotide sequences for the OrMV-Kr contained full-length CP cistron. The OrMV-K CP consists of 762 nucleotides, which encoded 253 amino acid residues with a mass of 32.1 kDa. The CP of OrMV-Kr has 94.1-98.0% amino acid sequence identities with that of three other isolates of OrMV (Fig. 6, Table 2). Twenty amino

acid alterations were present between OrMV-Kr and other strains of OrMV, and most were located in the central region of CP.

OrMV-Kr CP has two NT rich potential *N*-glycosylation motif sequences (NGTS and NWTM) in the central region. Similar motifs were also found in CP of ISMV (NVTR, NGTS, NGTS, NLTG and NLTD) (Park et al., 2000), *Dasheen mosaic virus* (Pappu et al., 1994), and *Potato virus X* (Tozzini et al., 1994). This motif is commonly found in glycoproteins of some animal viruses and is considered to be essential for host membrane recognition (Zaret and Sherman, 1982). The motif sequences and potential modifi-

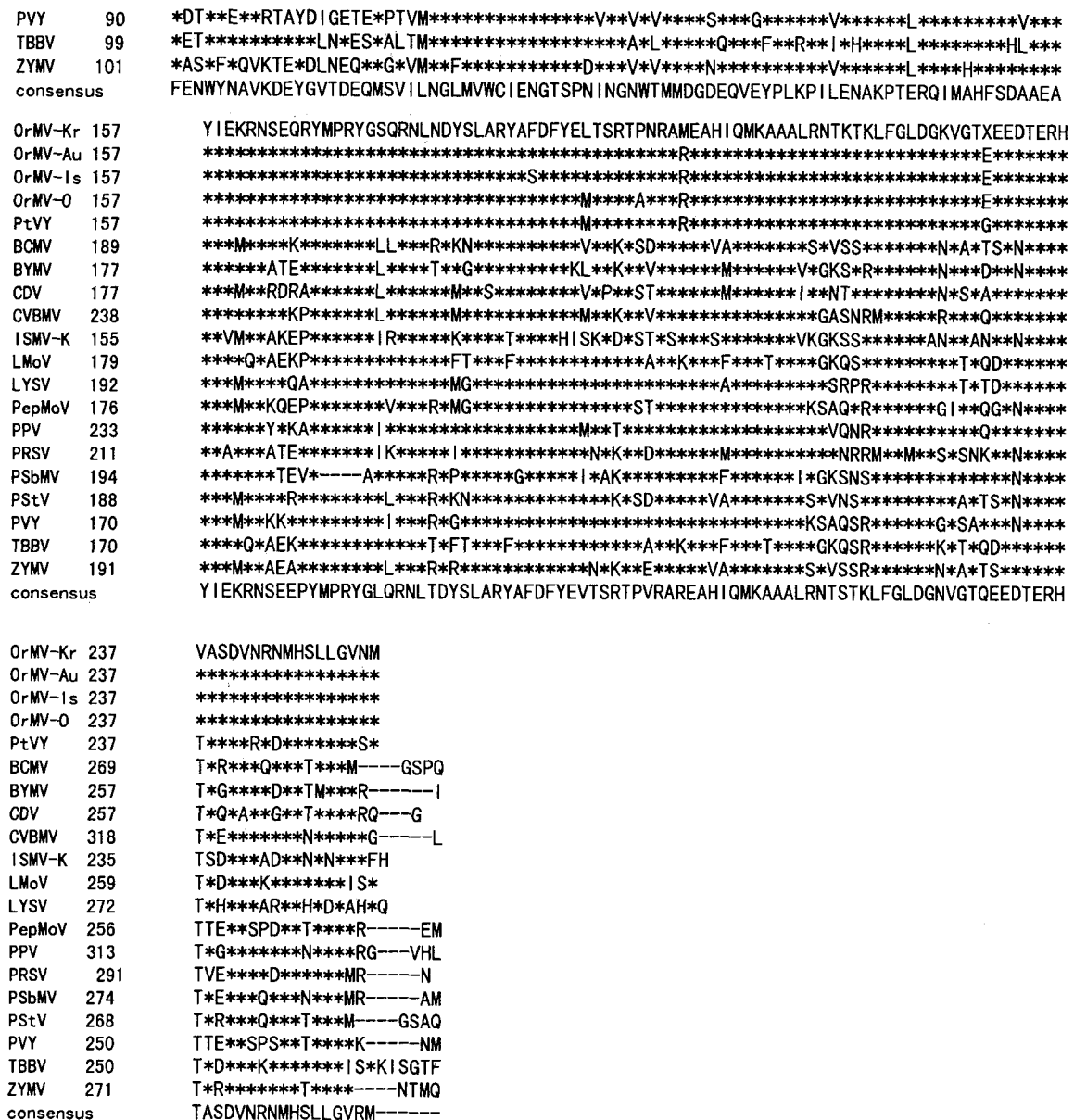


Fig. 7. Multiple alignment of the amino acid sequences of the coat protein of OrMV-Kr (in this study) and other members of the *Potyvirus*. References for sequences and abbreviations of the viruses are listed in Table 1.

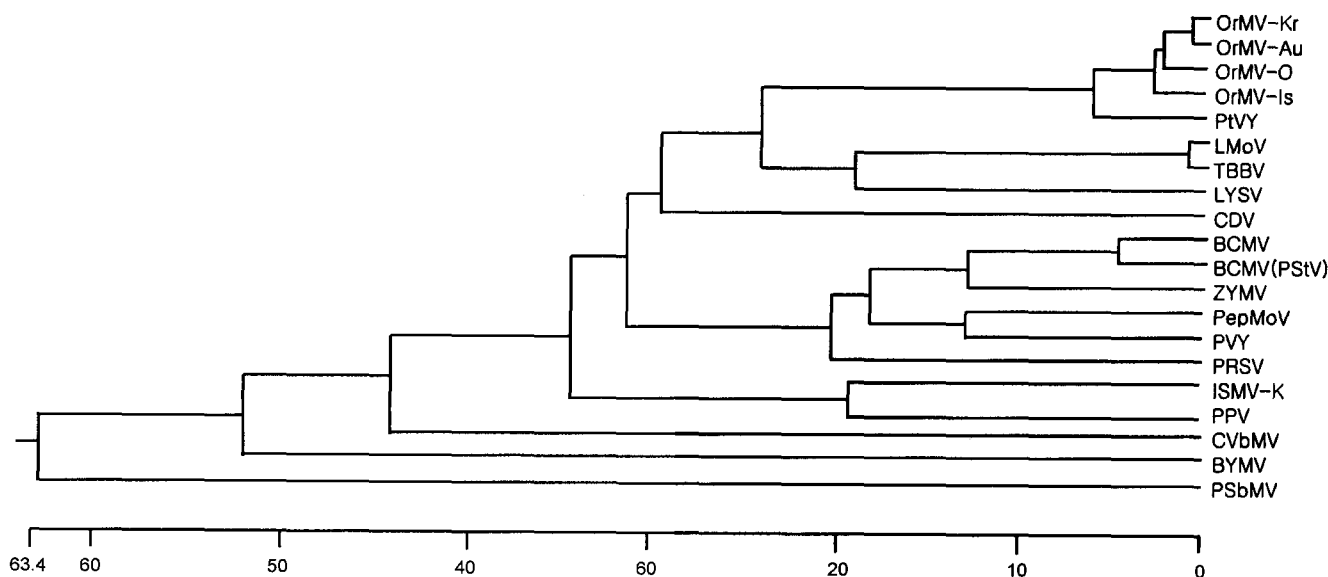


Fig. 8. Phylogenetic tree analysis of OrMV and other potyviruses based on the coat protein amino acid sequences. References for sequences and abbreviations of the viruses are listed in Table 1.

cation are responsible for the plant-virus interactions. A DAG amino acid triplet box, which is conserved in many aphid-transmissible potyviruses (Andrejeva et al., 1996; Atreya et al., 1990; Shukla et al., 1994), was found in the OrMV (Fig. 6).

Comparison and phylogenetic analysis of CP sequences at the genus level. Multiple sequence alignment of the CPs among OrMV-Kr and other potyviruses revealed that the conserved amino acid domains of the potyviruses are preserved in the corresponding locations; the N-terminal of the CPs vary considerably, however, two-thirds of the C-terminal was highly conserved (Fig. 7). The virus has 58.5-86.2% amino acid sequence identities with that of other 16 potyviruses, indicating OrMV to be a distinct species of the genus (Table 2, Fig. 7). OrMV-Kr was the most related with *Pterostylia virus Y* in the phylogenetic tree analysis of CP at the amino acid level (Fig. 8).

This is the first report on the occurrence of OrMV in *Iris* plants in Korea. Results of this study showed that OrMV is found in cultivated *Iris* plants, and may have mixed infection of OrMV and ISMV in Korea.

Acknowledgment

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