Molecular Characterization and Survey of the Infection Rate of *Orchid fleck virus* in Commercial Orchids

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(Received on February 1, 2010; Accepted on April 9, 2010)

Orchid fleck virus (OFV) is an unassigned plant virus in the family *Rhabdoviridae*. OFV was isolated from *Cymbidium* sp. showing oval necrotic lesions on their leaves in Korea, and designated as OFV-NHHS1. The complete nucleotide sequence of the RNA1 (6,413 nt) (GenBank accession no. AB516442) and RNA2 (6,001 nt) (GenBank accession no. AB516441) was determined in this study. RNA1 and RNA2 contained five and one ORF respectively. RNA1 encodes nucleocapsid (N) of 49 kDa, ORF2 of 26 kDa, ORF3 of 38 kDa, ORF4 of 20 kDa and glycoprotein (G) of 61 kDa proteins, whereas RNA2 encodes a single polymerase of 212 kDa. OFV-NHHS1 shared extremely high similarity of 98.6-100% and 98.9-99.6% in nucleotide and amino acid sequences with a Japanese isolate, OFV-so, respectively. However, the N, G and L of OFV-NHHS1 revealed 6.9-19.3%, 7.3-12.0%, and 13.4-26.6% identities to those of 29 *Rhabdoviruses*, respectively. To survey the infection rate of OFV in commercial orchids in Korea, 51 *Cymbidium* sp., 10 *Phalaenopsis* sp., 22 *Oncidium* sp. and 21 *Dendrobium* sp. plants that showed typical viral symptoms were collected. RT-PCR with specific primers for detection of *Cymbidium mosaic virus* (CymMV), ORSV and OFV showed high infection rate by ORSV alone and double infection by ORSV and CymMV. One of the orchids tested was infected with OFV. This is the first report of the complete nucleotide sequences of OFV isolated in Korea.

Keywords : *Cymbidium* sp., CymMV, *Orchid fleck virus*, *Rhabdovirus*, ORSV, RT-PCR, sequence analysis

Orchids are the largest family of flowering plants with more than 800 genera and over 25,000 species. It is one of the most important potted floricultural crops accounting for 25.8% in terms of cultivation area in Korea for the potted

floricultural crop viruses (Lawson and Hsu, 1995). Thirty kinds of viruses were reported on orchid worldwide, among them *Odontoglossum ringspot virus* (ORSV) and *Cymbidium mosaic virus* (CymMV) are the most common viruses (Lawson and Hsu, 1995). Since *Orchid fleck virus* (OFV) is reported first in *Cymbidium* spp. with chlorotic or necrotic ring spots and fleck symptoms from Japan (Doi et al., 1969), it is recognized as an important viral pathogen of orchids infecting more than 6 orchid genera (Chang et al., 1976). It has been reported in Australia, Brazil, Denmark, Germany, Korea, USA and Costa Rica (Blanchfield et al., 2001; Chang et al., 1991; Freitas-Astúa, 1999; Freitas-Astúa, 2002; Gibbs et al., 2000; Kitajima et al., 2001; Kondo et al., 2006).

OFV has non-enveloped, bacilliform particles of 40×150 nm in negatively stained preparations and is sap-transmissible to a few indicator species in the families *Chenopodiaceae*, *Solanaceae*, *Leguminosae* and *Aizoaceae* (Chang et al., 1976; Doi et al., 1977). OFV is known to be transmitted by *Brevipalpus californicus* Banks (Kondo et al., 2003) and *Brevipalpus phoenicis* (Hogenhout et al., 2008) in a persistent manner (Kondo et al., 2003). It is composed of negative sense, single stranded RNA molecules and bipartite; RNA 1 and 2 is 6,413 and 6,001 nucleotides long, respectively. RNA1 consists of five open reading frames (ORF) of nucleocapsid (N), glycoprotein (G) and three uncharacterized ORFs, and RNA2 consists of a single ORF of polymerase (L). The genome of OFV has common genes in the same order with other viruses in the family *Rhabdoviridae*, but is split between the G protein and L polymerase genes. Because of the bipartite genome, a new genus, *Dichorhabdovirus* was proposed (Kondo et al., 2006).

In Korea, OFV was first reported in 1991 but molecular characterization of OFV has never been studied (Chang et al., 1991). Therefore in this study we determined complete nucleotide sequences of OFV Korean isolate and compared them with those of OFV-so reported from Japan and other viruses in the family *Rhabdoviridae*. This result facilitated the detection of OFV by RT-PCR, which is sensitive,

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reliable and rapid method of detecting small amount of viral RNA.

Materials and Methods

Source of virus and plants. OFV-infected *Cymbidium* sp. was collected from a market selling orchids in Gyeongbuk Province, Korea. They showed oval necrotic lesions or necrotic line patterns on leaves (Fig. 1). Those leaves, neither infected with CymMV nor with ORSV (data not shown), were used as a source of nucleotide sequencing and host range test of virus, and they were designated as OFV-NHHS1 in this study. To survey the incidence of CymMV, ORSV and OFV in commercial orchids in Korea, 51 *Cymbidium*, 10 *Phalaenopsis*, 22 *Oncidium* and 21 *Dendrobium* plants showing typical viral symptoms were collected from Gyeonggi, Chungnam and Gyeongbuk provinces.

Host range test. Eight indicator plants including *Tetragonia expansa* were inoculated with sap of source orchid of OFV, NHHS1 in 0.05 M sodium phosphate buffer, pH 7.0. Three independent inoculation tests were conducted in each time course of May and September. Five plants were replicated with every indicator plant in every test. Symptom was determined at 15-20 days after inoculation and confirmed by RT-PCR using a primer pair specific to OFV.

Extraction of viral RNA. Viral RNAs were extracted from leaf tissue of orchids showing viral symptoms with RNeasy Plant Mini Kit (QIAGEN, Germany) according to the

manufacture's instructions.

Primer design. To determine the complete sequence of OFV-NHHS1 RNA1 and 2, sixteen pairs of primers were designed on the basis of OFV-so sequences (GenBank accession no. AB244417 and AB244418), and expected size of amplified each fragment is listed in Table 2. To survey the incidence of viral disease in orchids collected, three specific primer pairs were designed for detection of CymMV, ORSV and OFV on the basis of GenBank accession no. of NC_001812, DQ915440 and AB516441, respectively.

The sequence of the CymMVK-F was homologous to nucleotides 5462 to 5486 (5'-ACAATAATTTGAAATAATCATGGGA-3') of the NC_001812 and the sequence of CymMVK-R was complementary to nucleotides 6156 to 6180 (5'-AAAACCACACGCCTTATTAAGTTTG-3') of the NC_001812. The sequence of the ORSVK-F was homologous to nucleotides 26 to 49 (5'-ACGCACAATCTGATTCGIATTGAA-3') of the DQ915440 and the sequence of ORSVK-R was complementary to nucleotides 530 to 553 (5'-TATCAACGTTATTTTCCTAAATAT-3') of the DQ915440. The sequence of the OFVK-F was homologous to nucleotides 3758 to 3780 (5'-TACTGATGCTGATGCCACTCTTT-3') of the AB516441 and the sequence of OFVK-R was complementary to nucleotides 530 to 553 (5'-ACCCAACCTGGGAGAGACTCTATT-3') of the AB516441.

RT-PCR. Ten ng of RNA in 9 µl of nuclease free water and 1 µl of 10 pm reverse primer was heated at 70°C for 5 min followed by adding 4 µl of 5× reaction buffer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 1 µl of ImProm II reverse transcriptase (Promega, USA) and 1 µl of RNase inhibitor (1 U/µl) on ice, and incubating at 37°C for 1 hr. PCR amplification was performed in a 50 µl containing 20 µl of cDNA solution, 0.2 mM of each dNTP, 2 mM MgCl₂, 10 pM of each primer, 2.5 units of GoTaq DNA polymerase (Promega, USA), and 1× PCR buffer. Thirty five PCR cycles were conducted in PTC-0220 Perlitier Thermal Cycler (MJ Research, MA, USA). The thermal conditions were as follows: denaturation at 94°C for 30 sec (2 min for the first cycle), annealing at 50°C for 1 min and extension at 72°C for 1 min, and final extension at 72°C for 10 min. For detection of CymMV and ORSV, duplex RT-PCR was conducted.

Determination of complete nucleotide sequences of OFV. The amplified PCR products of the expected length were eluted and cloned into the pGEM-T easy vector. The ligation mixture was used to transform competent cells of *Escherichia coli* JM109. Nucleotide sequences of the

Fig. 1. Symptoms induced by natural infection with OFV-NHHS1 on *Cymbidium*. (A) Oval necrotic flecks; (B) Necrotic line pattern on leaves.

Table 1. Response of eight indicator plants to OFV-NHHS1 infection in comparison with OFV-so

Indicator plants	OFV-NHHS1 OFV-so ^a
<i>Tetragonia expansa</i>	NS/- ^b LL/-
<i>Chenopodium amaranticolor</i>	-/- LL/-
<i>C. quinoa</i>	-/- cLL/-
<i>Nicotiana clevelandii</i>	nLL, Y/- nt
<i>N. glutinosa</i>	-/- LL/-
<i>N. tabacum</i> cv. White Burley	-/- LL/-
<i>N. tabacum</i> cv. Xanthi-nc	-/- LL/-
<i>Vigna unguiculata</i>	nLL/- nt

^a Referred to Chang et al., 1976, Kondo et al., 2003, ICTV dB descriptions.

^b NS: necrotic spot, nLL: necrotic local lesions, Y: yellowing, LL: local lesions, cLL: chlorotic local lesions, nt: not tested. - : not infected.

cloned PCR products were determined using ABI Prism™ Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 377 Genetic Analyzer (Perkin Elmer, USA).

Sequence comparison and phylogenetic tree analysis.

The nucleotide sequences were assembled and analyzed with Lasergene package version 7 (DNASTar Inc., USA). Databases were searched using the Blast suite of programs from NCBI. Multiple sequence alignments and phylogenetic analysis were done using Clustal W of MegAlign program (DNASTar Inc., USA) based on its alignment obtained using default parameters. Values on the branches represent the percentage of trees containing each cluster of 1000 bootstrap replicates.

Table 2. Oligo nucleotide sequence for determination of complete full-length nucleotide sequences of OFV- NHHS1 RNA1 and RNA2 and expected amplified fragment size

OFV-NHHS1	Primer name	Nucleotide sequence	Size (bp)
RNA1	RNA1-1	F 5'-ACACAGGATAACCCGCTATTGGT-3' R 5'-CGCAGTGATGGCAAAGAGTTGCTT-3'	924
	RNA1-2	F 5'-TTCAATTACTCCTATTTCAGAAGGA-3' R 5'-GCTGTCAGTCACTGTTTCATGGCAT-3'	753
	RNA1-3	F 5'-TCCGCCAGACCTGCGCCCGTTGT-3' R 5'-TTAGTGAATCCGGGTGTTTCCTC-3'	643
	RNA1-4	F 5'-AGCTGGGGGAGATCTCCGGCCGA-3' R 5'-ACGACTTGCCGTCTCCCTGACCT-3'	712
	RNA1-5	F 5'-AACCATCCTGCAGGAGCTAAGAT-3' R 5'-TCTCATTCATATTGGTCTTGAAC-3'	732
	RNA1-6	F 5'-GTTCAAGACCAATATGAATGAGA-3' R 5'-ACATCGTGCCGGGCTGTTTCGTT-3'	1213
	RNA1-7	F 5'-AACGAAACAGCCCGGCACGATGT-3' R 5'-AGATGAGGACTCACATAGATGATGA-3'	879
	RNA1-8	F 5'-ACTCAGACGGATGACAATCAGAT-3' R 5'-ACACAGAGCCATGAGTAACTGTT-3'	1062
	RNA2-1	F 5'-ACACAGGACAACCAACTGTCTCA-3' R 5'-TAATCATCAGATGACACCAACTTC-3'	804
	RNA2-2	F 5'-ATGGGGAGGAAATTGGCCGATAA-3' R 5'-ATGCAAGACTGTATGTGGCCGGA-3'	822
	RNA2-3	F 5'-CCTATCACTTGAGAAAAGAGTTGGA-3' R 5'-TTCAACCCATGTTGTCTAGCTAC-3'	842
	RNA2-4	F 5'-GTTGGACCAATGACCCTTCTGGGA-3' R 5'-TCGATTCTTATAGCCAGAGGCTT-3'	852
	RNA2-5	F 5'-AGTGTCAACCATGACGCTCCCAT-3' R 5'-AGTGAGCGACATTGGGAACCTCC-3'	852
	RNA2-6	F 5'-TCTACGGAATGATGGAGTGGATT-3' R 5'-TCTTAGCACATATGACGTCATTG-3'	872
	RNA2-7	F 5'-TTCAGGTGGTTATGGATAACAGCA-3' R 5'-TTGCTTGTGGGATTTCTTACCAT-3'	882
	RNA2-8	F 5'-GGGCCAGAAGTGGGGAGATTTTCA-3' R 5'-ACACAGGACAGATCAACAGTACA-3'	650

Results and Discussion

Symptomatology and biological properties. To verify the biological properties of OFV-NHHS1, eight indicator plants were challenged with OFV-NHHS1 by mechanical inoculation and symptoms were compared with OFV-so reported by Chang et al., 1976. OFV-NHHS1 showed necrotic spots or lesions on inoculated leaves of *Tetragonia expansa*, *Nicotiana clevelandii* and *Vigna unguiculata* (Table 2) whereas, most of inoculated plants with OFV-so showed local lesion symptoms. However *Chenopodium amaranticolor*, *C. quinoa*, *N. gultinosa*, *N. tabacum* cv. White Burley and *N. tabacum* cv. Xanthi-nc were not infected with OFV-NHHS1 in this study (Table 1).

According to the report of Chang et al. (1976), transmission of OFV to the indicator plants only succeeded in summer when the temperature in the greenhouse was higher than 30°C, and they used both plant sap and partially purified virus preparation. In the present study, in the preliminary examination we conducted host range test in summer (higher than 35°C) with crude sap but we failed to infect those non-infected plants listed in Table 1 (data not shown), so we moved the test to the cooler season, May and September. However we still failed to infect them in May and September. Accordingly, we concluded the difference of host range between OFV-NHHS1 and OFV-so was

caused by difference in virus concentration used for inoculum.

Complete nucleotide sequence of OFV-NHHS1. Full-length nucleotide sequence of the genomic RNA1 and RNA2 of OFV-NHHS1 was determined with 16 pairs of primers designed. Each eight cDNA fragments from RNA1 and RNA2 of OFV-NHHS1 were amplified and sequenced (Fig. 2). Total number of nucleotides of OFV RNA1 and RNA2 were 6,413 and 6,001 nt respectively (Fig. 2). Computer assisted sequence analysis identified that there were significant six major open reading frames (ORFs) in the viral complementary-sense RNA1 and RNA2 cDNAs. The OFV-NHHS1 genome organizations in coding polarity were: 3'-leader-N-ORF2-ORF3-ORF4-G-trailer to RNA1 and 3'-leader-L-trailer to RNA2. The N protein positioned 228 to 1,580 nt of RNA1 was 49 kDa and may be the major protein forming the rhabdoviral nucleocapsid. ORF2 (26 kDa, 1,728 to 2,441 nt), ORF3 (38 kDa, 2,682 to 3,689 nt) and ORF4 (20 kDa, 3,830 to 4,381 nt) proteins showed no apparent similarities to other viral sequences in the BLAST database so far. But the ORF2 suggesting to phosphoprotein (P) contains consensus sites for phosphorylation by casein kinase II, which were deduced to be important for primary phosphorylation of phosphoprotein (Kondo et al., 2006). The ORF3 protein may correspond to the third non-

Fig. 2. Genome organization of OFV and schematic representation of nucleotide sequencing strategy. Horizontal lines represent templates for sequencing and expected amplified cDNA size were listed in Table 2. Arrows represent position and orientation of each primer for sequencing.

Table 3. Comparison of the homology percentage of nucleotide and deduced amino acid sequence of OFV-NHHS1 with OFV-soa

OFV-NHHS1	5'NCR	N		ORF2		ORF3		ORF4		G		L		3'NCR
		nt ^b	aa ^b	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	
RNA1	99.1	99.0	99.1	99.6	99.6	99.5	99.4	99.5	99.5	99.3	99.4			99.5
RNA2	100.0											98.6	98.9	99.5

^a OFV-so: GenBank accession no. NC_009608 for RNA1, and NC_009609 for RNA2).

^b nt=nucleotide sequence similarity, aa=deduced amino acid similarity

Table 4. Percentage of amino acid sequence identity of the nucleocapsid protein (N), glycoprotein (G) and polymerase (L) among OFV-NHHS1 and other Rhabdoviruses

Genus		Virus name	N	G	L
PLANT	Cytorhabdovirus	Barley yellow striate mosaic virus (BYSMV)	–	–	22.2
		Lettuce necrotic yellows virus (LNYV)	15.5	9.0	20.6
		Northern cereal mosaic virus (NCMV)	13.9	7.4	22.0
	Nucleorhabdovirus	Maize mosaic virus(MMV)	18.2	11.0	26.4
		Maize fine streak virus (MFSV)	16.9	10.0	26.6
		Potato yellow dwarf virus (PYDV)	17.8	–	–
		Rice yellow stunt virus (RYSV)	16.9	11.5	24.1
		Sonchus yellow net virus (SYNV)	18.1	9.3	24.7
		Taro vein chlorosis virus (TaVVCV)	19.3	12.0	25.0
	Lyssavirus	Australian bat lyssavirus (ABLV)	7.8	9.7	16.3
		Duvenhage virus (DUVV)	9.7	8.7	16.3
		European bat lyssavirus 1 (EBLV-1)	9.3	8.3	16.3
		European bat lyssavirus 2 (EBLV-2)	8.8	9.5	16.5
		Lagos bat virus (LBV)	9.0	9.3	16.5
		Mokola virus (MOKV)	8.8	11.1	16.6
		Rabies virus (RABV)	7.6	9.7	16.2
ANIMAL & FISH	Vesiculovirus	Chandipura virus (CHPV)	8.3	–	–
		Cocal virus (COCV)	8.8	8.5	16.7
		Isfahan virus (ISFV)	8.3	9.0	17.0
		Piry virus (PIRYV)	–	7.3	–
		Vesicular stomatitis Indiana virus (VSIV)	8.8	9.6	17.2
		Vesicular stomatitis New Jersey virus (VSNJV)	–	8.3	–
		Spring viremia of carp virus (SVCV)	7.9	9.3	15.7
	Ephemerovirus	Adelaide River virus (ARV)	7.1	8.0	–
		Bovine ephemeral fever virus (BEFV)	6.9	5.7	16.2
	Novirhabdovirus	Hirame rhabdovirus (HIRRV)	11.8	9.0	13.9
		Infectious hematopoietic necrosis virus (IHNV)	10.6	8.2	13.4
		Viral hemorrhagic septicemia virus (VHSV)	10.3	6.8	14.0
		Snakehead rhabdovirus (SHRV)	8.3	9.8	13.6

structural protein gene of plant Rhabdoviruses, which has been proved to be involved in viral cell-to-cell movement in plants and ORF4 protein may be a counterpart of the rhabdovirus matrix protein (M) (Huang et al., 2005). The G protein is the glycoprotein (61 kDa, 4580 to 6201 nt) which forms the surface spikes of the mature membrane-bound rhabdovirions (Jackson et al., 2005; Kondo et al., 2006). RNA2 encodes a single-large polymerase protein (L) of

212kDa which plays an essential role for the RNA polymerase activity (Schnell and Conzelmann, 1995).

Sequence alignment and phylogenetic tree analysis. The nucleotide and deduced amino acid sequence of OFV-NHHS1 were compared to those of OFV-so isolate from Japan and other Rhabdoviruses. OFV-NHHS1 shared extremely high identity of 98.6-100% and 98.9-99.6% in

Fig. 3. Phylogenetic relationship of amino acid sequence of nucleocapsid protein (A) and polymerase protein (B) among OFV-NHHS1 and other viruses in the family *Rhabdoviridae*. The scale beneath the tree represents the distance between sequences. Sequence alignment and phylogenetic tree were constructed by Clustal in the MegAlign program of DNASTAR Lasergene 7. The accession numbers and abbreviations for rhabdoviruses were as follows: *Barley yellow striate mosaic virus* (BYSMV), FJ665628; *Lettuce necrotic yellows virus* (LNYV), AJ867584; *Northern cereal mosaic virus* (NCMV), AB030277; *Strawberry crinkle virus* (SCV), AY331390; *Eggplant mottled dwarf virus* (EMDV), AM922321; *Maize mosaic virus* (MMV), AY618418; *Maize fine streak virus* (MFSV), AY618417; *Potato yellow dwarf virus* (PYDV), EU183122; *Rice yellow stunt virus* (RYSV), AB011257; *Sonchus yellow net virus* (SYNV), L32603; *Taro vein chlorosis virus* (TaVCV), AY674964; *Australian bat lyssavirus* (ABLV), NC_003243; *Duvenhage virus* (DUVV), EU293120; *European bat lyssavirus 1* (EBLV1), NC_009527; *European bat lyssavirus 2* (EBLV2), NC_009528; *Lagos bat virus* (LBV), EU293110; *Mokola virus* (MOKV), NC_006429; *Rabies virus* (RABV), NC_001542; *Chandipura virus* (CHPV), AY614731; *Cocal virus* (COCV), EU373657; *Isfahan virus* (ISFV), AJ810084; *Piry virus* (PIRYV), EU3733658; *Vesicular stomatitis Indiana virus* (VSIV), NC_001560; *Vesicular stomatitis New Jersey virus* (VSNJV), AF252252; *Spring viremia of carp virus* (SVCV), EU177782; *Adelaide river virus* (ARV), U10363 and AF234998; *Bovine ephemeral fever virus* (BEFV), AF234533; *Hirame rhabdovirus* (HIRRV), NC_005093; *Infectious hematopoietic necrosis virus* (IHNV), X89213; *Viral hemorrhagic septicemia virus* (VHSV), AF143863; *Snakehead rhabdovirus* (SHRV), AF147498.

OFV-NHHS1	588	VVNIDFRKWNQQMR		YSWTNDPSGKEGLRQKYWTIMTACDLM		LVGGGDNQVL		YD.HTTLSPLKQA
OFV-so	588	VVNIDFRKWNQQMR		YSWTNDPSGKEGLRQKYWTIMTACDLM		LVGGGDNQVL		YD.HTTLSPLKQA
MMV	596	IFNLDFIKWNLQMR		WAWAGFESGKEGLRQKGWTILT VVTIM		LMGGGDNQVL		KG.IPLRSPLKAI
TaVCV	597	IFNLDFVKWNLQMR		WSWTGDESGKEGLRQKGWTILT VVTIM		LMGGGDNQVL		KG.MPLRSPLKAI
RYSV	605	VINMDFVKWNLQMR		VCWIDDGAGKEGIRQKAWTITVCDIA		LVGGGDNQVL		KG.VPLRSPLKQV
MFSV	592	SINIDFKKWNQQMR		WSWTNDGSGKEGLRQKGWTITVCMIE		LIGGGDNQVL		YR.GTLRSCLKTA
SYNV	635	SMNIDFSKWNQNMNR		WSRTGDESGKEGLRQKGWTITTVCDIL		LIGGGDNQVL		YSGVPLRGRLLKVI
NCMV	560	CINMDFEKWNLNMR		KSYEGHIRGFEGRLRQKGWTVFTVVLIA		LMGGGDNQVL		YKGVPLCSSLKRI
LNIV	565	CMSLDFEKWNGHMR		YSFTGHKGGQEGRLRQKGWTFITVCLD		LMGGGDNQVL		LKGVPISSMDLKKI
		Motif A		Motif B		Motif C		Motif D

Fig. 4. Comparison of the conserved sequence motives of L-protein encoded by OFV-NHHS1 with other plant rhabdoviruses. The number indicates the location within the L-proteins. Conserved amino acids are boxed in dark grey.

nucleotide and amino acid sequences with OFV-so (Table 3). This sequence analysis suggests that the genome organization and ORF sizes of OFV-NHHS1 were typical of an OFV belonging to rhabdovirus. However, the N, G and L protein of OFV-NHHS1 revealed 6.9-19.3%, 7.3-12.0% and 13.4-26.6% identity to those of 29 Rhabdoviruses, respectively (Table 4). Based on amino acid sequence alignments of N and L protein, phylogenetic relationship of OFV suggested that OFV-NHHS1 was most closely related to *Nucleorhabdovirus*, especially *Maize mosaic virus* (MMV) and *Taro vein chlorosis virus* (TaVCV) (Fig. 3).

Amino acid sequence of L protein of OFV-NHHS1 and OFV-so was compared with other viruses in the genera *Cytorhabdovirus* (NCMV and LNYV) and *Nucleorhabdovirus* (MMV, TaVCV, RYSV, MFSV and SYNV). There were conserved sequences in 4 motives among them (Fig. 4).

Viral infection rate in commercial orchids. The incidence of CymMV, ORSV and OFV in commercial orchids, 51 *Cymbidium* sp., 10 *Phalaenopsis* sp., 22 *Oncidium* sp. and 21 *Dendrobium* sp. plants showing typical viral symptoms were surveyed using RT-PCR with specific primers The

expected PCR products were 718 bp for CymMV, 528 bp for ORSV and 873 bp for OFV, respectively. Incidence of either CymMV or ORSV showed 98% in *Cymbidium* sp., 90% in *Phalaenopsis* sp., 72.7% in *Oncidium* sp. and 66.7% in *Dendrobium* sp. investigated. Orchids mixed infected with CymMV and ORSV were 33.3% in *Cymbidium* sp., 60% in *Phalaenopsis* sp., 36.4% in *Oncidium* sp. and 38.1% in *Dendrobium* sp. whereas orchid infected by CymMV singly was not detected (Fig. 5 and Table 5). OFV positive orchid was found only in a *Cymbidium* sp. (Fig. 5 and Table 5). In this survey, both CymMV and ORSV were prevalent in most orchids cultivated in Korea. It looked like both viruses spread not only by virus-infected plants during mass propagation in tissue culture but also by plant sap through cutting tools or hands during cultivation, because both viruses are transmitted by sap (Franki, 1970; Paul, 1975). However OFV is not dominant in Korea so far.

In conclusion, we here report the molecular and biological properties of OFV Korean isolate, OFV-NHHS1. We found that OFV-NHHS1 was the closest to OFV-so on the basis of full-length nucleotide sequencing and align-

Fig. 5. Detection of CymMV, ORSV and OFV from 51 *Cymbidium* (A), 10 *Phalaenopsis* (B), 22 *Oncidium* (C) and 21 *Dendrobium* sp. (D) showing typical viral symptoms collected from Gyeonggi, Chungnam and Gyeongbuk Provinces. Duplex RT-PCR for CymMV and ORSV and simplex RT-PCR for OFV were used. The size of expected PCR products of CymMV, ORSV and OFV are 718 bp, 528 bp and 873 bp, respectively. Lane M is a 100 bp size marker (Invitrogen, USA) and lane PC and NC mean positive and negative control, respectively.

Table 5. The assessment of virus infection in 4 orchid genera showing typical viral symptom by RT-PCR

Genus	Symptom ^a	No. of plants investigated	No. of plant infected with (%)				
			OFV	CymMV	ORSV	CymMV & ORSV	CymMV or ORSV
<i>Cymbidium</i>	CS	15	0	0	10	5	15
	CS+BN	8	0	0	5	3	8
	CS+CH	1	0	0	1	0	1
	CS+Mo	4	0	0	4	0	4
	CS+Mo+BN	1	0	0	0	1	1
	BN	4	1	0	2	1	3
	Mo	3	0	0	3	0	3
	Mo+BN	1	0	0	1	0	1
	Mo+CS	3	0	0	2	1	3
	Mo+M+BN	1	0	0	1	0	1
	M	3	0	0	1	2	3
	Mo+M	1	0	0	1	0	1
	M+CS	3	0	0	2	1	3
	M+BN	3	0	0	0	3	3
	Total (%)	51	1 (2.0)	0 (0)	33 (64.7)	17 (33.3)	50 (98.0)
<i>Phalaenopsis</i>	Mo	6	0	0	3	3	6
	YS	2	0	0	0	2	2
	N	2	0	0	0	1	1
	Total (%)	10	0 (0)	0 (0)	3 (30)	6 (60)	9 (90.0)
	Mo	13	0	0	4	5	9
<i>Oncidium</i>	Mo+M	1	0	0	0	1	1
	M	6	0	0	3	1	4
	Mo+BN	1	0	0	1	0	1
	Mo+Y	1	0	0	0	1	1
	Total (%)	22	0 (0)	0 (0)	8 (36.4)	8 (36.4)	16 (72.7)
<i>Dendrobium</i>	CS	4	0	0	1	1	2
	SL	1	0	0	0	0	0
	BN	1	0	0	0	1	1
	Mo	1	0	0	0	1	1
	Mo+N	1	0	0	0	1	1
	Y	2	0	0	2	0	2
	YS	6	0	0	3	2	5
	YL	1	0	0	0	0	0
	N	4	0	0	0	2	2
	Total (%)	21	0 (0)	0 (0)	6 (28.6)	8 (38.1)	14 (66.7)

^a CS: chlorotic spot, N: necrosis, Y: yellowing, YL: yellow lesions, YS: yellow spot, BN: black necrosis, Mo: mottle, M: mosaic.

ment. This is the first report of complete nucleotide sequence of OFV isolated in Korea. Duplex RT-PCR for CymMV or/ and ORSV and simplex RT-PCR assay for OFV detection were developed and used successfully in this study. Occurrence of OFV in orchids cultivated in Korea was slight. We assumed the possible explanation of the low infection rate of OFV in orchids in Korea was the low number of OFV source orchids though the vector of OFV, *B. californicus*

Banks (false spider mite), was detected in *Aspidistra elatior* Blume, *Ruscus aculeatus* L. and *Hibiscus syriacus* L. in Korea in 2004 (Seo, 2004). Furthermore *B. californicus* is not a dominant mite in the glass house growing orchid. However if *B. californicus* Banks became prevalent due to the global warming and OFV-infected orchids increased, there would be a tentative possibility of prevalence of OFV in Korea. Further transmission of OFV by *Tenuipalpus*

pacificus Baker (Phalaenopsis mite) must be studied, as they are prevalent in *Dendrobium* sp.

Acknowledgments

This work was supported in part by the Korea Research Foundation Grants funded by the Korean Government (MOEHRD-KRF-2007-359-F00001 and MOEHRD, Basic Research Promotion Fund, KRF-2008-314-F00009) and by the Agenda Program Grants (200901010600310010200) of the Rural Development Administration in Korea.

References

- Blanchfield, A. L., Machenzie, A. M., Gibbs, A., Kondo, H., Tamada, T. and Wilson, C. R. 2001. Identification of Orchid fleck virus by reverse transcriptase-polymerase chain reaction and analysis of isolate relationships. *J. Phytopathol.* 149:713-718.
- Chang, M. U., Arai, K., Doi, Y. and Yora, K. 1976. Morphology and intracellular appearance of orchid fleck virus. *Ann. Phytopathol. Soc. Jpn.* 42:156-167.
- Chang, M. U., Chun, H. H., Baek, D. H. and Chung, J. D. 1991. Studies on the viruses in orchids in Korea. 2. Dendrobium mosaic virus, odontoglossum ringspot virus, orchid fleck virus, and unidentified potyvirus. *Korean J. Plant Pathol.* 7: 118-129.
- Doi, Y., Chang, M.U. and Yora, K. 1977. Orchid fleck virus. CMI/AAB Description of Plant Viruses No. 183.
- Doi, Y., Arai, K. and Yora, K. 1969. Distribution of bacilliform virus particles in Masaki mosaic disease and Cymbidium ring spot disease. *Ann. Phytopathol. Soc. Japan.* 35:388 (Abstract).
- Francki, R. I. B. 1970. Cymbidium mosaic potexvirus. CMI/AAB Description of Plant Viruses No. 27.
- Freitas-Astúa, J., Rezende, J. A. M. and Kitajima, E. W. 1999. Incidence of orchid viruses in the state of São Paulo, Brazil. *Fitopatol Bras* 24:125-130.
- Freitas-Astúa, J., Moreira, L., Rivera, C., Rodríguez, C. M. and Kitajima, E. W. 2002. First report of orchid fleck virus in Costa Rica. *Plant Dis* 86:1402.
- Gibbs, A., Mackenzie, A., Blanchfield, A., Cross, P., Wilson, C., Kitajima, E. W., Nightingale M. and Clements M. 2000. Viruses of orchids in Australia: their identification, biology and control. *Aust. Orchid Rev.* 65:10-21.
- Hogenhout, S. A. Ammar, E. D., Whitfield, A. E. and Redinbaugh, M. E. 2008. Insect vector interactions with persistently transmitted viruses. *Annu. Rev. Phytopathol.* 46:327-359.
- Huang, Y. W., Geng, Y., Ying, X. B., Chen, X. Y. and Fang, R. X. 2005. Identification of a movement protein of rice yellow stunt rhabdovirus. *J. Virol.* 79:2108-2114.
- Jackson, A. O., Dietzgen, R. G., Goodin, M. M., Bragg, J. N. and Deng, M. 2005. Biology of plant rhabdoviruses. *Annu. Rev. Phytopathol.* 43:623-660.
- Kitajima, E. W. Kondo, H., Mackenzie, A., Rezende, J. A. M., Gioria, R., Gibbs, A. and Tamada, T. 2001. Comparative cytopathology and immunocytochemistry of Japanese, Australian and Brazilian isolates of orchid fleck virus. *J. Gen. Plant Pathol.* 67:231-237.
- Kondo, H., Maeda, T. and Tamada, T. 2003. Orchid fleck virus: Brevipalpus californicus mite transmission, biological properties and genome structure. *Exp. Appl. Acarol.* 30: 215-223.
- Kondo, H., Maeda, T., Shirako, Y. and Tamada, T. 2006. Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. *J. Gen. Virol.* 87:2413-2421.
- Lawson, R. H. and Hsu, H. T. 1995. Orchid. In: *Virus and Virus-like Diseases of Bulb and Flower Crops*. Ed. by G. Loebenstein, R. H. Lawwon and A. A. Brunt, pp. 409-420, John Wiley & Sons, N. Y.
- Paul, H. L. 1975. Odontoglossum ring spot virus. CMI/AAB Description of plant viruses No. 155.
- Seo, J. W. 2004. A taxonomic study on the false spider mites (Acari: Tenuipalpidae) in Korea. MS Diss., Chonbuk National University, Chonbuk. (Diss. 1-59).