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The 52 kD Protein Gene of Odontoglossum Ringspot Virus Containing RNA-Dependent RNA Polymerase Motifs and Comparisons with Other Tobamoviruses

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Complementary DNA of the genomic RNA of odontoglossum ringspot virus *Cymbidium* strain (ORSV-Cy) was synthesized from polyadenylated viral RNA and cloned. Selected clones containing the viral RNA-dependent RNA polymerase gene of the virus has been sequenced by automated sequencing system. The complete nucleotide sequence of an open reading frame is 1377 base pairs in length, and encodes a protein of 458 amino acids about 52,334 D. The 52 kD protein of ORSV is 16 amino acids shorter than that of tobacco mosaic virus (TMV). The 52 kD protein of ORSV shares four sequence motifs characteristic of viral RNA-dependent RNA polymerase. Comparison of the ORSV 52 kD protein sequence with that of other five viruses in tobamovirus group showed 76.0 to 60.7% homologies at the amino acid level and the conservation of the four motifs between the viruses.

Keywords: ORSV, viral RNA-dependent RNA polymerase, cDNA, orchid, tobamovirus genus

Odontoglossum ringspot virus (ORSV) is a definite member of the tobamovirus genus, with a monopartite, positive-sense RNA genome (Edwardson and Zettler, 1986). It is one of the most economically important orchid pathogen in Korea (Park *et al.*, 1990a) as well as in worldwide (Paul, 1975; Zettler *et al.*, 1990). There are 13 definite members in the genus and 2 possible tentative viruses (Chara corallina virus, ChaCV; Maracuja mosaic virus, MarMV) which may be placed within the genus (Gibbs, 1986; Gibbs, personal communication, approved by ICTV, 1993). ORSV has been grouped in a subset of the tobamoviruses that includes tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), pepper mild mottle virus (PMMV) and U2 strain of TMV (synonym of tobacco mild green mosaic virus, TMGMV). The complete nucleotide sequences of five members, TMV (Goelet *et al.*, 1982), ToMV (Ohno *et al.*, 1984), TMGMV (Solis and Garcia-Arenal, 1990), cucumber green mottle mosaic virus (CGMMV) (Ugaki *et al.*, 1991) and PMMV (Alonso *et al.*, 1991) have been determined. And partial nucleotide sequence of sunn-hemp mosaic virus (SHMV) has been reported (Meshi *et al.*, 1982). More recently, the entire sequence of ToMV-Ob, a resistant-breaking strain, has been determined (Padgett and Beachy, 1993). The genome organization of TMV, the type member of the genus, has been fully characterized (Goelet *et al.*, 1982; Palukaitis and Zaitlin, 1986). It is generally agreed that single-stranded, positive-sense RNA encodes four proteins; two of which the 126 kD and 183 kD are viral replicases, and the rests are 30 kD movement protein and 17 kD coat protein. It has been proposed that a fifth protein, 54 kD polypeptide, is also encoded by TMV genomic RNA (Sulzinski *et al.*, 1985). The 54 kD protein sequence of TMV is in the frame of the 183 kD viral replicase sequence and shares RNA polymerase motifs (Goelet *et al.*, 1982; Bruenn, 1991).

Although the tobamovirus genus is one of the best-characterized plant virus groups, the taxonomic

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relationships among distinct viruses or even viral strains within one species are not always clear (Gibbs, 1986). The genus still remains an ungrouped genera with other plant virus groups.

We have cloned ORSV genomic RNA and the complete nucleotide sequence of upstream part of the movement protein (MP) gene, the 52 kD protein, has been determined. The sequences of the MP and viral coat protein genes of ORSV has been previously reported (Ryu *et al.*, 1995). Here, we report the complete nucleotide sequence of the 52 kD protein gene of the virus and compared with those of other tobamoviruses.

MATERIALS AND METHODS

Virus source and viral RNA

The ORSV-Cy strain, originally isolated from diseased *Cymbidium goeringii* Reichenbach in Korea, was used as a source of virus (Park *et al.*, 1990a). It was multiplied on tobacco plants (*Nicotiana tabacum* cv. Samsun) by mechanical inoculation of the virus source. Virus was purified and viral RNA was extracted from purified virion particles as described previously (Park *et al.*, 1990b; Ryu and Park, 1994a).

cDNA cloning and generation of subclones

Most of DNA manipulations were performed essentially as described by Sambrook *et al.* (1989). The synthesis of cDNA and cloning for the virus RNA was previously described (Ryu and Park, 1994b). Among recombinant clones harboring cDNAs of ORSV-Cy RNA, pORCY-068, -124 and -224 were subcloned into the pT7T3 18U phagemid vector (Pharmacia). Clone pORCY-124 (3.0 Kb) was digested with *Not*I and *Aat*II, and treated with exonuclease III. After digesting with S1 nuclease, treating with Klenow fragment of *E. coli* DNA polymerase and T4 DNA ligase, deleted clones were selected by the order of the deleted cDNA size. The resulting recombinants were transformed into competent *E. coli* strain NM522 cells by the $CaCl_2$ method (Sambrook *et al.*, 1989).

Nucleotide sequencing

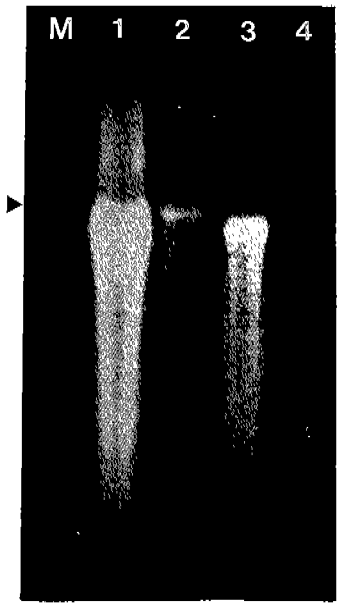

Fig. 1. Electrophoretic pattern of viral RNAs on 1.2% agarose gel. Lane 1, TMV RNA; 2, ORSV RNA; 3, PMMV RNA; 4, ToMV RNA; M, 1 kb ladder as size marker. Arrowhead indicates the site of ORSV RNA.

For DNA sequencing, double-stranded DNA templates of ORSV cDNA were prepared by modified alkaline-lysis minipreparation and Gene Clean Kit (BIO 101). The sequencing reactions were performed by PCR amplification in a DNA Thermal Cycler (Perkin Elmer Cetus) with a Taq Dye Primer Cycle Sequencing Kit (Applied Biosystems) as the manufacturer's instruction. The nucleotide sequences of both strands were determined by using an automated DNA Sequencer model 373A (Applied Biosystems). Analysis of the nucleotide sequence and amino acid sequence were performed by using PC /Gene Sequence Analysis Program (IntelliGenetics) for sequence homology and alignment and phylogenetic tree.

RESULTS

Viral RNA extraction and electrophoresis

The genomic RNA extracted from the purified ORSV-Cy revealed single band pattern about 6,600 nt in size on agarose gel electrophoresis compared with other tobamoviruses RNAs (TMV, ToMV and PMMV) as size markers (Fig. 1). It is the longest

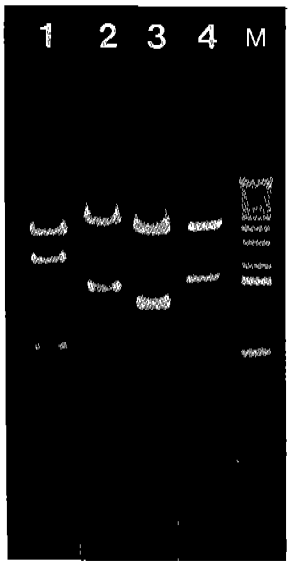

Fig. 2. Electrophoretic pattern of cDNA fragments of subclones for nucleotide sequencing of ORSV RNA. Lane 1, pORCY-124 *Kpn*I/*Eco*RI; 2, pORCY-068 *Kpn*I/*Pst*I; 3, pORCY-068 *Kpn*I/*Xba*I; 4, pORCY-224 *Kpn*I/*Xba*I; M, 1 kb DNA ladder as marker.

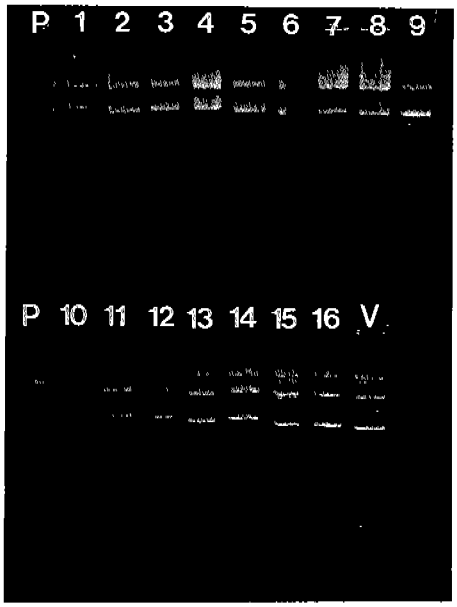

Fig. 3. Electrophoretic pattern of serially deleted cDNA clones from the pORCY-124 clone. Lane P, pORCY-124 parent type; V, pSPORT1 vector; and 1-16, deleted cDNA clones by exonuclease III from the 3'-terminal region.

viral RNA within the tobamovirus genus. The UV absorption pattern of the viral RNA preparation showed a maximum peak at 260 nm, a minimum peak at 240 nm, and a ratio of A280/A260 was 1.98 (data not shown).

Generation of deleted cDNA clones

Four subclones were generated from the pORCY-124, -068 and -224 for nucleotide sequencing (Fig. 2). pORCY-068 and -224 harbored 3'-terminal region of the virus and partially overlapped with the longest pORCY-124. Therefore, three clones were used to confirm sequence accuracy. Serially deleted subclones were generated from the clone pORCY-124 by the Erase-a-Base system (Promega Corp.). Successive unidirectional deleted clones differing in size by 250-300 bp were selected from the library of deleted clones which allowed overlaps of 50-100 bp in sequencing reactions (Fig. 3).

Nucleotide sequence

The nucleotide sequence and predicted amino acid sequence of the gene of ORSV is shown in Fig. 4 with numbering starting with the 5'-terminal region. The nucleotide sequence data would appear in the EMBL, GenBank and DDBJ databases under the accession number X82130. The sequence contained single open reading frame (ORF) of 1377 bp including the initiation (ATG) and termination codons (TAA). Analysis of the ORF revealed one coding region encoding protein of 458 amino acids. The molecular weight of the encoded polypeptides was calculated to be 52,344 D. The ORF, designed as 52 kD, was located upstream part of the viral movement protein (MP) gene and 48 nucleotides shorter than that of TMV (Goelet *et al.*, 1982). The ORF was overlapped in 47 bases with the following MP ORF (Ryu *et al.*, 1995).

Analysis of amino acid sequence and comparison within tobamoviruses

The amino acid sequences of the 52 kD protein between ORSV and other five tobamoviruses were compared and aligned (Fig. 5). The 52 kD protein of ORSV was 16 amino acids shorter than that of TMV. Sequences identities between ORSV and TMV were 67.6% and 72.9% in the nucleotide and amino acid levels, respectively. The nucleotide sequence homologies of the region of ORSV RNA to those of

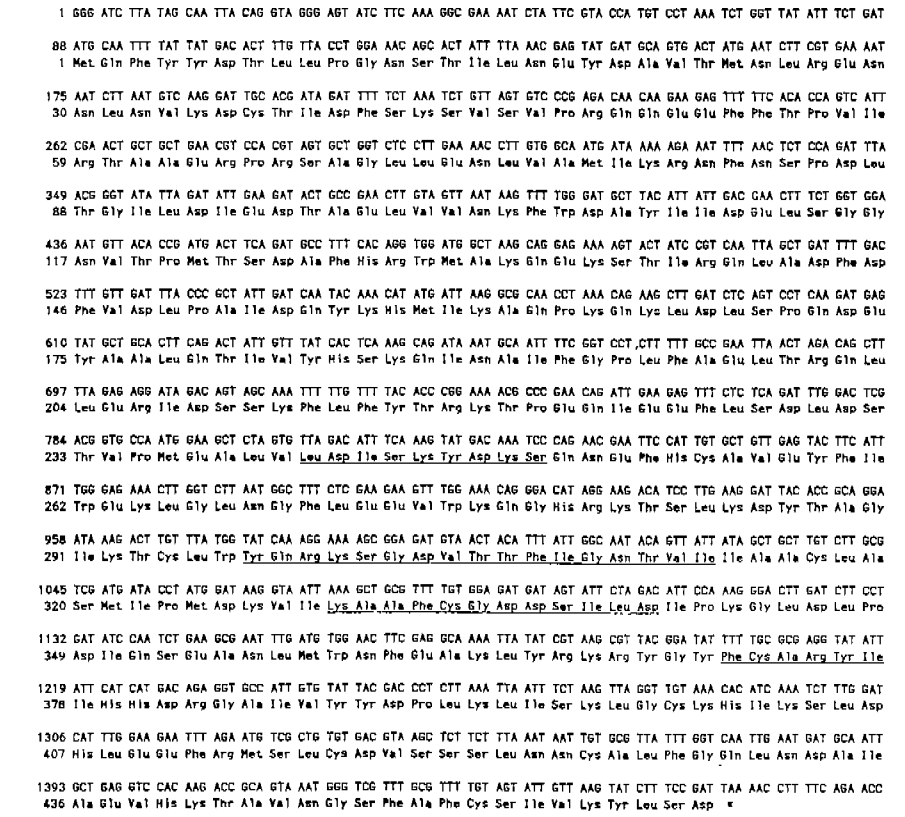

Fig. 4. Nucleotide sequence and amino acid sequence of the 52 kD protein gene of ORSV RNA. The triplet letter amino acid code is written below each codon triplet. An asterisk indicates the termination codon. The four underlines indicate the RNA-dependent RNA polymerase sequence motifs.

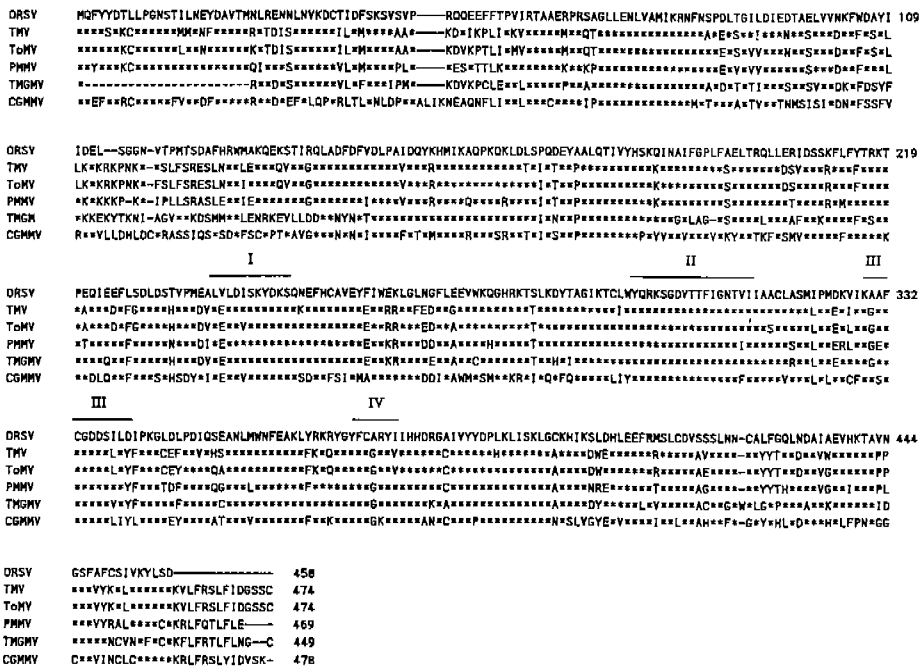

Fig. 5. Comparison of the amino acid sequence of ORSV 52 kD protein with those of five tobamoviruses. Hyphens are used to indicate deletions introduced for maximum alignment. Asterisks means perfectly conserved amino acid residues as compared with ORSV. The I, II, III, and IV indicate the portions of sequence motifs of viral RNA-dependent RNA polymerase.

Table 1. Comparison of amino acid composition of the 52 kD protein of odontoglossum ringspot virus (ORSV) with that of varuses of the tobamovirus group[a]

Code	ORSV		TMV		ToMV		TMGMV		PMMV		CGMMV	
	No	%	No	%	No	%	No	%	No	%	No	%
Ala	33	7.2	27	5.6	28	5.9	29	6.4	24	5.1	27	5.6
Arg	18	3.9	20	4.2	19	4.0	17	3.7	24	5.1	19	3.9
Asn	21	4.5	17	3.5	18	3.7	21	4.6	18	3.8	23	4.8
Asp	35	7.6	36	7.5	37	7.8	32	7.1	32	6.8	36	7.5
Cys	10	2.1	12	2.5	12	2.5	15	3.3	11	2.3	13	2.7
Gln	18	3.9	19	4.0	19	4.0	13	2.8	17	3.6	15	3.1
Glu	27	5.8	24	5.0	23	4.8	23	5.1	30	6.3	19	3.9
Gly	19	4.1	21	4.4	21	4.4	21	4.6	21	4.4	20	4.1
His	10	2.1	12	2.5	10	2.1	11	2.4	10	2.1	12	2.5
Ile	34	7.4	29	6.1	25	5.2	29	6.4	33	7.0	32	6.6
Leu	47	10.2	45	9.4	53	11.1	50	11.1	52	11.0	53	11.0
Lys	32	6.9	41	8.6	40	8.4	42	9.3	39	8.3	29	6.0
Met	11	2.4	13	2.7	11	2.3	11	2.4	9	1.9	12	2.5
Phe	25	5.4	29	6.1	27	5.6	28	6.2	24	5.1	37	7.7
Pro	16	3.4	19	4.0	19	4.0	17	3.7	20	4.2	21	4.3
Ser	31	6.7	33	6.9	33	6.9	24	5.3	27	5.7	39	8.1
Thr	23	5.0	22	4.6	22	4.6	19	4.2	26	5.5	19	3.9
Trp	6	1.3	7	1.4	6	1.2	5	1.1	5	1.0	5	1.0
Tyr	19	4.1	10	4.0	22	4.6	18	4.0	23	4.9	20	4.1
Val	23	5.0	29	6.1	29	6.1	24	5.3	24	5.1	27	5.6
Total	458		474		474		449		469		478	

[a]No, Number of each amino acid residues; %, molar percentage of amino acid residue. Data from Goelet *et al.* (1982) (TMV), Ohno *et al.* (1984) (ToMV), Solis and Garcia-Arenal (1990) (TMGMV), Alonso *et al.* (1991) (PMMV), and Ugaki *et al.* (1991) (CGMMV). ORSV data was from this studies.

Table 2. Comparison of charged amino acids of the 52 kD protein of odontoglossum ringspot virus (ORSV) with that of viruses of the tobamovirus group[a]

Virus	Number of amino acid							Terminal amino acid residue		Estimated isoelectric point (pI)
	(+) charge			(−) charge						
	Arg	Lys	His	Asp	Glu	Cys	Tyr	N	C	
ORSV	18	32	10	35	27	10	19	Met	Asp	5.22
TMV	20	41	12	36	24	12	19	Met	Cys	7.45
ToMV	19	40	10	37	23	12	22	Met	Cys	6.83
TMGMV	17	42	11	32	23	15	18	Met	Cys	7.90
PMMV	24	39	10	32	30	11	23	Met	Glu	7.46
CGMMV	19	29	12	36	19	13	20	Met	Lys	5.93

[a]Residues and pK values taken in acount in the computation by PCGENE software (Program CHARGPRO). pK values of each amino acid are as follows; Arg (12.48), Lys (10.79), His (6.00), Asp (3.65), Glu (4.25), Cys (8.35), Tyr (10.13). Data from Goelet *et al.* (1982) (TMV), Ohno *et al.* (1984) (ToMV, TMV-L), Solis and Garcia-Arenal (1990) (TMGMV, U2-TMV), Alonso *et al.* (1991) (PMMV), and Ugaki *et al.* (1991) (CGMMV).

ToMV, TMGMV, PMMV and CGMMV were 69.4%, 66.2%, 71.3% and 62.2%, respectively. And homologies at the amino acid level were 74.7%, 72.8%, 76.0% and 60.7%, respectively. Table 1 and 2 showed the comparisons of amino acid composition and charged amino acids properties of ORSV with those of other 5 tobamoviruses, respectively. Leucine (47 residues) and tryptophan (6 residues) were the most and the least amino acids, respectively, which are the same compared with that of other 5 tobamoviruses.

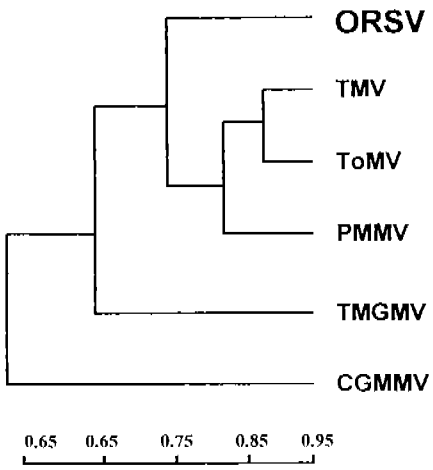

Fig. 6. The phylogenetic relationship of the 52 kD protein gene to viruses of the tobamovirus genus in the amino acid level.

Isoelectric point (pI) estimated by computer-aided analysis was 5.22, which was the lowest value among other tobamoviruses (pI 7.90-5.93). By the phylogenetic relationship of the 52 kD gene among viruses of the tobamovirus genus in the amino acid level, ORSV is more closely related to PMMV and TMGMV than other tobamoviruses (Fig. 6), which is the similar result with the coat protein comparison with the same viruses (Ryu *et al.*, 1995). The 52 kD protein of ORSV shares four sequence motifs (I; 241-249, II; 297-313, III; 329-340, and IV; 372-377 amino acids) which are characteristic of viral RNA-dependent RNA polymerase (Argos, 1988; Bruenn, 1991; Habili and Symons, 1989; Haseloff *et al.*, 1984; Hodgman, 1988). The Gly-Asp-Asp motif of GDD box (334-336 amino acids) is also found in the third motif.

DISCUSSION

This study was carried out to analyze the gene of ORSV RNA focusing on the viral polymerase. ORSV was first isolated from *Rossioglossum grande* (Helmut *et al.*, 1992) formerly called *Odontoglossum grande* (Jensen and Gold, 1951). We have constructed cDNA library of ORSV RNA and cloned to the independent recombinants which covered the entire region of the viral RNA (Ryu and Park, 1994b). Partial characterizations of ORSV genomic RNA have been determined by three groups (Dubs and Van Regenmortel, 1990; Isomura *et al.*, 1991; Ryu and Park, 1994b; Ryu *et al.*, 1995) but entire studies have not been reported as yet.

The 54 kD of TMV is in the same frame as the 183K readthrough product of 126 kD viral polymerase (Goelet *et al.*, 1982). The 183 kD polypeptide is produced by readthrough of the leaky-termination codon of the gene for the 126 kD polypeptide. These proteins are invloved in replication as a replicase or its components. They are found in cytoplasm and show sequence similarity with replicative proteins of alphalike supergroup RNA viruses. The C-terminal one-third of the 183 kD polypeptide has a motif common to RNA-dependent RNA polymerases. The sequence homologies of the ORSV 52 kD with other known tobamoviruses showed 62.2% to 71.3% and 60.7% to 76.0% in the nucleotide and amino acid levels, respectively. This region is more conserved than movement protein and coat protein within the genus. Especially, four sequence motifs are well conserved among the tobamoviruses. The positive strand RNA viruses can be grouped based upon amino acid sequence motifs of the putative NTP-binding domain of RNA helicase and the core domain of RNA-dependent RNA polymerase (POL) (Fraile and Garcia-Arenal, 1990; Haseloff *et al.*, 1984; Koonin, 1991). The percentage of amino acid sequence identities in the POL motif region between ORSV and TMV is 89.1%. And GDD box is also found in the third POL motif, which is best-conserved in the members of other virus groups as well as tobamoviruses. Interestingly, the 52 kD protein of ORSV shares 43.7% amino acid sequence homology with pea early browning virus (PEBV), a tobravirus (MacFarlane and Davies, 1992). The presence of four sequence motifs of POL suppose that the 52 kD protein of ORSV may be a third viral-coded replicase or a replicase-associated regulatory molecule.

Golemboski *et al.* (1990) and Carr *et al.* (1992) have reported that tobacco transformed with the 54 kD protein gene of TMV have been showed to be highly resistant to systemic infection with TMV. It was also exhibited against both TMV RNA and TMV particles, and did not appear to break down over time or with increasing concentrations of inoculum (Carr and Zaitlin, 1991, 1993). Interestingly, the expression of the 54 kD protein gene in transgenic tobacco plants rendered them highly resistant to TMV. Although ss and ds forms of a subgenomic RNA (I1

RNA) corresponding to the ORF for the 54 kD protein have been found in TMV-infected cells (Sulzinski *et al.*, 1985; Zelcer *et al.*, 1981) with the ss II RNA occurring on polysomes, no 54 kD protein has been detected *in vivo*. MacFarlane and Davies (1992) have reported that transgenic *Nicotiana benthamiana* plants with an analogous 54 kD protein sequence from PEBV renders these plants highly resistant to that virus.

Recently, we have determined the complete nucleotide sequence of ORSV. The 52 kD protein of ORSV is in the same frame of 181 kD readthrough product of 121 kD viral polymerase. The genome organization is similar to that of TMV and other tobamoviruses. Recognition of genes of virus is important for understanding the gene function as well as application of the gene for resistant transgenic plant and for understanding of pathogenesis in host plants by those viruses.

We currently start to clone the 52 kD gene of ORSV for plant transformation and to construct the *E. coli* expression system for detection of viral polymerase from virus-infected orchid plant. We expect that the gene, if succesfully introduced to orchid plants, would render virus-resistant transgenic plant.

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LITERATURE CITED

Alonso, E., I. Garcia-Luque, A. de la Cruz, B. Wicke, M. J. Avila-Rincon, M.T. Serra, C. Castresana and J.R. Diaz-Ruiz. 1991. Nucleotide sequence of the genomic RNA of pepper mild mottle virus, a resistance-breaking tobamovirus in pepper. *J. Gen. Virol.* **72**: 2875-2884.

Argos, P. 1988. A sequence motif in many polymerases. *Nucleic Acids Res.* **16**: 9909-9916.

Bruenn, J.A. 1991. Relationships among the positive strand and double-strand RNA viruses as viewed through their RNA-dependent RNA polymerases. *Nucleic Acids Res.* **19**: 217-226.

Carr, J.P., L.E. Marsh, G.P. Lomonossoff, M.E. Sekiya and M. Zaitlin. 1992. Resistance to tobacco mosaic virus induced by the 54-kD gene sequence requires expression of the 54-kD protein. *Mol. Plant-Microbe Interact.* **5**: 397-404.

Carr, J.P. and M. Zaitln. 1991. Resistance in transgenic tobacco plants expressing a nonstructural gene sequence of tobacco mosaic virus is a consequence of markedly reduced virus replication. *Mol. Plant-Microbe Interact.* **4**: 579-585.

Carr, J.P. and M. Zaitlin. 1993. Replicase-mediated resistance. *Seminars in Virology* **4**: 339-347.

Dubs, M.C. and M.H.V. Van Regenmortel. 1990. Odontoglossum ringspot virus coat protein: sequence and antgenic comparisons with other tobamoviruses. *Archives of Virology* **115**: 239-249.

Edwardson, J.R. and F.W. Zettler. 1986. Odontoglossum ringspot virus. *In* The Plant Viruses, Vol. 2, The Rod-shaped Plant Viruses. M.H.V. Van Regenmortel and H. Fraenkel-Conrat (eds.). Plenum Press, New York, pp. 233-247.

Fraile, A. and F. Garcia-Arenal. 1990. A classification of the tobamoviruses based on comparisons among their 126K proteins. *J. Gen. Virol.* **71**: 2223-2228.

Gibbs, A.J. 1986. Tobamovirus Classification. *In* The Plant Viruses, Vol. 2, The Rod-shaped Plant Viruses. M.H.V. Van Regenmortel and H. Fraenkel-Conrat (eds.). Plenum Press, New York, pp. 233-247.

Goelet, P., G.P. Lomonossoff, P.J.G. Butler, M.E. Akam, M.J. Gait, and J. Karn. 1982. Nucleotide sequence of tobacco mosaic virus RNA. *Proc. Natl. Acad. Sci. USA* **79**: 5818-5822.

Golemboski, D.B., G.P. Lomonossoff and M. Zaitlin. 1990. Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus. *Proc. Natl. Acad. Sci. USA* **87**: 6311-6315.

Habili, N. and R.H. Symons. 1989. Evolutionary relationship between leuteoviruses and other RNA plant viruses based on sequence motifs in their putative RNA polymerases and nucleic acid helicases. *Nucleic Acids Res.* **17**: 9543-9555.

Haseloff, J., Goelet, P., Zimmern, D., Ahlquist, P., Dasgupta, R. and Kaesberg, P. 1984. Striking similarities in amino acid sequence among nonstructural proteins encoded by RNA viruses that have dissimilar genomic organization. *Proc. Natl. Acad. Sci. USA* **81**: 4358-4362.

Helmut, B., P. Cribb and E. Launert. 1992. The Manual of Cultivated Orchids Species. 3rd ed. American Orchid Society Book Department, Florida, 585 pp.

Hodgman, T.C. 1988. A new superfamily of replicative proteins. *Nature* **335**: 22-23.

Isomura, Y., Y. Matumoto, A. Murayama, M. Chatani, N. Inouye and M. Ikegami. 1991. Molecular cloning, sequencing and expression in *Escherichia coli* of the odontoglossum ringspot virus coat protein gene. *J. Gen.*

Virol. **72**: 2247-2249.

Jensen, D.D. and A.H. Gold. 1951. A virus ring spot of *Odontoglossum* orchids: symptoms, transmission, and electron microscopy. *Phytopathology* **41**: 648-653.

Koonin, E.V. 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. Gen. Virol.* **72**: 2197-2206.

MacFarlane, S.A. and J.W. Davies. 1992. Plants trnasformed with aregion of the 201-kilodalton replicase gene from pea early browning virus RNA1 are resistant to virus infection. *Proc. Natl. Acad. Sci. USA* **89**: 5829-5833.

Meshi, T., T. Ohno and Y. Okada. 1982. Nucleotide sequence of the 30K protein cistron of cowpea strain of tobacco mosaic virus. *Nucleic Acids Res.* **10**: 6111-6117.

Ohno, T., M. Aoyagi, Y. Yamanashi, H. Saito, S. Ikawa, T. Meshi and Y. Okada. 1984. Nucleotide sequence of the tobacco mosaic virus (tomato strain) genome and comparison with the common strain genome. *J. Biochem.* **96**: 1915-1923.

Padgett, H.S. and R.N. Beachy. 1993. Analysis of tobacco mosaic virus strain capable of overcoming N gene-mediated resistance. *Plant Cell* **5**: 577-586.

Palukaitis, P. and M. Zaitlin. 1986. Tobacco mosaic virus: Infectivity and replication. *In* The Plant Viruses, The Rod-shaped Plant Viruses. M.H.V. Van Regenmortel and H. Fraenkel-Conrat (eds.). Vol. 2, Plenum Press, New York, pp. 105-132.

Park, W.M., K.E. Yoon, S.Y. Chung and K.H. Ryu. 1990a. Identification of odontoglossum ringspot virus isolated from *Cymbidium goeringii* Reichenbach in Korea. *Korean J. Plant Pathol.* **6**: 387-392.

Park, W.M., K.E. Yoon, S.Y. Chung and K.H. Ryu. 1990b. Purification and serological detection of odontoglossum ringspot virus isolated from *Cymbidium goeringii* in Korea. *Korean J. Plant Pathol.* **6**: 474-481.

Paul, H.L. 1975. Odontoglossum ringspot virus. CMI/AAB Description of Plant Viruses No. 155., Kew, England.

Ryu, K.H. and W.M. Park. 1994a. Complementary DNA cloning of genomic RNA in orchid strain of tobacco mosaic virus. *J. Plant Biol.* **37**: 349-355.

Ryu, K.H. and W.M. Park. 1994b. Construction of complementary DNA library and cDNA cloning for Cy strain of odontoglossum ringspot virus genomic RNA. *Korean J. Plant Pathol.* **10**: 228-234.

Ryu, K.H., C.W. Choi, J.K. Choi and W.M. Park. 1995. Cloning of the 3′-terminal region encoding movement and coat proteins of a Korean isolate of odontoglossum ringspot virus. *Archives of Virology* **140**: 481-490.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory, ColdSpring Harbor, New York.

Solis, I. and F. Garcia-Arenal. 1990. The complete nucleotide sequence of the genomic RNA of the tobamovirus tobacco mild green mosaic virus. *Virology* **177**: 553-558.

Sulzinski, M.A., K.A. Gabard, P. Palukaitis and M. Zaitlin. 1985. Replication of tobacco mosaic virus. VIII. Characterization of a third subgenomic TMV RNA. *Virology* **145**: 132-140.

Ugaki, M., M. Tomiyama, T. Kakutani, S. Hidaka, T. Higuchi, P. Nagata, T. Sato, F. Motoyoshi and M. Nishiguchi. 1991. The complete nucleotide sequence of cucumber green mottle mosaic virus (SH strain) genomic RNA. *J. Gen. Virol.* **72**: 1487-1495.

Zelcer, A., K.F. Weaber, E. Balazs and M. Zaitlin. 1981. The detection and characterization of viral-related double-stranded RNAs in tobacco mosaic virus-infected plants. *Virology* **113**: 417-427.

Zettler, F.W., N.J. Ko, G.C. Wisler, M.S. Elliott and S.M. Wong. 1990. Viruses of orchids and their control. *Plant Dis.* **74**: 621-626.

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RNA-dependent RNA 重合酵素 Motif를 包含하는 오돈토글로썸 윤문 바이러스 52 kD 遺傳子의 特性과 다른 Tobamovirus와의 比較

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적 요

오돈토글로썸 윤문 바이러스 심비디움 계통(ORSV-Cy) 게놈 RNA를 polyadenylation시켜 이로부터 cDNA를 합성하여 클로닝하였다. 바이러스 RNA-dependent RNA 중합효소 유전자를 포함한 클론을 선발하여 이의 염기서열을 결정하였다. 이 염기서열을 분석한 결과 1377 bp의 open reading frame(ORF)으로 총 458개 아미노산과 52,334 D의 단백질을 암호화하고 있었다. ORSV-Cy 52 kD 단백질은 담배 모자이크 바이러스의 54 kD 단백질보다 16개의 아미노산이 적었고, 네개의 바이러스 RNA-dependent RNA 중합효소 motif를 포함하고 있었다. Tobamovirus속에 속하는 5종의 다른 바이러스와 ORSV에서 52 kD 단백질을 비교한 결과, 76.0~60.7%의 높은 아미노산 상동성을 보였으며, 네개의 motif 모두 잘 보존되어 있었다.

주요어: 오돈토글로썸 윤문 바이러스, 바이러스 RNA-dependent RNA 중합효소, cDNA, Tobamovirus, 난

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