Plant Pathol. J. 21(2) : 167-171 (2005)

The Plant Pathology Journal

Isolation and Characterization of Watermelon Isolate of *Cucumber green mottle mosaic virus* (CGMMV-HY1) from Watermelon Plants with Severe Mottle Mosaic Symptoms

Chang Ki Shim[1], Ki Soo Han[1], Jung Han Lee[1], Dong Won Bae[2], Dong Kil Kim[3] and Hee Kyu Kim[3]*

[1]Division of Applied Biology & Environmental Sciences, Gyeongsang National University, Jinju 660-701, Korea

[2]Central Laboratory and [3]Research Institute of Life Science, Gyeongsang National University, Jinju 660-701, Korea

(Received on February 2, 2005; Accepted on February 22, 2005)

We isolated the *Cucumber green mottle mosaic virus* (CGMMV) particles from watermelon leaves and designated as CGMMV-HY1 as a watermelon isolate and attempted to characterize the pathogenic isolate responsible for such an epidemic in watermelon and also to monitor dominant viral isolates in greenhouse. The watermelon plants infected with CGMMV generally showed mottle mosaic, mosaic, growth stunting, necrosis and deformed fruit. The reactions of indicator plants to CGMMV-HY1 were the local lesions on *Nicotiana tabacum cv.* White Burley, *Nicotiana tabacum cv.* Samsun, and *Chenopodium amaranticola*, and the mosaic symptoms only on *Cucumis sativus*, but the CGMMV-HY1 did not infect *Nicotiana sylvesytis*, *Datura stramonium*, *Chenopodium quinoa*, and *Petunia hybrida*. Purified virus particles were rod-shaped and about 300 nm long. The coat protein (CP) of purified CGMMV-HY1 was single band with molecular weight of about 16.5 kDa which was confirmed by western blot analysis probed with monoclonal antibody of CGMMV-HY1. The genomic and subgenomic RNAs of 6.4 kb and 0.75 kb were revealed by the electrophoresis on 1.2% formaldehyde-denatured agarose gel. Viral and complementary CGMMV-specific primer sets were designed for spanning the genome using previously reported CGMMV sequences. A 464bp of CP gene of CGMMV-HY1 was amplified by RT-PCR and cloned into PGEM-T easy vector. The nucleotide sequence of CP gene of CGMMV-HY1 shared 98%, 99%, and 100% identities with that of CGMMV strains W, KOM, and KW respectively. Based on these results, we identified CGMMV-HY1 as a CGMMV isolate of watermelon, a member of *Tobamovirus*.

Keywords : *Cucumber green mottle mosaic virus*, CGMMV-HY1 isolate, coat protein, RT-PCR

Unexpectedly severe epidemics of watermelon in Korea resulted in significant economic losses as well as tremendous social impact in 1998, to date. Lee et al. (1990) identified, for the first time in Korea, watermelon strain of *Cucumber green mottle mosaic virus* (CGMMV) belongs to the genus *Tobamovirus* from leaves showing systemic mosaic symptom in watermelon being cultivated in greenhouse from the southern parts of Korea including Jinju, It was distinct from *Tobacco mosaic virus* (TMV) serologically and biologically for its reaction to indicator plants. As of 1998, acreage of 463 ha for watermelon cultivation was reported to be widespread epidemic of CGMMV (Choi et al., 2001; Lee et al., 1990), which was attributed to bottle gourd seed lots imported from China and drew the attention of public concern. The causal symptoms appeared on leaf, stem and fruit coat of watermelon and produced local lesions on *Chenopodium amaranticolor*, mosaic symptoms on *Cucumis sativus* and no symptoms on *Nicotiana glutinosa*, *Nicotiana tabacum* Turkish, *Petunia* and *Datura stramonium* by sap inoculation (Hollings et al., 1975; Lee et al., 1990).

In cucumber (*Cucumis sativus*) the type strain causes leaf mottling, blistering and distortion with stunted growth. Yield losses may be 15%; fruits are usually unmarketable, but some strains cause severe fruit mottling and distortion (Inoue et al., 1967). Some Asian strains show no leaf symptoms but suffer yield losses.

Transmission also occurs through foliage contact, when plants are handled during cultivation, or when infected rootstocks are used in watermelon or cucumber cultivation. Choi et al. (2004) estimated soil transmission rate of the virus in watermelon to be 0.2 to 3.5%.

In 1967, Inoue reported that CGMMV was first found in cucumber plants in Japan and formerly referred to as a new strain of CGMMV (CGMMV-C or CHMMV-Cu). However, Francki et al. (1986) renamed CGMMV-C as *Kyuri green mottle mosaic virus* (KGMMV) by serological and molecular hybridization analysis.

In Korea, Lee et al. (2000) reported that the virus causing green mottle and severe mosaic leaf symptoms and fruit distortion and malformation symptoms on zucchini squash

*Corresponding author.
Phone) +82-055-751-5443, FAX) +82-55-752-8204
E-mail) heekkim@nongae.gsnu.ac.kr

named as KGMMV-Z based on host range and comparison of nucleotide sequence between the agent virus and CGMMV isolates. However, Ryu et al. (2000) suggested that KGMMV-Z in zucchini (Lee et al., 2000) and KGMMV (Francki et al., 1986) was distinct species of the genus by analysis of coat protein genes between the two virus isolates and named it as *Zucchini green mottle mosaic virus* (ZGMMV). Choi et al. (2001) also proposed that KGMMV-Z (Lee, 2000) might be revised as ZGMMV because of difference in the gene sequence homology and serological specificity among these Tobamoviruses.

In this study, we have collected the watermelon plant infected with *Cucumber green mottle mosaic virus* (CGMMV-HY1) in the major greenhouse cultivation regions of Gyeongnam province during 5 years from 2000 to 2004 and attempted to characterize by inoculating to indicator plants and also by TEM. Coat protein and viral RNA were analyzed. Subsequently, coat protein gene was cloned and RT-PCR products were sequenced and similarities were compared.

In order to construct of antibodies for detection and identification of CGMMV, CGMMV-HY1 isolate was originally isolated from leaves of watermelon (Sambog-Honey) with severe mottle mosaic symptom in Hamyang 1999, and primarily inoculated in *Chenopodium amaranticola* to obtain single local lesions, which were individually macerated and proliferated in Cucumber (Fig. 1A, B, C).

Reference strains and species (CGMMV-W, ZGMMV, KGMMV, Fny-CMV, PMMoV) were kindly supplied by the Plant Virus GenBank (PVGB) of Seoul Women's University, Seoul, Korea.

We have collected the Cucurbit plant infected with *Cucumber green mottle mosaic virus* (CGMMV) in the major greenhouse cultivation regions of Gyeongnam province during 5 years from 2000 to 2004. CGMMV particles were detected from symptomatic Cucurbit samples by the monoclonal antibody based-DAS-ELISA system and sample was subjected to RT-PCR of coat protein for further confirmation. Of the Cucurbit plants, watermelon, melon, cucumber and zucchini were the host for CGMMV epidemics showing variety of symptoms such as mosaic, mottle mosaic, severe mottle mosaic, necrosis, fruit deforming and leaf rolling (Table 1).

Fig. 1. Symptomatology and particle morphology of *Cucumber green mottle mosaic virus* (CGMMV-HY1) particles: **A**, mottle mosaic; **B**, severe mottle mosaic; **C**, deformed fruit; and **D**, purified electron micrograph of CGMMV-HY1.

Purified CGMMV-HY1 particles were placed on carbon-coated copper grids and negatively stained with 2% sodium phosphotungstate (PTA), pH 7.0. The grids were examined in JEM-100SX (JEOL Ltd., UK) electron microscope. Transmission Electron microscopy revealed, CGMMV-HY1 particles rod-shaped with about 300 nm in length (Fig. 1D), which was the typical morphology of the genus

Table 1. The location, viral symptoms and hosts that were appeared on the Cucurbit plants infected with CGMMV in greenhouse of Gyeongnam province, during 5 years from 2000 to 2005[a]

Region	Host	**Viral Symptoms**[b]
Haman	Watermelon, Melon	M, MoM, SMM, N, DF
Uiryeong	Watermelon, Cucumber, Zucchini	M, MM, N, DF
Sancheong	Watermelon	M, MoM, SMM, N, DF
Changnyeong	Watermelon, Cucumber	M, SMM, N, DF
Gimhae	Watermelon, Cucumber	M, MoM, N,
Chinju	Watermelon, Zucchini, Cucumber, Melon	M, SMM, N, LR, DF
Hamyang	Watermelon, Melon	M, SMM, N
Changwon	Watermelon, Zucchini, Melon	M, MoM, SMM, N, DF

[a] CGMMV particles were detected from symptomatic cucurbit samples by the monoclonal antibody based-DAS-ELISA system and sample was subjected to RT-PCR.
[b] M, mosaic; MoM, mottle mosaic; SMM, Severe mottle mosaic; N, necrosis; DF, deformed fruit; LR, Leaf Rolling.

Table 2. Reactions of indicator plants mechanically inoculated with purified CGMMV-HY1

Indicator Plant	Host reaction of CGMMV[a]	
	CGMMV-HY1	CGMMV-W
Nicotiana sylvestris	—/—[b]	NT[c]
Nicotiana tabacum cv. White Burley	LL/—	LL/—
Nicotiana tabacum cv. Samsun	LL/—	LL/—
Datura stramonium	—/—	—/—
Chenopodium amaranticola	LL/—	LL/—
Chenopodium quinoa	—/—	—/—
Petunia hybrida	—/—	—/—
Cucumis sativus	I/M	I/M

[a]CGMMV-HY1: isolated from leaves of watermelon (Sambog-Honey) with severe mottle mosaic symptom, and primarily inoculated in *Chenopodium amaranticola* to obtain single local lesions, which were individually macerated and proliferated in cucumber. CGMMV-W: Reference strain that was kindly supplied from the Plant Virus GenBank (PVGB) of Seoul Women's University, Seoul, Korea.

[b]-/-, symptoms on inoculated leaves/upper leaves; LL, local lesion; M, mosaic; I, latent symptomless; —, not infected

[c]Not tested

Tobamovirus (Lee et al., 1990).

The local lesions were induced on the inoculated leaves of *Nicotiana tabacum cv.* White Burley, *Nicotiana tabacum cv.* Samsun, and *Chenopodium amaranticola* by CGMMV-HY1. The mosaic symptoms developed only on the upper leaves of *Cucumis sativus* without any symptom developed on inoculated leaves. But the CGMMV-HY1 did not infect in *Nicotiana sylvesytis*, *Datura stramonium*, *Chenopodium quinoa*, and *Petunia hybrida* on the inoculated and/or upper leaves (Table 2). This result was similar to that described for CGMMV-W by Choi et al. 1998.

The size of the viral coat protein of CGMMV-HY1 was determined by electrophoresis of 15% SDS-poly-acryl-amide gel electrophoresis (SDS-PAGE) gels with glycine-acetic acid buffers (pH 4.0) described by Laemmli (1970).

Electrophoretic pattern of purified coat protein of CGMMV-HY1 was analyzed along with that of reference viruses Fny-CMV, KGMMV, ZGMMV, and PMMoV. One major band about 16.5 kDa, distinguishable from those of other 4 viruses shown in Figure 2A.

Previously, coat protein of CGMMV-SH was reported to be 17.3 kDa by Ugaki et al. (1991) and those of ZGMMV and KGMMV was 17 kDa and 18 kDa, respectively, by Ryu et al. (2000). The molecular sizes of coat proteins for reference virus strains shown in Figure 2, A are agreeable with those previous reports of other groups. Viral coat protein bands were confirmed by Western blot analysis probed with monoclonal antibody of CGMMV-HY1 (Fig. 2B).

In RNA analysis, a single-stranded genomic RNA about

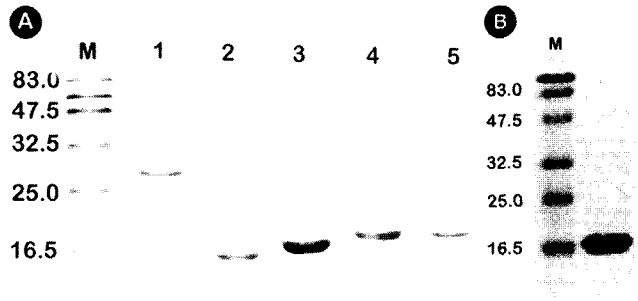

Fig. 2. SDS-PAGE analysis (**A**) and western blot analysis of the viral coat proteins of CGMMV-HY1 (**B**). Coat Proteins prepared from purified CGMMV-HY1 was electrophoresed on 15 % SDS-polyacryl amide gels. The protein band in gel was stained with Coomassie Blue R250 and electro blotted onto PVDF membrane and probed with CGMMV-HY1 Monoclonal antibody. Lanes 1-5 in panel A represent coat proteins purified from CMV-Fny, CGMMV-HY1, ZGMMV, KGMMV, and PMMoV, respectively. M indicates prestained protein standard markers.

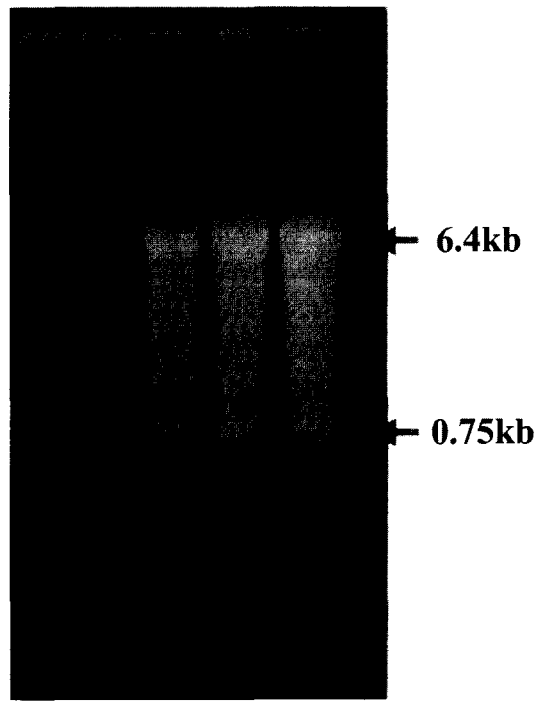

Fig. 3. Electrophoretic pattern of genomic RNAs of *Cucumber green mottle mosaic virus* (CGMMV-HY1) were analyzed on 1.2% formaldehyde-denatured agarose gel with MOPS buffer system and stained with ethidium bromide. Lane M, 100bp DNA ladder; 1, 2, and 3 lanes, CGMMV-HY1 genomic RNAs. The major genomic RNAs were about 6.4 kb and subunit genomic RNA was about 0.75 kb.

6.5 kb and a sub-genomic RNA of 0.75 kb were found in the electrophoresis patterns of CGMMV-HY1. The presence of the sub-genomic RNA in the virus particles demonstrates that the virion assembly origin of the virus is located in the CP region (Fig. 3).

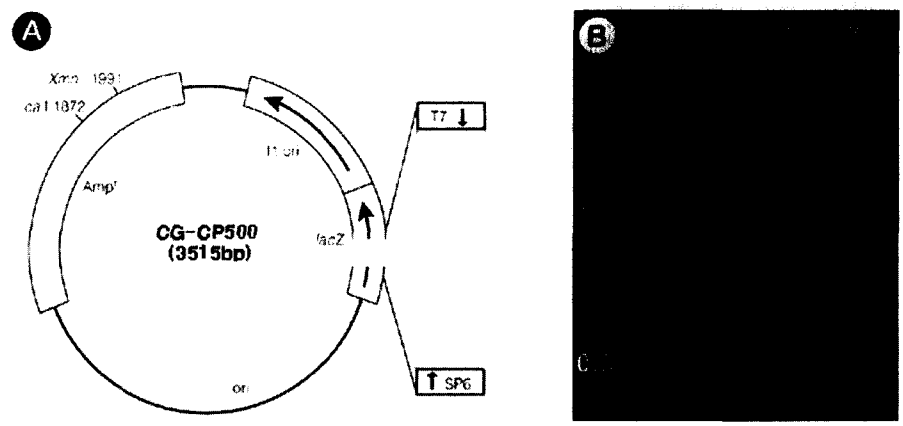

Fig. 4. Construction of cDNA (CG-CP500) of coat protein gene of *Cucumber green mottle mosaic virus*. A 500 bp of cDNA of CP gene was cloned into *EcoRI*/*SpeI* site of pGEM T easy Vector (3.015 bp). Lanes 1-3 in panel B represent pGEM T easy vector (3.015 kb), cDNA of CP gene of CGMMV-HY1 (464 bp), and transformants (3.5 kb), respectively.

The nucleotide sequences of primer sets for CGMMV-HY1 were designed on the basis of a comparative analysis of previously reported nucleotide sequences of CGMMV-W in GenBank by Tan et al. (2000). Reverse transcription-polymerase chain reaction (RT-PCR) was done as described previously by Ryu and Park (1995) and Choi et al. (2002).

An amplified RT-PCR product of 464 bp for CP gene of CGMMV-HY1 was successfully amplified from a viral RNA of CGMMV-HY1 by RT-PCR and cloned into pGEM-T easy vector (3 kb). About 3.5 kb of plasmids were obtained by cloning of 464 bp of RT-PCR products (Fig. 4) 464 bp of nucleotides sequences of CP gene of CGMMV-HY1 is shown in Figure 5. The nucleotide sequence of CP gene of CGMMV-HY1 shared 98%, 99%, and 100% similarities with CGMMV strains W, KOM and KW (Kim et al., 2003), respectively.

Based on above results, we identified CGMMV-HY1 as a

```
CGHY1:   1 atggcttacaatccgatcacacctagcaaacttattgcgtttagtgcttcttatgttccc  60
CGKOM: 5763 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5822
CG-KW: 5763 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5822
CG- W: 5763 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5822
CGHY1:  61 gtcaggactttacttaattttctagttgcttcacaaggtaccgctttccagactcaagcg 120
CGKOM: 5823 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5882
CG-KW: 5823 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5882
CG- W: 5823 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5882
CGHY1: 121 ggaagagattctttccgcgagtccctgtctgcgttaccctcgtctgtcgtagatattaat 180
```

Fig. 5. Similarities of nucleotide sequence of coat protein gene of CGMMV-HY1 compared with the other CGMMV strains, CG-W, CG-KOM and CG-KW (Kim et al., 2003). CG-W is an isolates reported by Tan et al., 2000.

```
CGKOM: 5883 ||||||||||||||||||||||||||||||||||||||||||||||||||c|||||||||||||||| 5942
CG-KW: 5883 ||||||||||||||||||||||||||||||||||||||||||||||||||c|||||||||||||||| 5942
CG- W: 5883 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 5942
CGHY1:  181 tctagattcccagatgcgggttttttacgctttcctcaacggtcctgtgttgaggcctatc 240
CGKOM: 5943 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6002
CG-KW: 5943 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6002
CG- W: 5943 |||||g|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6002
CGHY1:  241 ttcgtttcgcttctcagctccacggatacgcgtaatagggtcattgaggttgtagatcct 300
CGKOM: 6003 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6062
CG-KW: 6003 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6062
CG- W: 6003 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6062
CGHY1:  301 agcaatcctacgactgctgagtcgcttaacgccgtaaagcgtactgatgacgcgtctacg 360
CGKOM: 6063 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||a 6122
CG-KW: 6063 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||a 6122
CG- W: 6063 ||||||||||||||||||||||||||||||||t|||||||||||||||||||||||||||||||a|||||| 6122
CGHY1:  361 gccgctagggctgagatagataatttaatagagtctatttctaagggttttgatgtttac 420
CGKOM: 6123 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6182
CG-KW: 6123 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 6182
CG- W: 6123 ||||||||||||||||a|||||||||||||||||||||||||||||||||||||||||||||||||| 6182
CGHY1:  421 gatagggcttcatttgaagccgcgttttcggtagtctggtcaga 464
CGKOM: 6183 |||||||||||||||||||||||||||||||||||||||||| 6226
CG-KW: 6183 |||||||||||||||||||||||||||||||||||||||||| 6226
CG- W: 6183 |||||||||||||||||||||||||||||||||||||||||| 6226
```

Fig. 5. Continued.

CGMMV isolate of watermelon, a member of Tobamovirus.

Acknowledgment

This study was supported in part by a Research Grant from the Biological Industry Project, Gyeongnam provinces (2000-5). This work is partial fulfillment of senior authors for the Degree of Doctor of Philosophy in Plant Pathology, Gyeongsang National University.

References

Choi, G. S., Kim, J. H., Chung, B. N., Kim, H. R. and Choi, Y. M. 2001. Simultaneous detection of three Tobamovirus in Cucurbits by rapid immunofilter paper assay. *Plant Pathol. J.* 17:106-109.

Choi, J. G., Kang, S. G., Choi, Y. J., Jang, I. S., Suh, S. G. and Chung, H. D. 2004. Viral symptoms observed on Korean native Gourd (*Lagenaria siceraria*) plants and their causal viruses. *Kor. J. Hort. Sci. Technol.* 22:7-11 (in Korean with English summary).

Choi, J. K., Kim, H. J., Yoon, J. Y., Park, S. J., Kim, D. W. and Lee, S. Y. 1998. Detection of virus in fruit and seeds of vegetables using RT-PCR. *Korean J. Plant Pathol.* 14:630-635.

Choi, S. K., Choi, J. K., Ryu, K. H. and Park, W. M. 2002. Generation of infectious cDNA clones of a Korea strain of Tomato espermy virus. *Mol. Cells.* 13:52-60.

Frabki, R. I. B., Hu, J. and Palukaitis, P. 1986. Taxonomy of cucurbit-infecting obamoviruses as determined by serological and molecular hybridization analyses. Intervirology 26:156-163.

Hollings, M., Komuro, Y. and Tochihara H. 1975. *Cucumber Green Mottle Mosaic Virus*. Descriptions of Plant Viruses. No. 154. Kew, Surrey, England; C.M.I./A.A.B.

Inoue, C., Inoue, S., Asakoku, M. and Mitsuhata, K. 1967. Studies on *Cucumber green mottle mosaic virus* in Japan. *Art. Res.* (in Japan) 51:175-186.

Kim, S. M., Lee, J. M., Yim, K. O., Oh, M. H., Park, J. W. and Kim, K. H. 2003. Nucleotide sequences of two Korean isolates of *Cucumber green mottle mosaic virus*. *Mol. Cell.* 16:407-412.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.

Lee, K. W., Lee, B. C., Park, H. C. and Lee, Y. S. 1990. Occurrence of *Cucumber green mottle mosaic virus* Disease of Watermelon in Korea. *Korean J. Plant Pathol.* 6:250-255 (in Korean with English summary).

Lee, S. H., Lee, Y. G., Park, J. W., Choi, H. S., Kim Y. T. and Cheon J. U. 2000. Nucleotide sequence of coat protein gene of *Kyuri green mottle mosaic virus* isolatede from zucchini. *Plant Pathol. J.* 16:118-124.

Ryu, K. H., Min, B. E., Choi, G. S., Choi, S. H., Kwon, S. B., Noh, G. M., Yoon, J. Y., Choi, Y. M., Jang, S. H., Lee, G. P., Cho, K. H. and Park, W. M. 2000. *Zucchini green mottle mosaic virus* is a new Tobamovirus; comparison of its coat protein gene with that of *Kyuri green mottle mosaic virus*. *Arch Virol.* 145:2325-2333.

Ryu, K. H. and Park, W. M. 1995. Rapid detection and identification of Odontoglassum rinspot virus by polymerase chain reaction amplification. *FEMS Microbiol. Lett.* 133:265-269.

Tan, S. H., Nishiguchi, M., Murata, M. and Motoyoshi, F. 2000. The genome structure of *Kyuri green mottle mosaic* Tobamovirus and its comparison with that of *Cucumber green mottle mosaic* Tobamovirus. *Arch. Virol.* 145:1067-1079.

Ugaki, U., Tomiyama, M., Kakutami, T., Hidaka, S., Kiguchi, T., Nagata, R., Sato, T., Motoyoshi, F. and Nishiguchi, M. 1991. The complete nucleotide sequence of *Cucumber green mottle mosaic virus* (SH strain) genomic RNA. *J. Gen. Virol.* 71:1487-1495.

Yoon, J. Y., Min, B. E., Choi, S. H. and Ryu, K. H. 2001. Completion of nucleotide sequence and generation of highly infections transcripts to cucurbits from full-length cDNA clone of *Kyuri green mottle mosaic virus*. *Arch Virol.* 146:2085-2096.