Plant Pathol. J. 18(3) : 121-125 (2002)

The Plant Pathology Journal

©The Korean Society of Plant Pathology

First Report of *Zucchini yellow mosaic virus* on Hollyhock (*Althaea rosea*)

Seung Kook Choi, Ju Yeon Yoon[1], Ki Hyun Ryu[1], Jang Kyung Choi[2] and Won Mok Park*

Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

[1]Plant Virus GenBank, Department of Horticultural Science, Seoul Womens University, Seoul 139-774, Korea

[2]Division of Biological Environment, Kangwon National University, Chunchon 200-701, Korea

(Received on May 7, 2002)

This study was conducted to determine the causal virus that naturally infected hollyhock (*Althaea rosea*) plant showing mild mosaic symptom in 1999. Flexuous virus particles were found in the cytoplasm of plant tissue from infected hollyhock under transmissible electron microscopy. A virus from the genus *Potyvirus* under the family *Potyviridae* was isolated and was maintained on *Chenopodium quinoa* for three passages. Chlorotic local legions were used to inoculate 20 species of indicator plants. The virus infected all the tested cucurbit plants, but failed to infect *Nicotiana benthamiana*. Based on the host range test and RT-PCR analysis, the potyvirus was identified as a strain of *Zucchini yellow mosaic virus-A* (ZYMV-A), one of the major pathogens of cucurbits. Infectivity analysis showed that ZYMV-A induced faster systemic symptom than ZYMV-Cu on squash and other cucurbit plants, suggesting that ZYMV-A was a more severe strain. To better characterize ZYMV-A, Western blot assay was carried out to the coat protein (CP) of the virus using ZYMV-specific antiserum with ZYMV-Cu and other potyviruses. The CP of the virus reacted strongly with the antiserum against ZYMV, and other tested antisera did not react with the CP of ZYMV-A. Results strongly suggest that the potyvirus infecting hollyhock was a novel strain of ZYMV. This is the first report on ZYMV as the causal virus infecting hollyhock in Korea.

Keywords : hollyhock, host range, *Potyvirus*, *yellow mosaic virus*, *Zucchini*.

Zucchini yellow mosaic virus (ZYMV), a species of the genus *Potyvirus* in the family *Potyviridae*, was first reported in Italy and France by Lisa et al (1981). The virus is transmitted from infected plants to healthy ones by several kinds of aphids in a non-persistent manner (Gal-On et al., 1995). ZYMV has caused devastating epidemics in a number of commercial cucurbits worldwide, and several distinct biological strains of ZYMV have been described (Desbiez and Lecoq, 1997; Lecoq and Pitat, 1984; Provvidenti et al., 1984).

Like other potyviruses, ZYMV has a positive sense single-stranded RNA genome translated as one polyprotein precursor which is cleaved subsequently to yield eight to nine functional proteins by virus-encoded protein proteases: P1, HC-Pro, and NIa. P1 and HC-Pro proteinases are located at the N-terminal region of the protein and act as suppression factor to post-transcriptional gene silencing (PTGS) (Brigneti et al., 1998). NIa interacts with NIb containing the activity of viral RNA-dependent RNA polymerase (Hong et al., 1995), while VPg is one of the determinant factors in pathogenicity. Other potyvirus-encoded proteins include that of CI protein acting as ATPase/ RNA helicase, and coat protein (CP) of potyvirus which has multifunctional activities involved in aphid transmission, cell-to-cell movement, systemic movement, encapsidation of the viral RNA, and regulation of viral RNA amplification (Gal-On et al., 1992; Dolja et al., 1993; Varrelamann and Maiss, 2000; Hong et al., 1995).

To date, there have been several reports on different strains of ZYMV (Lee and Wong, 1998; Wisler et al., 1995; Kundu et al., 1997; Yoon and Choi, 1998). However, all the known strains (or isolates) of ZYMV were originally isolated from cucurbit plants such as squash and cucumber. In this report, biological properties of a novel ZYMV strain isolated from a hollyhock (*Althaea rosea*) plant were investigated.

Materials and Methods

Virus source and isolation. In 1999, hollyhock (*Althaea rosea*) showing mild mosaic symptoms on the leaves was observed in Seoul (Fig. 1A). The virus from the diseased plant was detected by electron microscope. The crude sap containing the virus from hollyhock was inoculated to *Chenopodium quinoa*, and after three passages, a single local legion was transferred to squash (*Cucurbita pepo*) and cucumber (*Cucumis sativus*). The ZYMV-Cu (Yoon and Choi, 1998) was obtained from the Plant Virus GenBank (Seoul, Korea; http://www.virusbank.org) and used as a control in this study. The viruses were inoculated into two independent plants simultaneously as analyzed symptomatology

*Corresponding author.
Phone) +82-2-3290-3412, FAX) +82-2-923-9923
E-mail) wmpark@korea.ac.kr

Fig. 1. Systemic foliar symptoms caused by ZYMV-A infecting hollyhock. (A) The leaf symptom of naturally infected hollyhock plant infected by the virus as source of virus in this study. (B) Blistering mosaic and yellowing symptoms of leaves in squash (*Cucurbita pepo*) (left) caused by ZYMV-A isolated from hollyhock, and healthy plant (right).

and accumulations of virus in infected tissues.

Host range and RT-PCR. Virus inoculum maintained in squash or cucumber plants was mechanically inoculated into 20 species of test plants using Carborundum with 10 mM phosphate buffer (pH 7.0). The plants were grown in a greenhouse at 26°C. Symptom assessment was conducted for 4 weeks, and the virus characteristics were compared with that of ZYMV-Cu strain.

For analysis of RT-PCR, total RNA was extracted from either diseased leaves of hollyhock or from plants maintained by sodium dodecyl sulfate (SDS)-phenol method of Choi et al. (1999). A pair of primers, forward primer (ZYU: 5'-GCTCCATACATAGCT-GAGACAGC-3') and reverse primer (ZYD1186: 5'TAGGCTTG-CAAACGGAGTCTAATC), was used in RT-PCR (Yoon, 1999). The cDNA was synthesized using Superscript II RNase H^- with the reverse primer at 42°C. PCR conditions were 94°C for 1 minute, 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 5 minutes.

Virus purification and analysis of coat protein. Leaves of squash plants showing systemic symptom were ground with phosphate buffer (0.1 M sodium phosphate [pH 7.0], 5 mM EDTA, and 0.1% 2-mercaptoethanol). The extract was centrifuged at 15,000×*g* for 10 minutes at 4°C. The collected supernatant was filtered through three layers of Miracloth (Calbiochem-Behring), and the resulting filtrate was loaded on a 20% sucrose cushion in phosphate buffer and was pelleted by twice differential ultracentrifugation at 150,000×*g* for 3 h at 4°C, according to the method by Gal-On et al. (1995). The purified virus was mixed with 2x protein sample buffer (Laemmli, 1970), denatured by boiling for 5 minutes, and chilled on ice. The sample was subjected to SDS-polyacrylamide gel electrophoresis described by Laemmli (1970).

For Western blot assay, the gel was incubated with transfer buffer (39 mM glycine, 48 mM Tris base, 0.037% SDS, 20% methanol), and electro-blotting on the nitrocellulose membrane was performed based on the manufacturer's instruction (Bio-Rad) and standard protocol (Sambrook et al., 1987). The blotted proteins were immunodetected with the antiserum against ZYMV CP (diluted in 1:1000). Subsequently, the membranes were incubated with the solution of goat anti-rabbit IgG (diluted in 1:7,500, Promega) conjugated with alkaline phosphatase as the secondary antibody. Color reaction was developed with Western Blue Stabilizer Substrate (Promega).

Results and Discussion

Isolation of causal virus and its host reaction. Crude sap from hollyhock showing mild mosaic symptom on leaves was detected by electron microscopy. Filamentous-shaped virus particles of approximately 800×12 nm were observed in the cytoplasm of the infected host (data not shown). It is

Fig. 2. Electron micrograph of purified particles of ZYMV-A The bar represents 100 nm.

Table 1. Reactions of indicator plants infected by *Zucchini yellow mosaic virus* strain A

Indicator plant	Host reaction
Chenopodium quinoa	CL/–[a]
Gomphrena globosa	–/mM
Datura stramonium	–/–
Physalis floridana	–/–
Capsium annuum	–/–
Lycopersicon esculentum	–/–
Brassica campestris	–/–
Citrullus lanatus	–/M
Cucumis melo	–/M,Vb
Cucumis sativus	–/M, Vb
Cucurbita maxima	–/M, Y, Vb
Cucurbita pepo cv. BlackBeauty	–/M, Y, Vb
Sacheol	–/M, Y, Vb
Cucurbita moschata	–/M,Y
Luffa cylindrical	–/M,Y, Vb
Phaseolus vulgaris	–/CS
Nicotiana tabacum cv. Samsun	–/–
Xanti-nc	–/–
Burley 21	–/–
Nicotiana benthamiana	–/–
Nicotiana rustica	–/–
Rhapanus sativus	–/–

[a] Inoculated leaf/systemic leaf.
CL = chlorotic local legion; CS = chlorotic spot; mM = mild mosaic; M = mosaic; Vb = vein banding; Y = yellowing; – = no symptom.

assumed that the virus belonged to the genus *Potyvirus*. Purified virus preparations were examined and the typical filamentous-type morphology in the *Potyvirus* was observed and the same as that of the original diseased host (Fig. 2).

To identify the isolate of the potyvirus, crude sap extract was used to inoculate *Chenopodium quinoa*. After three passages on *C. quinoa*, the generated chlorotic local legion on the leaf was mechanically inoculated onto 20 test plants as listed in Table 1. The potyvirus did not infect *Datura stramonium*, *Physalis floridana*, *Capsium annuum*, *Lycopersicon esculentum*, *Nicotiana tabacum* (cv. Samsun, Xanti-nc, Burley 21), *N. benthamiana*, *N. rustica*, *Brassica campestris*, and *Raphanus sativus*. Meanwhile, the virus infected most of the cucurbits namely, *Citrullus lanatus*, *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. pepo* (cv. BlackBeauty, Sacheol), *C. moschata*, *Luffa cylindrica*, *Gomphrena globosa*, and *Phaseolus vulgaris*.

Major potyviruses infecting cucurbits are *Watermelon mosaic virus*-2 (WMV-2), *Papaya ringspot virus* (PRSV) and ZYMV, according to previous reports (Thomson et al., 1995). The first two viruses, WMV-2 and PRSV, were able to systemically infect *N. benthamiana*, but ZYMV could not (Brunt, 1996). This suggests that the virus could be a strain of ZYMV, and we designated as ZYMV-A strain.

To better identify the virus, RT-PCR was carried out using a pair of primers to ZYMV-specific. A single band of RT-PCR product generated from a sample containing RNAs of ZYMV-A is shown in Fig. 3A (lane 4). Identical result was obtained from the RNA sample of ZYMV-CU, used as positive control (Fig. 3A, lane 5). There was no synthesis of cDNAs from samples containing RNAs of other potyviruses. The results further suggest that the virus is a strain of ZYMV at molecular level.

In cucurbit plants, ZYMV-A induced yellow-mosaic systemic symptom and its initial expression was quite faster than that of ZYMV-Cu. However, systemic symptoms induced by both strains were shown to have similar patterns at 7-10 days post-inoculation (Fig. 1B). This indicates that the accumulation of ZYMV-A in the infected plant increased rapidly on the host cells at an earlier stage than

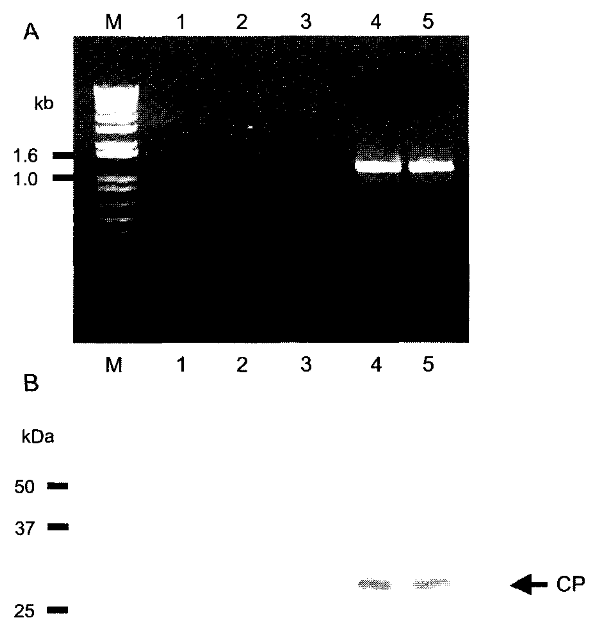

Fig. 3. Electrophoresis analysis of amplified RT-PCR products (A) and Western blot assay for capsid protein of potyviruses and ZYMV-A strain (B). (A) Lane M: 1 kb puls ladder (BRL), 1: *Cucumber mosaic virus* (Fny strain), 2: *Turnip mosaic virus* (TuMV), 3: *Potato virus Y* (PVY), 4: ZYMV-A, 5: ZYMV-Cu. The bands were anlyzed on agarose gel electrophoresis (1.2%) and stained with ethidium bromide. (B) Lane M: protein size marker (Bio-Rad); 1: healthy squash, 2: TuMV, 3: PVY, 4: ZYMV-A, 5: ZYMV-Cu. The total protein was extracted from the upper leaves at 14 days post-inoculation and separated on SDS-polyacrylamide gel electrophoresis, and were immuno-detected by Western blot assay using antiserum specific to ZYMV capsid protein. The positions of the coat protein are indicated (CP).

that of ZYMV-Cu. Otherwise, since the potyviral movement mechanism in host plants was related with various virus-encoded proteins, such as CI protein, P1/HC-Pro, and CP as host factors (Carrrington et al., 1998), the facilitation of virus movement by these factors might affect the expression of systemic symptom on squash or cucumber plants.

Immunological analysis of coat protein for ZYMV-A. ZYMV-A, which was originally isolated from hollyhock, was characterized by Western blot analysis using ZYMV CP-specific antiserum. In the Western blot assay, the protein-blotted membrane showed a positive reaction to the original infected hollyhock and ZYMV-Cu with antiserum to ZYMV CP. Meanwhile, no reactions from the other two *potyviruses* as controls were detected (Fig. 3B). This indicates that the virus in hollyhock (ZYMV-A) and ZYMV-Cu belonged to the ZYMV species. The protein band demonstrated that the molecular weight for CP of ZYMV-A was approximately 34 kDa, similar to that of ZYMV-Cu and other isolates.

Although the complete nucleotide sequences of viral RNA of ZYMV-A were detected in this study, the possibility of variation at genomic RNA level was not completely excluded based on the biological characteristics of ZYMV-A such as rapid induction of symptom, as compared with that of ZYMV-Cu. In addition, it is interesting to note that ZYMV-A strain could infect hollyhock plant, which is a weed plant, as well as cucurbits, while infectivity of most ZYMV is limited to cucurbit plants. In the early development stage of host plants, the systemic symptom induced by ZYMV-A was more severe than that of ZYMV-Cu originally isolated from cucumber, which is the common host of ZYMV (Yoon and Choi, 1998). It was demonstrated that P1/Hc-Pro of the potyvirus interfered with host defense mechanism, referred to as PTGS (Brigneti et al., 1998; Li et al., 1999; Kasschau and Carrington, 1998). By processing the suppression to PTGS, viruses can have better adaptations to host plants. In this category, HC-Pro of ZYMV-A copes with host defense factors more effectively than ZYMV-Cu during early stages of virus infection.

ZYMV-A can be considered as a quasi-species of ZYMV population in host plants or vector transmission for effective viral RNA replication, movement and transmission resulting in rapid genetic virus evolution (Schneider and Roossinck, 2001).

Acknowledgment

This study was supported by the BK21 program of the Ministry of Education in Korea to SKC under WMP guidance.

References

Brigneti, G., Voinnet, O., Li, W.-X., Ding, S.-W. and Baulcombe, D. 1998. Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J.* 22:6739-6746.

Brunt, A., Crabtree, K., Dallwits, M., Gibbs, A. and Watson, L. 1996. Viruses of plants: Descriptions and lists from the VIDE database, CAB International, Wallingford, United Kingdom.

Carrington, J. C., Jensen, P. E. and Schaad, M. C. 1998. Genetic evidence for an essential role for potyvirus CI protein in cell-to-cell movement. *Plant J.* 14: 393-400.

Choi, S. K., Choi, J. K., Park, W. M. and Ryu, K. H. 1999. RT-PCR detection and identification of three species of cucumoviruses with a single pair of primers. *J. Virol. Methods* 83:67-73.

Desbiez, C. and Lecoq, H. 1997. Zucchini yellow mosaic virus. *Plant Pathol.* 46:809-829.

Dolja, V. V., Herndon, K. L., Pirone, T. P. and Carrington, J. C. 1993. Spontaneous mutagenesis of a plant potyvirus genome after insertion of a foreign gene. *J. Virol.* 67:5968-5975.

Gal-On, A., Antignus, Y., Rosner, A. and Raccah, B. 1992. A zucchini yellow mosaic virus coat protein gene mutation restores aphid transmissibility but has no effect on multiplication. *J. Gen. Virol.* 73:2183-2187.

Gal-On, A., Meiri, E., Huet, H., Hua, W. J., Raccah, B. and Gaba, V. 1995. Particle bombardment drastically increases the infectivity of cloned DNA of zucchini yellow mosaic potyvirus. *J. Gen. Virol.* 76: 3223-3227.

Hong, Y., Levay, K., Murphy, J. F., Klein, P. G., Shaw, J. G. and Hunt, A. G. 1995. A potyvirus polymerase interacts with the viral coat protein and VPg in yeast cells. *Virology.* 214:159-166.

Kasschau, K. D. and Carrington, J. C. 1998. A counterdefective strategy of plant viruses: Suppression of posttranscriptional gene silencing. *Cell* 95:461-470.

Kundu, A. K., Oshima, K. and Sako, N. 1997. Nucleotide sequences of the coat protein genes of two japanese zucchini yellow mosaic virus isolates. *Acta Virol.* 41:297-301.

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

Lecoq, H. and Pitrat, M. 1984. Strains of zucchini yellow mosaic virus in muskmelon. *Phytopathol. Z.* 111:165-173.

Lee, K. C. and Wong, S. M. 1998.Variability of P1 protein of zucchini yellow mosaic virus for strain differentiation and phylogenetic analysis with other potyviruses *DNA Sequence* 9:275-293.

Li, H. W., Lucy, A. P., Guo, H. S., Li, W. X., Ji, L. H., Wong, S. M. and Ding, S. W. 1999. Strong host resistance targeted against a viral suppressor of the plant gene silencing defense mechanism. *EMBO J.* 18:2683-2691.

Lisa, V., Boccardo, G., DAgostino, Dellavalle, G. and dAquilio, M. 1981. Characterization of a potyvirus that causes zucchini yellow mosaic. *Phytopathology.* 71:667-672.

Provvidenti, R., Gonsalves, D. and Humaydan, H. S. 1984.

Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. *Plant Dis.* 68: 443-446.

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

Schneider, W. L. and Roossinck, M. J. 2001 Genetic diversity in RNA virus quasi-species is controlled by host-virus interactions. *J Virol.* 75:6566-6571.

Thomson, K. G., Dietzgen, R. G., Gibbs, A. J., Tang, Y. C., Liesack, W., Teakle, D. S. and Stackebrandt, E. 1995. Identification of zucchini yellow mosaic potyvirus by RT-PCR and analysis of sequence variability. *J. Virol. Methods* 55:83-96.

Varrelamann, M. and Maiss, E. 2000. Mutations in the coat protein gene of plum pox virus suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of *Nicotiana benthamiana. J. Gen. Virol.* 81:567-576.

Wisler, G. C., Purcifull, D. E. and Hiebert, E. 1995. Characterization of the P1 protein and coding region of the zucchini yellow mosaic virus. *J. Gen. Virol.* 76:37-45.

Yoon, J. Y. 1999. Construction of full-length cDNAs for genomic RNAs of Zucchini yellow mosaic potyvirus (ZYMV-Cu) isolated from cucumber. M.S. thesis. Kangwon National University.

Yoon, J. Y. and Choi, J. K. 1998. Nucleotide sequence of 3-terminal region of zucchini yellow mosaic virus (cucumber isolate) RNA. *Korean J. Plant Pathol.* 14:23-27.