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Occurrence of Mosaic Disease of *Hosta* Plants Caused by *Hosta virus X*

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Systemic virus symptoms caused by a *Potexvirus* were observed on leaves of infected hosta (*Hosta* spp.) plants cultivated in Seoul, Korea. Symptoms on diseased hosta plants include mosaic, mottle, irregular blotchy patches, and chlorotic spots on or distortion of the leaves. No other viruses, such as *Cucumber mosaic virus*, *Lily symptomless virus,* or *Potyvirus,* were detected from the same plants by electron microscopy and by Western blot and RT-PCR analyses, indicating that they were singly infected by the potexvirus. The symptoms differed among cultivars and species of hosta, and affected the quality of plants for commercialization, as well as, plant growth and flowering of susceptible cultivars. Most of the cultivars and species investigated were susceptible to the virus, while some were not infected by the virus at all. Purified virus particles were of filamentous type with unaggregated forms 540 nm in length, which is a typical potexviral morphology. The virus consisted of a single-stranded RNA molecule of 6 kb long for genome and single component of coat protein (CP) about 27 kDa. The CP strongly reacted with the antiserum against *Hosta virus X* (HVX), suggesting that the virus is an isolate of HVX. This is the first report of the occurrence and identification of HVX from hosta plants in Korea.

Keywords **:** *Hosta minor*, *Hosta virus X*, identification, *Liliaceae*, *Potexvirus*, serology.

Hosta (*Hosta* spp.) is a member of the family *Lilliaceae* and has become a popular perennial shade-tolerant garden plant worldwide. Six native hostas, namely, *Hosta capitata* Nakai, *H. clausa*, *H. longipes*, *H. minor,* and *H. yingeri,* inhabit the southern areas of Korea. Recently, a number of varieties of hosta have been introduced in Korea for garden plants. So far, there is no known disease infecting the plants in the country. Some virus diseases have been reported in cultivated hostas, such as *Hosta virus X* (HVX), *Arabis mosaic virus,* and *Tomato ringspot virus* in the world. HVX, a species of the genus *Potexvirus*, was first reported in the USA (Brunt et al., 2000; Currier and Lockhart, 1996). HVX is considered as the main pathogen of the plants. HVX usually induces mosaic and mottle symptoms in cultivated hostas, although the incidence of the virus in the USA did not seem to be as serious as once thought (Currier and Lockhart, 1996).

No virus-like symptoms were observed in wild-type native species of hostas in Korea, although some imported hostas showed virus-like symptoms. In 2001, systemic virus-like symptoms were observed in leaves of cultivated hostas (*Hosta* spp.) in Seoul, Korea. The symptoms exhibited quite diverse patterns depending on cultivars and species of hostas examined. This study identified the causal pathogen as *Hosta virus X* (HVX) in the country, and determined some of its properties.

Materials and Methods

Sources of plants, viruses, and antisera. One native hosta (*Hosta minor* Nakai) and three imported hosta (*Hosta sieboldii* 'Ginko Craig', *Hosta* 'Blue Cadet', and *Hosta* 'Geisha') plants from the Netherlands showing systemic mosaic and mottle leaf symptoms were collected from cultivation fields of the Seoul Women's University (Fig. 1). The diseased plants were maintained in a light/temperature-controlled glasshouse and used as sources of virus. *Hosta virus X* (HVX), denoted as HVX-U in this study, was kindly provided by Dr. B.E.L. Lockhart (University of Minnesota, MN, USA) and was used as control (Currier and Lockhart, 1996). Antiserum against HVX obtained from Dr. Lockhart, and three kinds of antisera against *Lily symptomless virus* (LSV), *Cucumber mosaic virus* (CMV), and *Potato virus Y* (PVY) from the Plant Virus GenBank (Seoul, Korea; http://www.virusbank.org), were used for serology.

Electron microscopy. Crude saps extracted from leaves of diseased hostas and partially purified virus preparations were mounted on carbon-coated grids and negatively stained with 2% sodium phosphotungstic acid (pH 7.0).

Host range test. Inoculation for host range tests was conducted in a glasshouse at 25±2°C. The inoculum was extracted from naturally infected leaves of hosta in 0.01 M phosphate buffer (pH 7.0), and was mechanically inoculated onto Carborundum (350 mesh)-dusted leaves of 13 tested plants, namely: *Nicotiana tabacum* cv. Samsun, *N. tabacum* cv. Xanthi-nc, *N. benthamiana*,

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Fig. 1. Mottle and severe mosaic symptoms produced in leaves of *Hosta virus X* (HVX)-infected hosta plants. HVX-infected *Hosta sieboldii* 'Ginko Craig' (**A**) and *H. minor* (**B**).

N. glutinosa, *N. clevelandii*, *Chenopodium amaranticolor*, *C. quinoa*, *Datura stramonium*, *Cucumis sativus* cv. Baekdadagi, *Cucurbita pepo* cv. Black Beauty, *Vigna unguiculata*, *Gomphrena globosa*, and *Hosta minor*. The plants were maintained for visual inspection of symptoms for at least 5 weeks and confirmed by serology and electron microscopy.

Purification of virus and extraction of viral RNA. The virus was partially purified by extraction and clarification with chloroform, followed by differential centrifugation method as described by Ryu et al. (2002). The total genomic RNA of the virus was extracted from purified virus particles by SDS-proteinase K digestion and phenol extraction procedure (Yoon et al., 2002). RNA was denatured with formamide and formaldehyde, and separated in 1.0% formaldehyde-denatured agarose gel (Sambrook et al., 1989).

Electrophoresis of coat protein and Western blot. Purified virus preparation (1 mg/ml) was mixed with an equal volume of sample treatment buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 1% 2-mercaptoethanol, and 0.02% bromophenol blue) and heated for 5 minutes at 100°C. The sample was electrophoresed at 30 mA for 1.5 hours on 10% polyacrylamide slab gel containing 0.1% SDS following the method of Laemmli and Favre (1973). Coat protein (CP) band on the gel was stained with Coomassie brilliant blue R 250 solution (Bio-Rad, USA). For the Western blot analysis, CP was separated on the same gel and transferred onto a nitrocellulose membrane using an electroblot apparatus (Bio-Rad). The membrane was treated with 5% skim milk solution and then immuno-probed with antibodies (1:1,000, v/v) for the Western blot analysis (Yoon et al., 2002).

Results

Occurrence and biological properties of HVX. Systemic virus symptoms caused by a *Potexvirus* were observed on leaves of infected hosta (*Hosta* spp.) plants cultivated in Seoul, Korea. The virus was frequently detected in diseased leaves of hostas showing mosaic and mottle symptoms (Fig. 1). In the newly emerging leaves, symptoms were more severe and were systemically spread on the upper leaves in cultivated hostas. Symptoms produced on diseased hosta plants include mosaic, mottle, irregular blotchy patches, and chlorotic spots on or distortion of the leaves. The natural virus infection rate of the virus disease in hosta-cultivated fields was over 24%.

Four out of the 22 species and cultivars of cultivated hostas, namely, *H. minor*, *H. sieboldii* 'Ginko Craig', *Hosta* 'Blue Cadet', and *Hosta* 'Geisha', showed visible virus symptoms. The symptoms differed among cultivars and species of hosta, and the symptoms in susceptible hostas affected the quality of plants for commercialization, as well as, plant growth and flowering. The virus was transmitted to *H. minor* mechanically and symptoms appeared in newly emerged leaf parts with mild mottle and mosaic symptoms 6 weeks after inoculation. All the 13 tested plants did not produce any symptoms, and virus was not detected by serology and molecular detection techniques (data not shown). Also, no local symptoms were observed in the tested plants.

Particle morphology. Purified virus particles, as well as, particles from crude sap extracts, as observed by electron microscopy, were of filamentous type about 540 nm in length and 13 nm in width (Fig. 2), which is a typical morphology of the members of the genus Potexvirus. No other virus particles were observed in the same plants.

RNA and CP properties of HVX-Kr. The virus consisted of a 6 kb single-stranded RNA for genome (Fig. 3) and single component of coat protein (CP) about 27 kDa (Fig. 4A). The CP strongly reacted with the antiserum against HVX-U, suggesting that the virus is an isolate of HVX,

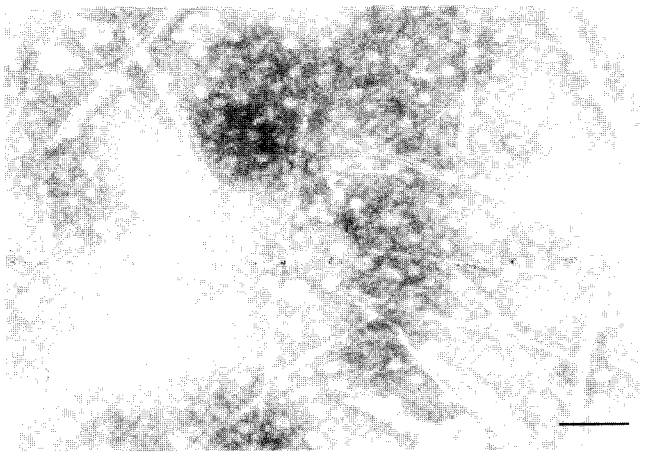

Fig. 2. Electron micrograph of *Hosta virus X* partially purified from *Hosta minor* negatively stained with phosphotungstic acid. Scale bar represents 200 nm.

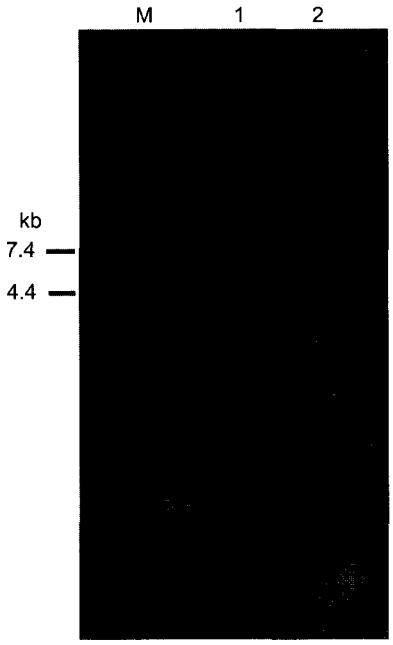

Fig. 3. Electrophoretic pattern of genomic RNA of *Hosta virus X* separated in 1.0% formaldehyde-denatured agarose gel. Lane M, RNA ladder; Lane 1, HVX-Kr; Lane 2, HVX-U.

denoted as HVX-Kr (Fig. 4B).

Discussion

In this study, a potexvirus was identified from cultivated hostas and the causal pathogen was identified as an isolate of *Hosta virus X* (HVX) based on host range, particle morphology, and serology. Initial symptoms were observed in cultivars imported from the Netherlands in the investigated field in Seoul, Korea. The virus has spread in some healthy native species and, thereafter, in imported hosta

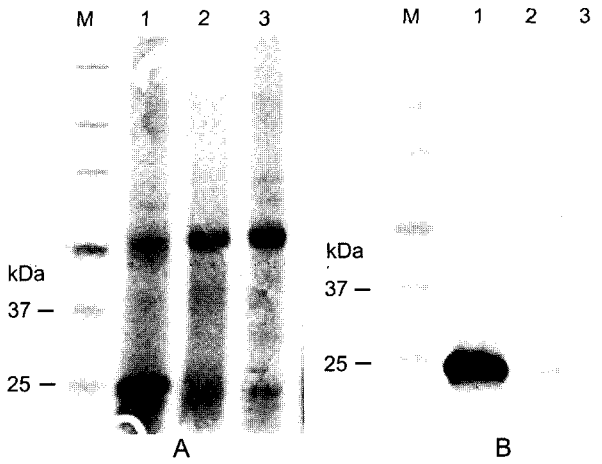

Fig. 4. Electrophoretic pattern of HVX-Kr in SDS-PAGE (**A**) and Western blot analysis of HVX immunoprobed with antiserum against HVX-U (**B**). Lane M, pre-stained SDS protein markers; Lane 1, partially purified HVX-Kr; Lane 2, total protein from leaves of HVX-U-infected hosta; Lane 3, total protein from leaves of healthy hosta (*H. minor*).

varieties. Virus infection was neither uniform nor correlated with the density of planting and source of plants; the higher the densities, the higher infection rates were observed (data not shown). Therefore, the virus might be transmitted from the diseased plants to healthy ones by cutting practices for massive propagation and breeding program, and probably by virus-contaminated soils. HVX was first identified from hostas in the USA (Currier and Lockhart, 1996). Currier and Lockhart (1996) analyzed the biological properties of the virus and the serological relationships among the potexviruses. Biological properties of the HVX presented in this study and that of Currier and Lockhart (1996) indicate that the virus has very narrow host ranges, probably limited to hosta plants. Members of the *Potexvirus* cause mosaic or ringspot symptoms in a wide range of monocotyledonous and dicotyledonous plants; the natural host range of individual viruses, however, is rather limited (Koenig and Lesemann, 1978).

HVX is still considered as a tentative member of the genus *Potexvirus*. HVX reacted well with the antiserum against *Clover yellow mosaic virus*, reacted weakly with the antiserum against *Hydrangia ringspot virus*, and did not react at all with the other 14 potexviral antisera (Currier and Lockhart, 1996), suggesting that the virus is a distinct species. However, there is no available genome information, such as nucleotide and amino acid sequences, even for HVX CP gene. This lack of sequence information makes the virus a tentative species of *Potexvirus*.

This study clearly indicates that based on biological and serological properties, the potexvirus isolated from hostas is HVX. This is the first report of the identification of HVX in Korea. To determine whether the HVX is really a distinct

species of the genus *Potexvirus*, we have recently analyzed the 3'-terminal nucleotide sequences of the HVX-U and HVX-Kr. The analysis showed over 99% similarity in CP gene and shared homologies with other known potexviruses (Park, M. H. and Ryu, K. H., manuscript in preparation). This follow-up study is expected to provide the direct evidence that the species is a *Potexvirus*, and is useful for the development of molecular detection such as PCR technique. Most of the investigated cultivars and species of hosta were susceptible to the virus, while some cultivars were not infected at all. These resistant hostas are good candidates for virus-resistance sources for breeding program. For the control of the virus in hostas, diseased plants must be separated from susceptible cultivars in order to minimize mechanical transmission. Elimination of infected plants, screening of resistance cultivars and species, and breeding program are also necessary.

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