Presence of *Hosta virus X* in New Zealand

J. Tang · C. Hardy · B. S. M. Lebas · L. I. Ward

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Abstract Leaf mosaic and deformation symptoms were observed on hosta plants (*Hosta* spp.) in a nursery in Kihikihi in the North Island of New Zealand. The virus associated with these symptoms has been identified as *Hosta virus X* (HVX), using electron microscopy, mechanical transmission, ELISA and RT-PCR techniques. This is the first report of HVX in New Zealand although this virus is believed to have been present and widespread in the country for more than 10 years.

Keywords *Hosta virus X* · Presence · New Zealand

Hostas (*Hosta* spp.) are common garden plants in many countries including New Zealand. These species were reported to be infected with *Hosta virus X* (HVX) for the first time in 1996 in the USA (Currier and Lockhart 1996). HVX has been subsequently reported in Korea (Ryu et al. 2002), the Netherlands (Verhoeven et al. 2006), Poland (Cajza and Zielinska 2007) and France (Jullien and Chauvel 2007) and has become a problem to hosta growers. HVX has been confirmed to be a distinct species of the genus *Potexvirus* in the family *Flexiviridae* (Park and Ryu 2003;

Fajolu et al. 2009). In nature, this virus infects only hostas and is most likely to be spread by vegetative propagation or mechanical contact (Currier and Lockhart 1996), and through seed (Ryu et al. 2006).

In February 2011, a plant of *Hosta* sp. cv. Thomas Hogg with leaf mosaic and deformation symptoms (Fig. 1) was collected from a nursery in Kihikihi in the North Island of New Zealand. Herbaceous indicator plants, *Gomphrena globosa* and *Nicotiana benthamiana*, which had been inoculated with sap from symptomatic hosta leaves, developed necrotic local spots and systemic chlorotic lesions, respectively. No symptoms were observed on other inoculated herbaceous species (*Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *N. clelandii*, *N. occidentalis*, *N. sylvestris*, *N. tabacum* and *Phaseolus vulgaris*). Electron microscopy of crude sap preparations from the hosta and symptomatic herbaceous plants revealed the presence of filamentous virus particles approximately 550 nm long (no virus particles were observed from other inoculated herbaceous plants which were symptomless or in healthy control plants). These viruliferous plants tested positive using HVX polyclonal antiserum in a double-antibody sandwich (DAS) - enzyme-linked immunosorbent assay (ELISA) (Agdia Inc., Elkhart, USA). An RNeasy Plant Mini Kit (Qiagen, Melbourne, Australia) was used to extract total RNA from the leaves of the symptomatic hosta and herbaceous plants. The RNA was used in one-step reverse transcription-polymerase chain reaction (RT-PCR) tests with novel forward (5'- CCCGATGTGCTCAACCAGAT -3') and reverse (5'- GTGGAGGCGAAGTCGTTTCT -3') primers specific to a 464-bp fragment of the HVX coat protein gene. RT-PCR reactions were performed in a total volume of 20 µL, containing 250 nmol of each primer and using SuperScript™ III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase (Invitrogen), as per the manufacturer's

J. Tang (✉) · B. S. M. Lebas · L. I. Ward
Plant Health and Environment Laboratory,
Investigation and Diagnostic Centres,
Ministry of Agriculture and Forestry, New Zealand,
PO Box 2095, Auckland 1140, New Zealand
e-mail: joe.tang@maf.govt.nz

C. Hardy
Inursion Investigation Plants and Environment,
Investigation and Diagnostic Centres,
Ministry of Agriculture and Forestry, New Zealand,
PO Box 2095, Auckland 1140, New Zealand

Fig. 1 Leaf mosaic and deformation symptoms on *Hosta* sp. cv. Thomas Hogg

instructions. Thermocycling conditions were 50°C for 30 min and 94°C for 5 min then 40 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, followed by a final extension of 5 min at 72°C. PCR products of the expected size were obtained using RNA extracted from the three symptomatic species tested. An amplicon derived from the hosta RNA was cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, USA) and sequenced. The consensus sequence from four clones was deposited in the GenBank (Accession number JF732917). A BLASTn search in GenBank showed 99% nucleotide sequence identity to 47 isolates of HVX (e.g. GenBank accession FJ903405).

In order to confirm that other known hosta-infecting viruses were not the cause of the symptoms observed, the sample was screened for the presence of *Arabis mosaic virus*, *Cucumber mosaic virus*, *Impatiens necrotic spot virus*, *Tobacco rattle virus*, *Tobacco ringspot virus*, *Tomato ringspot virus* and *Tomato spotted wilt virus*. The hosta tested negative for all these viruses by ELISA or PCR. Therefore, it was concluded that the symptoms observed in the hosta were very probably caused by HVX alone.

A further 20 leaf samples of *Hosta* spp. with suspect disease symptoms were subsequently collected from the same nursery. Sixteen plants, representing 15 cultivars, tested positive for HVX using RT-PCR as described. HVX is thought to be widespread in New Zealand as the nursery investigated is the main supplier of hosta plants for garden stores throughout the country. Furthermore, HVX may have established in New Zealand for more than 10 years. In December of 2000, six hosta samples that had been collected from a park in Christchurch, in the South Island, were tested by the University of Minnesota, USA. Five out of the six samples were suggested to contain HVX particles after an examination of immunosorbent electron microscopy (B. Lockhart pers. comm., Mar 2011). This study confirms the presence of HVX for the first time in New Zealand.

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