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LETTER TO THE EDITOR

First report of *Hosta virus X* infecting hosta plants in Ukraine

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Summary. – In September 2011, the leaf samples of hosta cultivar 'Sum and substance' were collected from the collection of Gryshko' National Botanical Garden in Kyiv. The leaves showed dark green streaking and puckering along the leaf veins. Transmission electron microscopy revealed the presence of filamentous viral particles 13 nm in diameter and 470-580 nm in length. Reverse transcription PCR (RT-PCR) analysis confirmed the presence of *Hosta virus X* (HVX). The sequencing of the complete genome revealed 99% identity to HVX-37 and 97.5% identity to HVX-Kr. Notably, ORF4 initiation codon presented a non-conventional start codon (UUG) like it was previously identified in HVX-37.

Keywords: Hosta virus X; potexvirus; Ukraine

Hosta species (*Hosta* spp.) are native to northeast Asia. Nowadays, they are among the most popular ornamental garden plants worldwide represented by more than 7000 varieties. In Ukraine, the most complete hosta collection is situated in Gryshko´ National Botanical Garden in Kyiv.

The list of viruses known to infect hosta plants include *Hosta virus X*, *Tobacco rattle virus*, *Tobacco ringspot virus*, *Tomato ringspot virus*, *Tomato spotted wilt virus*, *Impatiens necrotic spot virus* and *Arabis mosaic virus*.

Hosta virus X (HVX), a member of the genus *Potexvirus*, is the most economically significant virus infecting hostas. Vegetative propagation of infected material is the main source of virus transmission. There is no evidence of biological vectors. Natural host range of HVX is restricted to hosta species *(1)*. HVX causes wide variety of symptoms including mosaic or mottling, ringspots, irregular blotchy patches, enations, necrosis, stunting and dieback *(2)*. Because of wide range of phenotypes among the healthy hosta cultivars, the HVX-symptoms may be difficult to distinguish from normal plant appearance. HVX has been identified in Korea, USA, Europe and New Zealand *(3)*.

In September 2011, we collected the samples of hybrid hosta (*H. x hybridum*) 'Abiqua', 'August Moon', 'Lady Guinevere', 'Old Faithful', 'Sum and Substance', 'Ultraviolet Light', 'Wide Brim'; *H. fortunei* 'Gold Standard', 'Striptease', 'Twilight', 'Whirlwind'; *H. x tardiana* 'Halcyon'; *H. sieboldiana* 'Great Expectation', 'Paul Glory'; *H. undulata* 'Mediovariegata'; and the samples of *H. crispula, H. venticosa, H. undulata* and *H. sieboldiana* exhibiting virus-like symptoms from Gryshko' National Botanical Garden in Kyiv, Ukraine. The plants displayed the symptoms characteristic of possible virus infection – dark green streaking and puckering along the leaf veins. Transmission electron microscopy revealed the presence of filamentous viral particles 13 nm in diameter and 470–580 nm in length in partially cleared plant sap. Viral particles were further purified by clarification in chloroform, precipitation using PEG-6000 and differential centrifugation.

Total RNA was extracted from plant material and a 706-bp fragment corresponding to nt pos. 5722–6448 in HVX-Kr

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Abbreviations: HVX = Hosta virus X

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HVX-Kr    -GAAAACAAAACCAATTTTAACCAACTTCAAACAAAGAAGTTTAATTTCGCTT 52
HVX-37    -GAAAACAAAACCAATTTTAACCAACTTCAAACAAAGAAGTTTAATTTCGCTT 52
HVX-Ukr   GGAAAAGAAAACCAAACGAACCTAACTTCAAACAAAGAAGTTTAATTTCGCTT 53
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HVX-Kr    ACAAAC-CATTCGCAAACAGATCGATCGGAG-GGACTCCTAGATCTTAAGCA 102
HVX-37    ACAAACCCATTCGCAAACAGATCGATCGGAGGGACTCCCTAGATCTTAAGCA 104
HVX-Ukr   ACAAAC-CATTCGCAAACAGATCGATCGGAGGG-ACTCCTAGATCTTAAGCA 103
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Fig. 1

Nucleotide sequence comparison of HVX 5' UTR regions of isolates from Korea (HV-Kr), United States (HVX-37) and Ukraine (HVX-Ukr)
Sequences were aligned using ClustalO ver. 1.2.4 (http://www.ebi.ac.uk/Tools/msa/clustalo/).

was amplified in RT-PCR from the samples of 'Sum and Substance', 'Ultraviolet Light', 'Gold Standard', 'Halycon', 'Great Expectations' and *H. crispula*. Presence of HVX was confirmed by sequencing.

All isolated RNA samples were pooled to synthesize a cDNA library for the next generation sequencing using TruSeq siRNA kit (Illumina) in order to identify all possible viruses. Sequencing was carried on HiSeq2500 rapid flow-cell with 50 bp single reads (Illumina). Reads were subsequently trimmed to remove kit-derived adaptor sequences using FastX toolbox. *De novo* contig assembly was done using Oases 0.2.08. The assembled contigs were analysed using BLASTn program 2.2.28+. The ends of the HVX genome were verified by RACE using RNA purified from virus particles as a template.

The assembled sequence representing the complete genome of HVX-Ukr (Ukrainian isolate) was deposited in GenBank (KX033798). In complete genome ClustalO alignments, HVX-Ukr presented 99 % homology to HVX-37 and 97.5 % homology to HVX-Kr. Notably, HVX-Ukr genome 5' end showed some differences from other two isolates (Fig. 1). Also, the region of ORF4 initiation codon was additionally analysed by Sanger sequencing to confirm the presence of nonconventional start codon (UUG) previously identified in HVX-37 (U.S. isolate; JQ911698) but not in HVX-Kr (Korean isolate; AJ620114) *(4)*.

This is the first complete genome sequence of HVX isolate from Europe and the first evidence of HVX occurrence in Ukraine. Earlier, HVX was reported in several European countries like Czech Republic *(6)*, France *(7)*, Netherlands *(8)* and Poland *(9)*.

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