cells of such morphology in the available scientific literature) [8]. The cells which had developed no complete monolayer survived during 29 days, their death causes being unknown. A primary cell culture prepared using *Lazerta agilis* kidneys and testicles survived at 29°C during 16 days.

Conclusions. The analysis of available published data proves the spreading of dangerous pathogens among reptiles, these pathogens being able to cause viral diseases of warm-blooded animals and humans. It is extremely necessary to pay attention to reptilian viruses investigation among Ukrainian reptilian populations to maintain and increase the biosafety in our country.

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Надійшла до редколегії 25.04.12

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FIRST DETECTION OF HOSTA VIRUS X IN UKRAINE

В статье представлены результаты мониторинга Х вируса хосты (HVX) в Национальном ботаническом саду Украины им. Н.Н. Гришка. На растениях хосты наблюдали симптомы мозаики и деформации листьев. Присутствие HVX-инфекции в растениях хосты сорта 'Sum of and substance' было подтверждено результатами ELISA, TEM и RT-PCR. HVX был изолирован из больных растений хосты, концентрация очищенного препарата HVX составляла приблизительно 3 мг/мл.

The results of Hosta virus X (HVX) monitoring in M.M. Hryshko National botanical garden of Ukraine (NAS of Ukraine) are presented. Leaf mosaic and deformation symptoms were observed on hosta plants. The presence of HVX in hosta cultivar 'Sum of and substance' was confirmed by ELISA, TEM and RT-PCR. HVX was isolated from diseased hosta. Purification of HVX from hosta leaves yielded about 3 mg/ml.

Hostas or (funkias) are very popular in Ukraine and worldwide, hardy herbaceous perennials grown primarily for their beautiful foliage. Hosta plants generally reache full maturity in 4-8 years, and their size depends on the cultivar [1]. Hosta foliage can be blue, gold (yellow), or green and are often variegated. They are easy to grow and shade-tolerant plants. The plants are low maintenance and are widely available in nurseries and garden centers. In Ukraine hosta settled in private collections, but the biggest collection has been mounted in the National botanical garden by M.M. Hryshko. Certain hosta cultivars are susceptible to a number of viruses, including Hosta Virus X (HVX). Despite its relatively recent discovery, HVX had a significant impact on the hosta industry resulting from the infection and subsequent destruction of many HVX-infected hostas [2]. Symptoms of this virus include mosaic, chlorosis, and necrosis on leaves; severely affected leaves may wither and die. HVX can be transmitted during vegetative propagation of plants and mechanically. Diagnostic laboratories and nursery surveys of symptomatic plantshave confirmed a number of HVX-infected hosta cultivars worldwide [3]. New HVX-infected hosta cultivars continue tobe detected. Investigations of distribution of HVX in Ukraine have not been conducted. The aim of this work was monitoring of HVX in M.M. Hryshko National botanical garden of Ukraine.

Materials and methods. During autumn 2011, the samples of hostas exhibiting virus-like symptoms were collected from M. M. Gryshko National Botanical Garden (NAS of Ukraine). Several samples were collected from symptomless plants. A total 21 samples from 4 hosta cultivars ('Sum and substance', 'Striptease', 'Lady Guinevere', 'Old Faithful') were analyzed for the presence of the virus.

Taking into account serological relation between HVX and PVX (potato virus X) indirect ELISA tests of hosta samples were carried out using antiserum to PVX.

For EM examination, partially purified plant sap was mounted on formvar grids and negatively stained with 2% uranyl acetate. The preparations were viewed under the electron microscope at an instrumental magnification of 20,000.

The method used or virus purification was adapted from those of Goodman (1975) for PVX. The virus was purified by clarification in chloroform, precipitations using polyethylene glycol 6000, and differential centrifugation.

Total RNA was extracted from plant material and subjected to RT-PCR using primers a6448 and s5722 corresponding to nucleotide sequences 6448-6428 and 5722-5742, respectively of HVX from Korea (HVX-Kr) (GenBank Accession No. AJ620114)[1,3].

Results and discussion. The main objective of the present investigation was diagnostic of hosta plantings for infection with HVX in Ukraine. For this purpose four varieties of hosta were tested. Visual monitoring of hosta plantings revealed the presence of plants with symptoms of viral disease. On hosta plants of cultivar 'Sum of and substance' we observed dark, green streaking and puckering along the leaf veins (Fig. 1.). The plants of cultivars 'Striptease' and 'Lady Guinevere' with symptoms of chlorosis and leaf desiccation were collected for farther analysis (Fig. 2,3). Hosta 'Old Faithful' displayed puckering and distortion on leaves (Fig. 4.). According to the literature data the symptoms of HXV-infection can vary depending on cultivar, plant age and growth condition. The viral symptoms also can be confused with symptoms cased by other ethiological agents. To reveal viral etiology of diseases more specific serological methods should be used.

Fig.1. Virus-like symptoms on hosta of cultivar 'Sum of and substance'

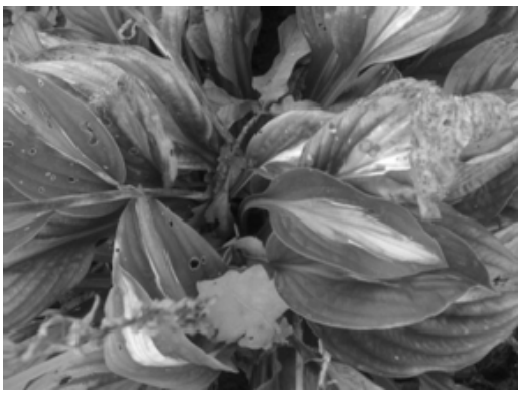

Fig.2. Chlorosis and leaf desiccation on hosta of cultivar 'Striptease'

Fig. 3. Color blotches and leaf desiccation of hosta 'Lady Guinevere'

Fig. 4. Puckering and distortion on leaves of hosta 'Old Faithful'

HVX belongs to the genus potexvirus and is serologicaly related to PVX so it was a good chance that newly isolated HVX would react with antiserum raised against PVX. Given this we used anti-PVX antiserum for primary identification of HVX in collected samples. According to ELISA results HVX was detected in pants of 'Sum of and substance' cultivar (Fig. 5), however absorbance value in this case was low. The samples of other hosta cultivars gave negative results is ELISA.

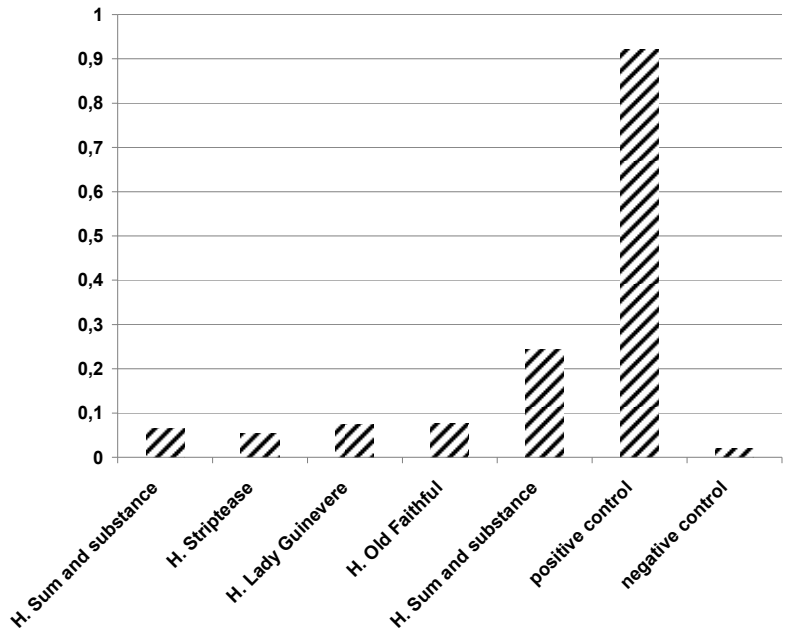

Fig.5. ELISA results for HVX detection in the samples of hosta

Transmission electron microscopy revealed the presence of filamentous viral particles in partially cleared plant sap obtained from hosta of cultivar 'Sum of and substance' (fig.6). The modal length and diameter of such particles were 470-580 and 13 nm, respectively. According to virion morphology and size observed viral particle correspond to HVX.

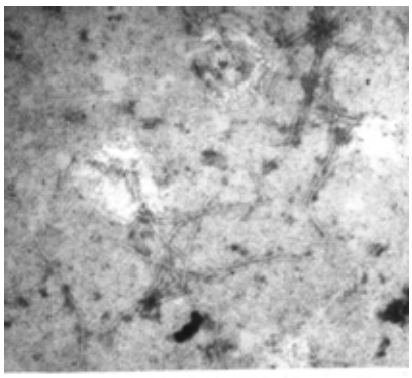

Fig.6. Electron micrographs of virus isolated from hosta (instrumental magnification 20,000)

The virus was purified from systematically infected of hosta plants (cultivar 'Sum of and substance'). An yield of 3,1 mg /ml was thus obtained. The 260/280 ratio of the purified preparation was 1.37. Purified virus will be used for the future research of isolated virus and for obtaining diagnostic antiserum.

Taking into account low absorbance values obtained under ELISA we performed RT-PCR for detection of HXV in collected samples. At raising of RT – PCR used kit SYPER – SKRIPT – 2(Invitrogen, USA), and pair of primers that amplification product by molecular mass 706[9]. We modified methodology of raising for reaction. On the first stage conducted a selection to the virus(but not RNA). After warming up, the standards of virus were used for raising of RT – PCR (without a previous selection to RNA). As a result by the method of electrophoresis in 1,5% agarose gels are educed product of amplification of corresponding molecular mass (fig. 7).

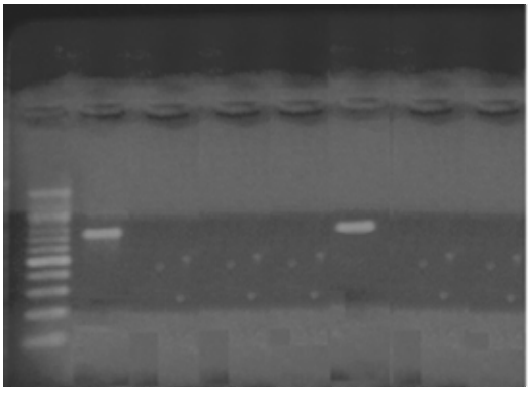

Fig.7. Agarose gel electrophoresis of RT-PCR products from hosta plants.
M – markers, 1 -Hosta ' Sum and substance', 2 – hosta 'Striptease', 3 – hosta 'Lady Guinevere', 4 – Hosta 'Old Faithfu', 5 – is positive control, 6 is negative control

The presence of HVX in hosta cultivar' Sum and substance' was confirmed by RT-PCR using a specific primer pair that amplifies the HVX coat protein (fig. 7). The amplified product with corresponding molecular mass (706 bp.) was obtained and will be used for the nucleotide sequence determining of CP-gene of HVX Ukrainian isolate and for phylogenetic analysis. The virus was not detected in samples of another hosta cultivars.

Conclusions. Monitoring of hosta virus X in M. M. Gryshko National Botanical Garden revealed the presence of this virus in at least one hosta cultivar. According ELISA and RT-PCR results another three investigated hosta cultivars were free from HVX. However all this cultivars displayed the symptoms of viral infection. Hosta plants can be infected with several viruses including impatiens necrotic spot virus, tomato ringspot virus, tobacco rattle virus. The causative agent of virus-like symptoms in this case should be established using specific methods.

Identified HVX was purified from plant material and will be used for obtaining antiserum with in turn will enable a diagnostic of this virus in Ukrainian collections of hosta. Hosta is a popular perennial ornamental plant in Ukraine. Hence, measures should be taken to reduce HVX spread. Screening of hostas for HVX prior to distribution is the first important step in control of this virus.

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Надійшла до редколегії 26.04.12