Thus, the Ukrainian PMMoV isolate belongs to a cluster containing virus strains found in Spain and Korea.

Conclusions. According to the DAS-ELISA, tobamoviruses PMMoV and ToMV are prevalent among vegetable crops in the studied regions of Ukraine. Electron microscopic studies confirmed the presence of rod-shaped virus particles typical of *Tobamovirus* genus, namely, for the *Pepper mild mottle virus* and *Tomato mosaic virus* whose size is approximately 310±3 x 15±3 nm and 300±3 x 19±3 nm, respectively. We have generated a cDNA of 387 bp corresponding to the coat protein gene of Ukrainian PMMoV isolate. Phylogenetic analysis of the coat protein gene of PMMoV showed that Ukrainian PMMoV isolate groups into a cluster with strains detected in Spain and Korea.

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НОВИЙ ПРЕДСТАВНИК РОДУ *TOBAMOVIRUS*, ВИДІЛЕНИЙ З ОВОЧЕВИХ КУЛЬТУР В УКРАЇНІ

Знайдено новий для України вірус овочевих культур, зокрема рослин родини Solanaceae, визначено розмір та морфологію його вірусних часток. Встановлено приналежність українського ізоляту PMMoV до кластерів штамів вірусів Іспанії та Кореї.

Ключові слова: Tobamovirus, полімеразна ланцюгова реакція, філогенія, PMMoV.

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НОВЫЙ ПРЕДСТАВИТЕЛЬ РОДА *TOBAMOVIRUS*, ВЫДЕЛЕНЫЙ ИЗ ОВОЩНЫХ КУЛЬТУР

Найден новый для Украины вирус овощных культур, в частности растений семейства Solanaceae, определен размер и морфология вирусных частиц. Установлено принадлежность украинского изолята PMMoV к кластерам штаммов вирусов Испании и Кореи.

Ключевые слова: Tobamovirus, полимеразная цепная реакция, филогения, PMMoV.

UDK 578

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PHYLOGENETIC ANALYSIS OF HOSTA VIRUS X ISOLATED IN UKRAINE

Hosta virus X (Potexvirus, HVX) was identified by ELISA in different cultivars of hosta selected from M.M. Hryshko National botanical garden of Ukraine (NAS of Ukraine). Coat protein gene sequences of Ukrainaian HVX isolate was determined and compared with all known strains and isolates from Gene Bank. Phylogenetic trees were constructed using neighbour-joining and maximum likelihood methods. The CP of Ukrainian isolate shared 99–100% nucleotide and amino acid sequence identity with American isolates.

Key words: Hosta virus X, ELISA, Gene Bank.

Introduction. Hosta spp. are popular herbaceous perennial plants with more than 7000 varieties, and widely cultivated due to their diversity in leaf shape and color patterns, shade tolerance and pest resistance. Hosta virus X *(Potexvirus*, HVX) is a very serious problem for hosta growers. This virus was first identified and described in Minnesota, USA in 1996. Since then, HVX has been reported from other US states, Canada, Europe as well as from other continents. HVX is generally considered to be the most economically important virus infecting hostas [1]. As HVX is an emerging disease that is causing problems for growers, garden centers, and gardeners, the EPPO Secretariat felt that HVX could usefully be added to the EPPO Alert List. As HVX is sap-transmissible, it is easily transmitted during vegetative plant propagation. Hostas can also be propagated by seeds, but the possible seed transmission of HVX needs to be clarified. As is the case for other potexviruses, HVX is also spread by mechanical contact. Therefore, it is easily transmitted from plant to plant on hands and tools (e.g. pruning tools when removing old leaves or flowers). Over long distances, trade of infected plants has probably been the most significant source of the disease. In addition, it is suggested that some cultivars which have been selected and commercialized because of their 'interesting foliage' were in fact infected by HVX, which has contributed to further spreading the virus. There is no evidence that HVX might be transmitted by insects or other vectors [2, 3].

As is the case for other viruses, the control of the disease is difficult and essentially based on the use of resistant cultivars and of prophylactic measures to minimize the possibility of mechanical transmission of HVX. The production of virus-free planting material through the implementation of certification schemes could also contribute to limiting the spread of HVX [4,5].

We have previously shown that HVX infected some varieties of Hosta plants from the M. M. Gryshko National Botanic Gardens. The aim of this study was to carry out a phylogenetic analysis of Ukrainian isolate of HVX.

Materials and methods:

The samples of various hosta cultivars were collected from M. M. Gryshko National Botanical Garden (NAS of Ukraine). The investigated hosta cultivars included: Hosta Sum and substance, Hosta Striptease, Hosta Lady Guinevere, Hosta spp, Hosta Venticosa, Hosta Udulata, Hosta. Halcyon, Hosta. Crispula Maek, Hosta Gold Standart, Hosta Great Expectation, Hosta Ultraviolet light, Hosta Mediovariegata, Hosta spp, Hosta, Hosta August Moon, Hosta Twilight, Hosta Paul Glory, Hosta Siboldiana, Hosta Whirlwind, Hosta Abigua, Hosta Wide Brim. The samples were selected from plants with the following symptoms: systemic chlorosis, veinal chlorosis interveinal chlo-

rosis, leaf discoloration, leaf rolling and curling, necrotic lesions. Indirect ELISA tests of hosta samples were carried out using antiserum obtained in our laboratory earlier. The results were measured automatically with ELISA reader Stat Fax 2100 (Awarennes Technology, USA) at 405 (for alkaline phosphatase conjugated antibodies). Reverse transcription polymerase chain reaction (RT–PCR) was accomplished using kit SuperScript II (Invitrogen, USA) and a primer pair that amplified a 706 bp fragment [6].

RT–PCR was carried out according to manufacturer recommendations. The amplified product from hosta cultivar *Sum and substance* was sequenced on Applied Biosystems 3730x1 DNA Analyzer using Big Dye terminators, version 3.1 (Applied Biosystems, USA). Obtained sequences were identified and compared using BLAST-analysis (http://www.ncbi.nlm.him.gov).

Phylogenetic analysis was realized through program packages of MEGA 5 [7]. Bootstrep test with 1000 bootstrep replications was applied to check the reliability of phylogenetic trees. Phylogenetic trees were constructed using neighbour-joining (NJ) and maximum likelihood (ML) methods. Statistical analyses of data obtained was carried out using MS Excel software with performing Student's *t* test.

Results and discussion:

HVX was identified by ELISA in the samples from the next cultivars of hosta: Sum and substance,Halcyon, Crispula Maek, Gold Standart, Great Expectation, Ultraviolet light. Further, the samples were used to optimize the conditions of RT-PSR for hosta virus X detection. The first phase of investigations included the extraction of the virus from plant material (non RNA extraction). After heating, the samples of isolated virus were subjected to RT-PCR (without prior RNA extraction). Agarose gel electrophoresis revealed the presence of amplified products with corresponding molecular weight (Fig. 1).

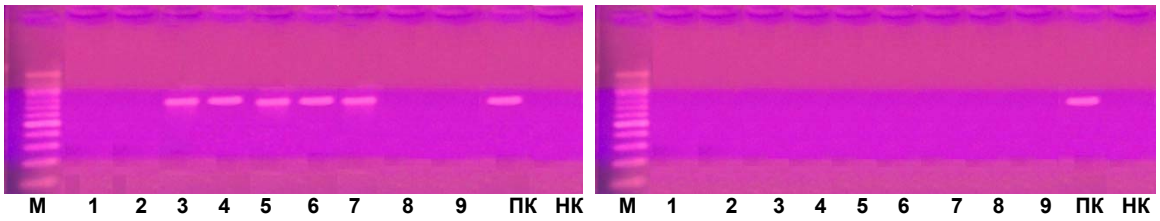

Fig. 1. Electrophoretic studies of RT-PCR products in agarose gel:
M – markers, Fermentas 100 bp, 1 – sample isolated from *Hosta Venticosa*, 2 – sample isolated from *H. Udulata, 3 – H. Halcyon, 4 – H. Crispula Maek, 5 – H. Gold Standart,*6 – *H. Great Expectation.,* 7 – *H. Ultraviolet light,* 8 – *H. Mediovariegata,* 9 – *Hosta spp.*, 10 – *Hosta spp*, 11 – *H. August Moon,* 12 – *H. Twilight,* 13 – *Hosta Paul Glory* 14 – *H. Paul Glory,* 15 – *H. Siboldiana,* 16 – *H. Whirlwind,* 17 – *H. Abigua,* 18 – *H. Wide Brim*, ПК- positive control (Sum and substance), НГ – negative control

As can be seen from Figure 1. five amplified products with expected size (706 b.p.) were obtained. Amplified product from hosta *Sum and substance* further was used for sequencing and construction of phylogenic trees.

Comparison of Ukrainian isolate nucleotide sequences with available in databases sequences of known HVX strains and isolates revealed high percent of their similarity (near 99% for nucleotide sequences and near 98% for amino acid sequences) to American isolates. Thus Ukrainian isolate and American isolates probably belong to the same strain (Table 1).

***Table* 1. Comparison of amino acid and nucleotide sequences of HVX isolated in Ukraine with different strains and isolates from GenBank**

Isolate	Nucleotide sequense, %	Amino acid, %
HVX_USA_Sum_and_Substance	99,1	97
HVX_Poland_Sum_it_Up	99,1	97
HVX_Korea	98,6	95,2
HVX-Kr	98,6	95,2
HVX_Poland_Vim_and_Vigor	98,9	96,4
HVX_Poland_Sum_and_Substance1	98,8	97
HVX_USA_Striptease	99,7	98,8
HVX_Poland_Sum_and_Substance	98,6	96,4
HVX_USA:_Tennessee	99,5	98,8
HVX_USA_Sum_and_Substance_2	99,2	97,6
HVX_USA_Sugar_and_Cream	99,2	98,2
HVX_USA_Hosta_fortunei_Antioch	99,2	98,2
HVX_USA_Gold_Standard	99,2	97,6
HVX_USA_Sum_and_Substance1	99,1	97
HVX-37_USA_Sum_and_Substance	99,1	97
HVX-36_USA_Sum_and_Substance	99,1	97
HVX_USA_HVX-U	99,1	97
HVX_China	98,9	97
HVX_Czech_Republic	98,9	97,6

Construction of phylogenetic tree by NJ method (Fig.2) demonstrated the similarity of Ukrainian isolates with other HVX isolates that is confirmed by date available in Table1.

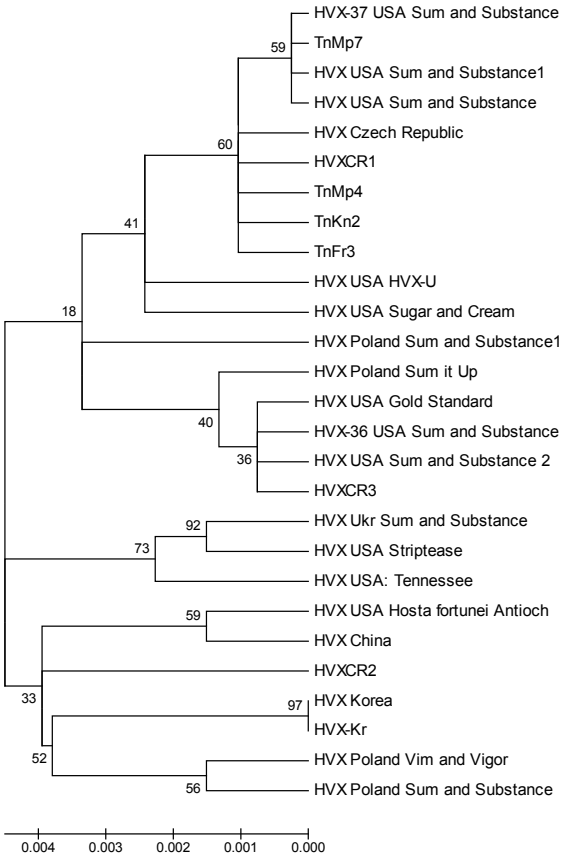

Fig.2. Evolution relationships of Ukrainian HVX isolate with known from Gene bank isolates (NJ is 1000 bootstrap replications)

To determine possible origin of Ukrainian isolate another approach to analysis of obtained nucleotide was applied. Maximum Likelihood method was chosen as discrete method and was performed using model JC+I. The chose of model for calculation was accomplished with program software MEGA5. As we can see from obtained phylogenetic tree (Fig.3), Ukrainian and American isolates are placed in the same cluster (have a joint origin) and possibly have a common ancestor.

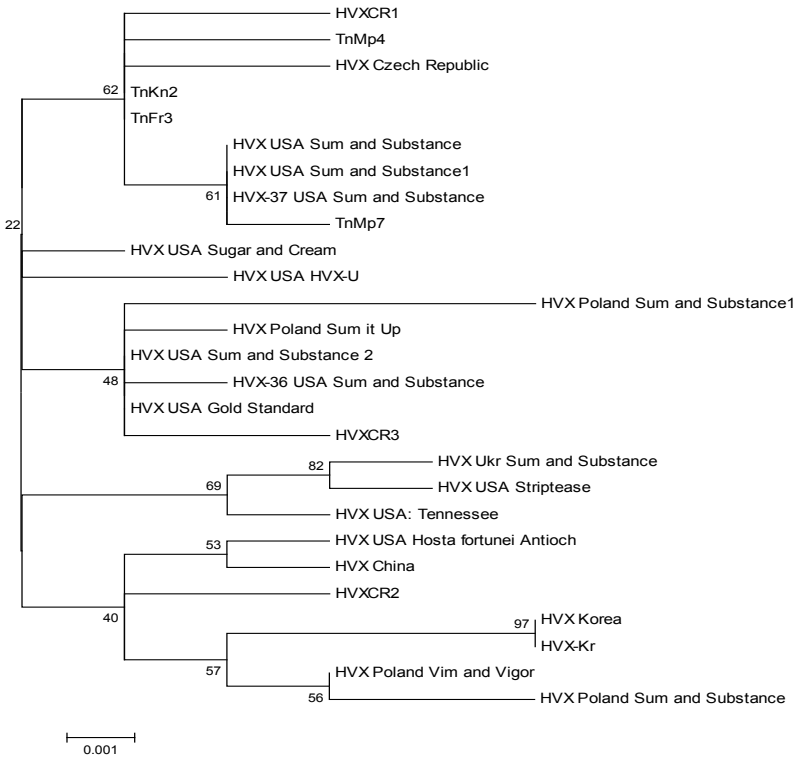

Fig.3. Molecular phylogenetic analysis of HVX capsid protein gene, constructed by Maximum likelihood method (ML is 1000 bootstrap replications)

We can see from phylogenetic tree that cluster separation depends neither on geography of virus isolation (Ukrainian isolate are similar to American) nor on plant cultivar as Ukraine isolate was extracted from *Sum and substance* hosta cultivar whereas similar American isolate was selected from hosta *Striptease*.

Our data is in agreement with literuture data according to with analisys of CP і TGB1 aminoacid sequenses of all known HVX strains confirmed their monophyletic origin. Never the less the relationships between different isolates of HVX are still unclear [8].

It was also shown that the HVX-CP gene is less variable than TGB1, which suggests that CP is possibly under more stringent selection pressure than TGB1. The substitutions observed among the isolates in their respective sequences of the two genes were irregularly distributed. The 3'-proximal part of CP was the least variable region. This is probably due to its critical role in mediating essential functions such as interaction with the genomic RNA, movement and encapsidation [9,10].

Observed HVX genetic variability possibly has biological value. There are many instances where it has been shown that a single amino acid changes in the CP of a plant virus has a significant impact on virus/host interactions. Hence, additional investigations are required to determine the biological significance of the observed amino acid sequence diversity in CP and TGB1 of HVX.

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ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ Х ВІРУСУ ХОСТИ ДЕТЕКТОВАНОГО НА ТЕРИТОРІЇ УКРАЇНИ

Х-вірус хости (Potexvirus, HVX) був ідентифікований методом ІФА в різних сортах хоста з колекції Національного ботанічного садаНАН України ім. М.М. Гришка. Отримано сиквенс гена білка оболонки українського ізоляту HVX. Отримана послідовність порівнювалася з відомими штамами з Генбанка. Филогенетические дерева були побудовані за допомогою методу об'єднання сусідів і максимальної правдоподібності. Ген капсидного білка українського ізоляту на 99-100% подібні за нуклеотидним і амінокислотним послідовностей з американськими изолятами.

Ключові слова: х-вірус хости, імуноферментний аналіз, генбанк.

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Х-вирус хосты (Potexvirus, HVX) был идентифицирован методом ИФА в различных сортах хосты из коллекции Национального ботанического сада НАН Украины им. Н.Н. Гришка. Получен сиквенс гена белка оболочки украинского изолята HVX. Полученная последовательность сравнивалась с известными штаммами из генбанка. Филогенетические деревья были построены с помощью метода объединения соседей и максимального правдоподобия. Ген капсидного белка украинского изолята на 99-100% подобен по нуклеотидным и аминокислотным последовательностям с американскими изолятами.

Ключовые слова: х-вирус хосты, иммуноферментный анализ, генбанк.

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COMPLEMENTARY RESULTS OF LUMINESCENT AND TRANSMISSION ELECTRON MICROSCOPY PROVIDE STRIKING EVIDENCE OF HEAVY METAL IONS' EFFECT ON THE FORMATION OF AGGREGATES OF *TOBACCO MOSAIC VIRUS* VIRIONS BOTH *IN VITRO* AND *IN VIVO*

In vitro electron microscopy studies showed that virus particles treated with heavy metals aggregate creating either 'typical' lateral (side-to-side) aggregates of virions or star-like ones not reported previously. Luminescent light microscopy of epidermal cells of virus-infected tobacco plants demonstrated that metal treatment has led to the appearance of mostly amorphous and noncompact inclusion bodies, which were not typical for cells of plants not stressed with a heavy metal. Finally, electron microscopy of thin sections of tissues of virus-infected tobacco plants showed that metal-affected cells contained higher numbers of larger crystalline multilayered inclusions consisting of virus particles.

Key words: virus-infected tobacco, metal-affected cells.

Introduction. Previously we have shown that heavy metal contamination of ecosystems favours plant virus spread [1, 2], more intense accumulation of viruses by systemically infected plants and delay in the onset of virus-specific symptoms [3]. We have also demonstrated positive correlation between the heavy metal content in soil and virus concentration in tissues of plants grown in such soil [4]. Long-term virus passaging in heavy metal-stressed plants has been shown to affect neither virus infectivity nor the appearance of local virus-specific symptoms [4, 5].

According to the proposed hypothesis (partially confirmed by the outcomes of laboratory and small-scale field